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**“DEVELOPMENT OF INNOVATIVE
EXPERIMENTAL PROTOCOLS AND
COMPUTATIONAL TOOLS FOR ASSESSING
NEURAL CONNECTIVITY IN ARM AND
SHOULDER MUSCLES”**

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Abstract

Il sistema neuromuscolare è gerarchico, con componenti strettamente interconnesse che operano in modo coordinato e sinergico. La generazione del movimento ha origine da segnali provenienti dal sistema nervoso centrale, che vengono trasmessi ai muscoli attraverso i motoneuroni spinali. L'unità motoria, costituita da un motoneurone alfa e dalle fibre che esso innerva, agisce come un trasduttore convertendo l'input neurale in un segnale elettrico (potenziale d'azione) che raggiunge il muscolo. Questa attività elettrica produce una risposta meccanica, espressa come forza.

La forza generata dal muscolo è strettamente legata alla scarica dei potenziali d'azione e riflette le proprietà intrinseche e sinaptiche dei motoneuroni. Pertanto, analizzando l'attività elettrica muscolare è possibile ottenere informazioni indirette sui meccanismi neurali alla base del controllo motorio. Tecniche non invasive come l'elettromiografia di superficie ad alta densità (HD-sEMG) consentono di investigare questi segnali neurali a livello di unità motoria, offrendo uno strumento per studiare l'organizzazione e la modulazione del drive neurale nell'uomo.

La prima parte della tesi introduce gli obiettivi della ricerca e illustra come gli studi condotti durante il dottorato siano concettualmente collegati tra loro. Nella seconda parte (capitoli 2-4) viene rivisto lo stato dell'arte, fornendo il quadro teorico di riferimento per il lavoro sperimentale. La terza parte della tesi presenta gli studi sperimentali, che nel loro insieme mirano a investigare come il controllo neurale dei muscoli sia organizzato e modulato in diversi contesti. In particolare, questi studi analizzano il drive neurale al muscolo attraverso l'analisi delle unità motorie in differenti condizioni, tra cui apprendimento motorio, contesto (azioni isolate vs sinergiche) e livelli di forza.

Gli studi sono stati condotti e presentati in modo progressivo, permettendo di caratterizzare il controllo neurale e la connettività a partire da muscoli ben descritti in letteratura fino ad arrivare a muscoli meno caratterizzati e metodologicamente più complessi. L'analisi comprende la valutazione della frequenza di scarica (rate coding), della variabilità della forza e della scarica delle unità motorie, della distribuzione spaziale delle unità motorie (territorio) e dell'analisi dell'input comune, tutti parametri derivati dalla decomposizione HD-sEMG.

La tesi si conclude integrando i principali risultati e discutendone le implicazioni.

Part 1: Introduction

1.1 General introduction

The neuromuscular system is hierarchical, with components closely interconnected and operating in a coordinated, synergistic manner. Movement generation originates from signals within the central nervous system, which are transmitted to the muscles via spinal motoneurons. The motor unit, consisting of an alpha motoneuron and the fibres it innervates (Sherrington, 1925), acts as a transducer by converting the neural input into an electrical signal (action potential) that is delivered to the muscle. This electrical activity ultimately produces a mechanical response, expressed as force output (Heckman & Enoka, 2012).

The force generated by the muscle is strictly related to the discharge of action potentials in motoneurons and reflects their intrinsic and synaptic properties. Therefore, by analysing muscle electrical activity, it is possible to gain indirect insight into the neural mechanisms underlying motor control. Non-invasive techniques such as high-density surface electromyography (HD-sEMG) allow investigation of these neural signals at the motor unit level, providing a powerful tool for studying the organization and modulation of neural drive in humans (Farina & Negro, 2015).

This first part of the thesis introduces the research objectives and outlines how the studies conducted during the PhD program are conceptually connected.

In the second part (chapters 2-4), the current state of the art is revised, providing the main theoretical framework underlying the experimental work. Chapter 2 describes the anatomical and physiological mechanisms involved in movement generation, with a focus on how different structures interact to ensure the accurate execution of motor tasks. This chapter is essential for contextualizing the experimental work in this thesis, as it provides the conceptual basis for interpreting the modulation of motor unit behaviour and neural connectivity investigated across the different studies. Chapter 3 presents the principles of HD-sEMG, the primary methodological approach employed in all the studies included in this thesis to investigate neural control. This chapter explains why this technique is suited to quantify motor unit discharge behaviour and neural connectivity, and it clarifies how the methodological framework adopted in this thesis enables the investigation of the neuromuscular systems. Chapter 4 describes the anatomy and biomechanics of the shoulder joint, which represents the main target of the experimental investigation of this thesis. This chapter highlights the anatomical and functional characteristics that make the shoulder an underexplored region in motor unit research and explains why addressing this gap is a central

aim of the thesis. Together, these chapters form a coherent state-of-the-art section that supports the experimental work, providing the foundations and guiding the reader toward understanding the motivation behind the studies presented and the interpretation of their results.

The third part of the thesis presents the experimental studies, which collectively aim to investigate how neural control of muscles is organized and modulated across different contexts. Specifically, these studies investigate the neural drive to muscle with motor unit analysis under different conditions, including motor learning, task context (isolated versus synergistic actions), and force gradation. These studies were conducted and presented progressively, allowing the characterization of neural control and connectivity starting from muscles that are well described in the literature and moving toward those that are less characterized and methodologically more complex. This analysis includes the assessment of discharge rate (rate coding), variability in force and motor unit discharge, motor unit spatial distribution (territory), and common input analysis, all derived from HD-sEMG decomposition. The specific objectives of each study and their conceptual interconnections are presented in Section 1.2.

Finally, this thesis concludes with a discussion of the main findings from the experimental studies, highlighting their contributions to the existing neurophysiological literature and outlining potential directions for future research.

1.2 Research aims

1.2.1 General aim

This project aims to investigate how neural control and connectivity to spinal motoneurons are organized and modulated during voluntary contractions across different functional contexts and body regions. Specifically, the project seeks to develop and apply innovative HD-sEMG protocols to assess neural drive and motor unit behaviour, with a particular focus on extending these approaches to the shoulder joint complex, a body region that remains underexplored due to its biomechanical and anatomical complexity.

1.2.2 Specific aims

The overall objective of this project was structured into a series of interconnected studies conducted throughout the three years of the doctoral program. These studies were

designed to progressively address increasing levels of neuromuscular complexity, ultimately converging on the investigation of the shoulder joint.

The overarching goal was to elucidate the neural control mechanisms underlying movement in the shoulder complex, a region characterized by high anatomical, biomechanical, and functional complexity. To achieve this, a stepwise research strategy was adopted. This approach began by establishing a general understanding of motor unit behaviour across muscles, then progressively moved to more complex motor tasks and anatomical regions, culminating in the investigation of the shoulder.

The first step of this research strategy addressed a fundamental gap in the literature: the lack of a comprehensive synthesis of motor unit behaviour assessed with HD-sEMG across different muscles and isometric force levels. Therefore, the initial study consisted of a systematic literature review aimed at summarizing the existing evidence on motor unit discharge behaviour during voluntary isometric contractions. This review revealed that the literature is heavily biased toward lower-limb muscles, while shoulder-movement muscles remain largely unexplored. These findings highlighted the need to extend motor unit investigations to more complex and less-studied anatomical regions of the human body.

Building on this foundation, the subsequent experimental studies were designed to investigate how neural control is modulated across different contexts. Importantly, the progression of these studies followed a rationale of progressively increasing complexity, both in terms of motor task and anatomical structure.

The first experimental study focused on well-characterized monoarticular muscles of the lower limb (vastus medialis and vastus lateralis). This model was selected to investigate how neural connectivity between motoneurons is modulated during motor learning, while minimizing methodological and anatomical complexity. This step provided a controlled framework to examine adaptations in neural drive associated with skill acquisition.

The second experimental study extended this investigation to the upper limb, specifically to the hand, which represents a more complex system involving multiarticular muscles. This study examined how neural control is shaped by behavioural context, by comparing isolated finger movements with a synergistic action (grasping). This approach enabled investigation of task-dependent modulation of neural connectivity in a system of greater functional relevance and complexity.

Finally, the last experimental study focused on the shoulder complex, specifically on the deltoid muscle. At this stage, the investigation targeted both anatomical and functional complexity, analyzing motor unit discharge behaviour across various force levels and at two different joint angles. Given the low number of studies on deltoid motor units, all of which utilized exclusively intramuscular EMG (Alpaydin Baslo et al., 2024a; de Luca et al., 1982; Doherty & Stashuk, 2003), this work aimed to characterize rate coding strategies and their modulation as a function of biomechanical conditions, providing novel insights into the neural control of this muscle.

Collectively, these studies form a coherent research project that progresses from general characterization to context-dependent modulation and, ultimately, to the investigation of a complex and underexplored anatomical region. This integrative approach enables a more comprehensive understanding of how neural control strategies are organized and adapted across different muscles, tasks, and functional demands.

1.3 Flow Diagram

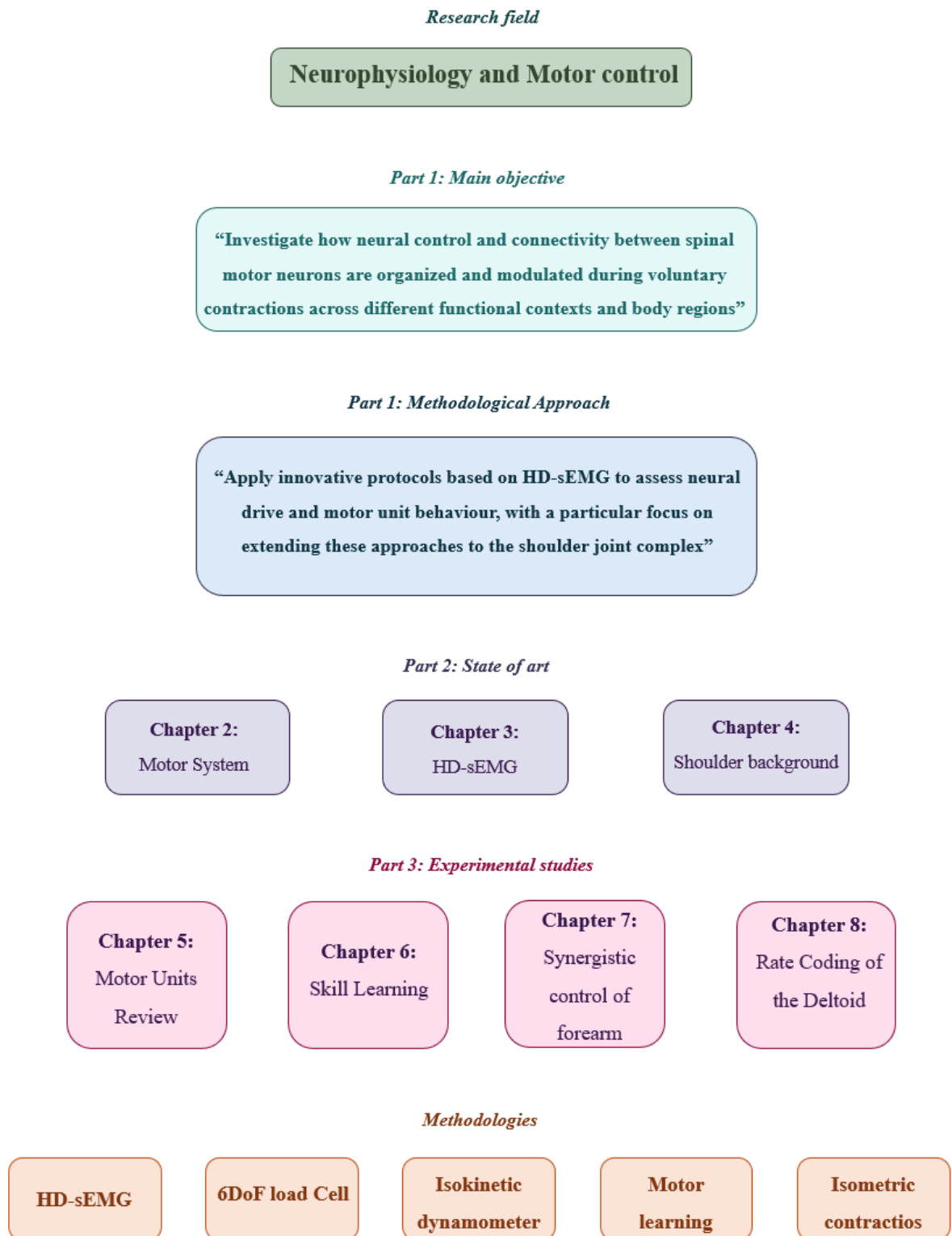


Figure 1.1: Flow diagram of the research project

Part 2: State of the art

2. General overview of the motor system

In this chapter, the organization of the neuromuscular system is described, with particular emphasis on how its components contribute to the transmission of synaptic input from the central nervous system (motor cortex) to the muscle, where contraction is ultimately generated. This overview provides the conceptual foundation required to interpret the modulation of motor unit behaviour examined throughout this thesis.

First, the principal components of the neuromuscular system are introduced, including the neural structures, the motoneuron, the muscle fibres, the motor unit, and the sensory feedback system. This background is essential for understanding how neural drive is generated and how it can be quantified through motor unit analysis, which represents the core methodological approach of the experimental studies.

The chapter then outlines the processes underlying movement generation, from the initiation of neural signals within the central nervous system to their propagation to the muscle fibres, where they are translated into mechanical force through mechanisms of muscle contraction. This framework is crucial for interpreting the experimental work of this thesis, which investigates how neural connectivity and motor unit discharge behaviour are modulated across different conditions, force intensities, and anatomical regions.

Finally, both neural and intrinsic muscular factors involved in the modulation of force production are discussed, with particular relevance to the analysis of motor unit behaviour and neural drive, which represent the central focus of this thesis.

2.1 Hierarchical Organization of the Motor System

The human motor system is a complex hierarchical system that enables the generation of a wide repertoire of movements. These movements are achieved through the coordinated interaction of neural, muscular, articular, and sensory components (Kandel et al., 2021).

This hierarchical organization simplifies motor control by distributing functions across different levels. At the highest level, the cognitive stage, which is primarily associated with the prefrontal cortex, defines the goal of the movement (Wise, 1985). This is followed by the planning stage, involving the posterior parietal and premotor areas, where proprioceptive and sensory information is integrated and transformed into motor planning

(Andersen & Cui, 2009). Finally, the execution stage, which comprises the primary motor cortex, brainstem, and spinal cord, is responsible for translating these plans into coordinated motor output (Farina & Negro, 2015). Notably, this system does not operate in a strictly serial manner but relies on parallel processing and continuous sensory feedback to modulate motor output based on information received from the environment (Kandel et al., 2021).

As stated by Heckman and Enoka (2004), for the system to function effectively, several requirements must be met, including the ability to generate forces across a wide range of magnitudes and speeds, maintain stability and precision, and allow for flexible adjustments when necessary (Heckman & Enoka, 2004). These properties are achieved through the integration of neural commands and peripheral feedback mechanisms.

Furthermore, as described in detail in Windhorst's (2007) review, the production of movement involves a complex sequence of events that convert neural signals into mechanical output. Initially, to execute a movement, the nervous system constructs a body schema, which is continuously updated during task execution based on sensory inputs (Windhorst, 2007). Proprioceptive receptors, such as muscle spindles and Golgi tendon organs, provide information about muscle length and muscle tension, respectively (Hultborn, 2006). Subsequently, sensory information is integrated across different reference frames, requiring spatial transformations to generate appropriate motor commands (Scott, 2004). These commands are ultimately translated into force output at the level of the motoneurons, which act as the interface between the nervous and muscular systems, a concept that will be explored in detail in the following sections.

2.2 Principal components of the neuromuscular system

The neuromuscular system comprises several elements that work together to generate and control movement. The principal components are: (i) the neural control system, responsible for the generation of motor commands (Kandel et al., 2021); (ii) the motoneuron, which integrates synaptic inputs and acts as a transducer of neural signals (Sherrington, 1906); (iii) the neuromuscular junction, which transmits the neural signals to the muscle fibres (Sanes & Lichtman, 1999); (iv) the motor unit, which converts synaptic inputs into muscle force output (Heckman & Enoka, 2012). In addition, the sensory feedback system plays a fundamental role, continuously adjusting motor output via afferent inputs.

2.2.1 The neural component

The neural component comprises cortical and subcortical structures involved in the organization and planning of movement. These structures correspond to higher levels of the motor system hierarchy, including the prefrontal cortex, supplementary motor areas, and premotor regions, and generate motor commands that are transmitted through the corticospinal tract (Graziano, 2006).

2.2.2 The motoneuron

Motoneurons were historically considered a homogeneous population. However, it is now well established that they exhibit substantial heterogeneity in their structural, electrical, and functional properties (Binder et al., 1995; Burke et al., 1970; Manuel & Zytnicki, 2011).

Early studies demonstrated that different axons innervate distinct types of muscle fibres, leading to the classification of motoneurons into alpha and gamma (Eccles & Sherrington, 1930; Langley, 1922; Matthews, 1972). Alpha motoneurons have a low electrical threshold due to lower internal resistance, allowing for fast conduction of the action potential (Erlanger et al., 1926). They innervate extrafusal muscle fibers and are directly responsible for force production. Gamma motoneurons, in contrast, are smaller and characterized by slower potential propagation due to their high threshold; they innervate intrafusal fibers within muscle spindles and regulate their sensitivity (Leksell, 1945).

Motoneuron recruitment depends on their intrinsic properties and the synaptic input they receive. According to size-related principles (Henneman, 1957), smaller motoneurons require less synaptic current to reach the threshold for action potential generation due to lower membrane conductance, whereas larger motoneurons require greater input (Manuel & Zytnicki, 2011). This relationship plays a fundamental role in the graded control of force.

2.2.3 The alpha motoneuron

Alpha motoneurons are the 'final common pathway' of the neuromuscular system, as originally introduced by Sherrington in 1906 (Sherrington, 1906). They integrate and convert synaptic inputs from different pathways (efferent, interneurons, afferent) into trains of action potentials that drive muscle contraction (Manuel & Zytnicki, 2011).

The alpha motoneurons are located in the ventral horn of the spinal cord and brainstem (Heckman & Enoke, 2004), and a single motoneuron may innervate from as few

(e.g., 5-10) to several thousand muscle fibres. This innervation ratio is closely related to the muscle's functional requirements, directly affecting the maximal force the motor unit can produce (Heckman & Enoka, 2012; Klein et al., 2001; Van Cutsem et al., 1997).

Structurally, motoneurons are composed of dendrites, a soma, and an axon (Figure 2.1). The dendrites, which are arranged in a branching pattern, receive and integrate synaptic inputs from various neural sources (Moore & Appenteng, 1991). These inputs are transmitted to the soma and initial axon segment, where action potentials are generated through the coordinated activity of voltage-gated ion channels (Powers & Binder, 2001). The axon then propagates these signals toward the muscle fibres via saltatory conduction, enabled by myelination (Black et al., 1990).

Overall, alpha motoneurons act as integrators of neural inputs, determining the timing and pattern of motor unit discharge (Heckman et al., 2003).

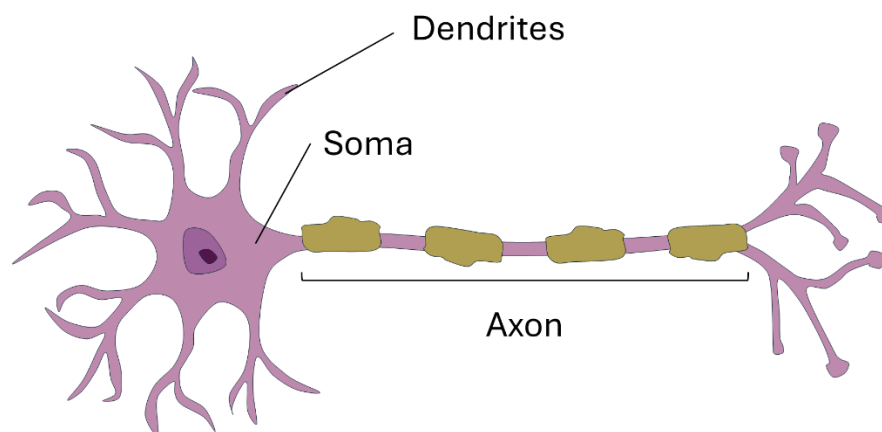


Figure 2.1. The alpha motoneuron. Schematic representation of the main structural components of the alpha motoneuron, including dendrites, soma and axon.

2.2.4 The muscle unit

The muscle unit is considered the motor of movement; it comprises all the muscle fibres innervated by a single motoneuron. Its functioning is rooted in four principles described by Heckman and Enoka (2004):

- I. Muscle mechanics: The muscle unit is responsible for converting the action potential received from the motoneuron into mechanical tension. A more detailed description of the sarcomere and the mechanical contraction process is provided in subsection 2.3.2.
- II. Number of cross-bridges: The force that can be exerted is proportional to the number of formed cross-bridges, which in turn depends on several factors

(subsection 2.4.2) such as calcium concentration, the force-length relationship, the force-velocity relationship and muscle stretch.

III. Energy substrates: To form cross-bridges and consequently generate movement, the muscle fibre utilizes ATP. Since ATP is limited, the muscular system regenerates it through different metabolic pathways: the anaerobic alactic system for maximal-intensity, very short-duration efforts; the anaerobic lactic system for high-intensity, short-duration efforts; and the aerobic system for low-intensity, prolonged efforts.

IV. Mechanical properties of the muscle unit: The mechanical properties of a motor unit are directly derived from the specific characteristics of the muscle fibres that constitute the muscle unit (subsection 2.4.2).

2.2.5 The motor unit

The motor unit, introduced by Sherrington in 1925, is the functional unit through which the nervous system controls movement (Liddell & Sherrington, 1925). This structure represents the core element analysed experimentally in the studies of this thesis. As detailed in the next chapter (chapter 3), HD-sEMG decomposition enables the extraction of motor unit discharge patterns during voluntary force production, allowing direct investigation of the neural drive to muscle. Anatomically, a motor unit consists of an alpha motoneuron and all the muscle fibres it innervates (Heckman & Enoka, 2012), acting as a neuromechanical transducer that converts neural inputs into force (Farina & Negro, 2015). Typically, skeletal muscles contain from a few dozen to over a thousand motor units (Enoka & Fuglevand, 2001).

Motor units are organized into motoneuron pools, which comprise all motoneurons innervating a given muscle (Heckman & Enoka, 2012; Routal & Pal, 1999; Tomlinson & Irving, 1977). These units vary in size and properties, with smaller motoneurons innervating fewer muscle fibres, whereas larger motoneurons innervate a greater number (Purves et al., 2001). These fibres, however, are not distributed homogeneously throughout the muscle and remain within a limited portion of the muscle cross-sectional area, which is referred to as motor unit territory (Enoka, 2008). This territory extends across approximately 10-30% of the cross-sectional area, depending on the specific function of the muscle or its sub-portions. Importantly, fibres from different motor units are interdigitated, allowing for smooth force

production and distribution (Butler et al., 2005; English, 1984; Falla & Farina, 2008; Fleckenstein et al., 1992; Saboisky et al., 2006).

Consequently, the expressible force depends on both the innervation ratio and the number of motor units recruited through their behaviour, but it can also vary based on their intrinsic properties (subsection 2.4.2).

2.2.6 The sensory feedback system

As mentioned previously, the neural drive does not reside solely at the cortical level, but results from the integration of descending inputs and afferent feedback (Dideriksen et al., 2015). Therefore, the sensory feedback plays a fundamental role in modulating motor output, enabling continuous adjustments during movement execution (Hagbarth & Young, 1979; Prochazka & Ellaway, 2012).

Key structures involved in this feedback, as presented in section 2.1, include the Golgi tendon organs and muscle spindles (Hultborn, 2006). Golgi tendon organs primarily detect muscle tension and can exert inhibitory or excitatory effects depending on the motor context, contributing to force regulation (Houk & Henneman, 1967; Jami, 1992). For instance, during locomotion, they send an excitatory signal to the motoneurons of the agonist (extensor) muscle (McCrea, 2001), which generates positive force feedback while simultaneously inhibiting the antagonist (flexor) muscle (Conway et al., 1987; Dietz & Duysens, 2000; Hultborn & Nielsen, 2007; Pearson, 2004; Pearson & Misiaszek, 2000). Muscle spindles, in contrast, detect changes in muscle length and contribute to stretch reflexes and movement stabilization through reciprocal inhibition mechanisms (Matthews, 1964).

The sensitivity of these receptors is modulated by gamma motoneurons (Henneman et al., 1965; Prochazka & Ellaway, 2012). Experimental evidence shows that sensory feedback mechanisms influence neural drive depending on task conditions, such as the presence or absence of visual feedback (Laine et al., 2013, 2014). Specifically, it has been demonstrated that spindle-mediated activity increases when visual feedback is removed or distorted (Laine et al., 2014).

Therefore, understanding motor control requires considering not only descending neural commands but also the interaction with sensory feedback. These mechanisms can be investigated experimentally through the analysis of motor unit discharge behaviour and the frequency content of neural signals, including frequency-domain measures (e.g., coherence)

derived from HD-sEMG. These methodologies are further detailed in subsection 3.2.4 and 3.2.5.

2.3 Generation of movement

The generation of movement originates within the central nervous system (Heckman & Enoka, 2012). Three main categories of movement can be distinguished: reflexes, automatic behaviours, and voluntary actions (Enoka, 2008). This section focuses on the generation of voluntary movements, as the studies conducted during the PhD program and described in this thesis exclusively involved the execution of voluntary tasks.

Following the action planning process, a synaptic input is generated by the pyramidal cells, descending from the motor cortex through the brainstem along the efferent pathway, reaching the corticospinal axons and, ultimately, the alpha motoneuron (Farina & Negro, 2015). When the summation of these inputs reaches the activation threshold, the motoneuron fires an action potential along the efferent axon toward the motor-end plate at the neuromuscular junction (Sanes & Lichtman, 1999). The subsequent chemical synapse occurring at the neuromuscular junction initiates muscle contraction, ultimately leading to the generation of movement (Liddell & Sherrington, 1925; Sherrington, 1925).

2.3.1 Generation and propagation of an action potential

Motoneurons operate according to the all-or-none principle: an action potential is triggered and propagated only when the synaptic input received is strong enough to reach the critical threshold (Adrian, 1928). This action potential is characterized as a rapid (1-4 ms) and large (100 mV) alteration in membrane potential, which can recur at very high frequencies (Kandel et al., 2021).

At rest, the membrane potential is approximately -70 mV, referred to as resting potential. This state is maintained by the sodium-potassium gradient and regulated by leak channels in the motoneuron membrane (Forsythe & Redman, 1988). These channels are selectively permeable to potassium and, to a lesser extent, to sodium and chloride. When a stimulus arrives, ionotropic synaptic channels contribute to bringing the membrane potential to the critical threshold of -50 mV, the minimum value required for action potential generation and the onset of depolarization (Hodgkin & Horowicz, 1959).

Ionotropic synaptic channels are ligand-gated and mediate the synaptic transmission of neural input. Their function depends on the neurotransmitter to which they bind: they may

be excitatory, as in the case of glutamate, or inhibitory, as in the case of glycine or GABA (Fyffe, 2001). Binding occurs when a neurotransmitter released from the presynaptic neuron interacts with receptors on the postsynaptic alpha motoneuron, opening specific channels and generating ionic currents (Coombs et al., 1955).

Once the threshold is reached, voltage-gated channels are activated to generate the action potential (Hille, 2001). Sodium channels open rapidly, allowing an influx of sodium ions that causes depolarization. As a result, the membrane potential rises to approximately +30 mV and the action potential propagates along the motoneuron axon. During this process, voltage-gated potassium channels open more slowly, allowing potassium efflux and thereby initiating repolarization and the return to resting membrane potential (Sanes & Lichtman, 1999).

2.3.2 Mechanical generation of movement

The structure that enables the mechanical process of muscle contraction is the sarcomere, which is considered the functional unit of the muscle. It is bounded by two Z-lines, which defines its ends, and is stabilized by the protein titin, which contributes to its passive stiffness (Bloch & Gonzalez-Serratos, 2003; Gordon et al., 1966; Linke, 2018). A central M-line divides the sarcomere into two symmetrical halves (Figure 2.2).

Within the sarcomere, two types of filaments are present: thick myosin filaments and thin actin filaments. The latter also contain the regulatory proteins tropomyosin and troponin. In resting conditions, tropomyosin covers the binding sites of actin. When an electrical signal triggers contraction, calcium binds to troponin, causing a conformational change that shifts tropomyosin and exposes the actin binding sites (Gordon et al., 2000). This enables the myosin heads to bind to actin, forming cross-bridges (Rayment et al., 1993). The myosin heads then undergo a conformational change, pulling the actin filaments toward the centre of the sarcomere (Huxley, 1957).

Because actin filaments are anchored to the Z-lines, their inward movement brings the Z-lines closer together, resulting in shortening of the muscle fibre and, consequently, muscle contraction. This mechanism in which filament sliding leads to sarcomere shortening without actual shortening of the filaments themselves, is known as the sliding filament theory (Huxley & Niedergerke, 1954; Huxley & Hanson, 1954).

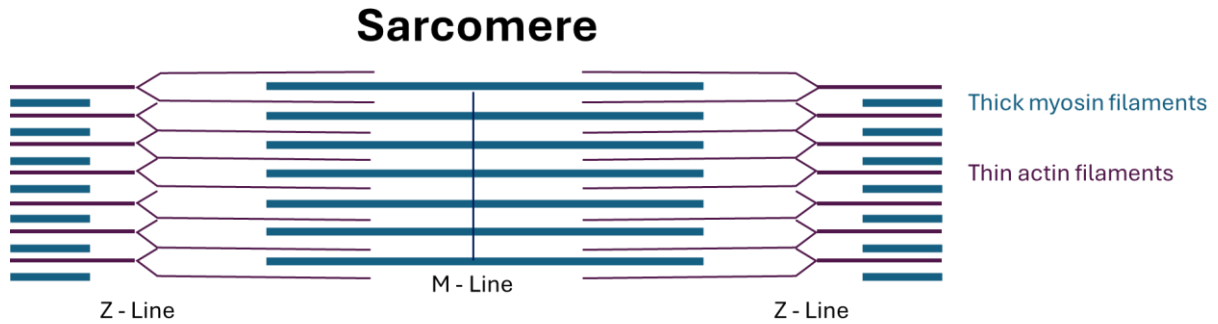


Figure 2.2. *The sarcomere. Schematic representation of the sarcomere.*

2.4 Mechanisms underlying force modulation

This section is of particular relevance to the aims of this thesis, as the mechanisms underlying force generation across different contexts directly correspond to the neural strategies investigated in all experimental studies presented in part three.

The human body and its muscles respond to a wide range of functional demands through highly diverse movements. Depending on the motor goal, tasks may require different levels of speed, force, and precision, ranging from fine to gross motor patterns. Consequently, muscles exhibit distinct characteristics at the level of motor units and muscle fibres, reflecting their specific functional role (Ariano et al., 1973).

The mechanisms underlying force modulation are addressed in the following subsections. They can be divided into neural factors (subsection 2.4.1), which describe how the central nervous system modulates motor unit properties to achieve the desired force output, and intrinsic factors (subsection 2.4.2), which are related to the mechanical and physiological properties of muscle fibres.

2.4.1 Neural factors

The neural factors described in the following subsections are central to this thesis, as they are the focus of the experimental investigations presented in chapters 5, 6, 7, and 8.

Recruitment and rate coding

In addition to the intrinsic properties of motor units, as briefly described in the next subsection (2.4.2), the neuromuscular system modulates force output through two primary neural strategies: motor unit recruitment and rate coding. The relative contribution of these mechanisms varies across muscles, force levels and task conditions.

Motor unit recruitment refers to the number of active motor units during a contraction. According to Henneman's size principle, motor units are recruited in an orderly manner based on motoneuron size (Henneman, 1957). Smaller motoneurons, which typically innervate Type S motor units, are characterized by a smaller surface area. Due to their higher input resistance, these smaller motoneurons reach the depolarization threshold more easily and are therefore recruited first (low-threshold units). As the synaptic input increases, larger motoneurons, which typically innervate Type FF motor units and present lower input resistance, are progressively recruited (high-threshold units). This hierarchical organization allows for precise force control at low intensities and efficient scaling of force as task demands increase. In addition, such organization saves energy expenditure by limiting the use of motor units that are expensive in terms of energy only to when it is strictly required (Henneman, 1957).

Rate coding, in contrast, regulates force output by modulating the discharge frequency of already active motor units. An increase in discharge rate results in greater force production due to temporal summation of muscle twitches. However, the relationship between synaptic input and discharge rate is not fixed, as it can be influenced by intrinsic motoneuron properties and neuromodulatory inputs (Heckman et al., 2007).

Different models have been proposed to describe how the central nervous system modulates rate coding in motor units. Earlier studies suggested that larger motor units, recruited last, discharge at higher rates to achieve tetanic fusion (Kernell, 1965; Oya et al., 2009). However, subsequent work introduced the "Onion Skin" phenomenon, which posits that motor units recruited earlier tend to discharge at higher frequencies than those recruited at higher force levels (De Luca & Erim, 1994; Hoffer et al., 1981; Monster & Chan, 1977; Person & Kudina, 1972). This behaviour has been interpreted as a mechanism to ensure smooth force production. Furthermore, since larger fibres are inherently more susceptible to fatigue, a higher discharge frequency would accelerate their exhaustion (De Luca & Contessa, 2014).

Both rate coding and recruitment contribute to the execution of a motor task. A computational model proposed by Fuglevand et al. (1993) suggested that rate coding may account for approximately 75% of total force generation. (Fuglevand et al., 1993a). Moreover, experimental evidence indicates that different muscles adopt distinct strategies. For example, smaller and more distal muscles of the upper limb, such as the first dorsal interosseous, may complete motor unit recruitment at approximately 65% of maximal

voluntary contraction, relying more on rate coding thereafter (De Luca & Hostage, 2010). Conversely, larger muscles of limb and trunk, can extend recruitment up to 85% MVC (De Luca & Hostage, 2010). For instance, the deltoid muscle, which is investigated in the experimental study of chapter 8, may recruit motor units up to 80% of maximal voluntary contraction (de Luca et al., 1982).

In summary, these mechanisms to modulate force output are non-linear, muscle-specific, and task-dependent. Although motor units within a pool receive a dominant common synaptic input, their individual responses dependent on intrinsic properties and functional context.

Among these two neural strategies, rate coding plays a particularly central role in this doctoral project. The first study (chapter 5) specifically investigated motor unit discharge behaviour through a systematic review and meta-analysis, examining the discharge rate modulation across different force levels and muscles. This analysis revealed substantial gaps in the literature, particularly the limited evidence available for shoulder muscles, which motivated the final experimental study (chapter 8). In that study, rate coding behaviour was characterised in the deltoid using HD-sEMG, providing the first reference values, with a non-invasive technique, for a muscle that had previously been explored almost exclusively through intramuscular recordings

Neural drive and common input

Force generation is orchestrated by synaptic input from the central nervous system to the motoneuron pool, which transforms these inputs into neural command (i.e., neural drive) for muscles, enabling contraction. The neural drive spans a wide frequency range; however, the muscle acts as a low-pass filter, reflecting only lower frequencies in the force output (Mannard & Stein, 1973). Consequently, approximately two-thirds of the force output is determined by frequencies below 2 Hz (Negro et al., 2009).

The synaptic input to motoneurons consists of both common and independent inputs. Common input is defined as the shared component across motoneurons (sum of excitatory and inhibitory inputs), whereas independent input reflects variability that is not shared among units (De Luca & Erim, 1994). When considering the entire pool rather than individual motoneurons, the pool functions as a linear filter system that cancels out independent input components. Common components, instead, are summed and transmitted unaltered to the muscle. As a result, the force output is primarily determined by the common

component of the neural input within the frequency band relevant for force production. This component is referred to as the effective neural drive (Farina & Negro, 2015)

The common component of the neural drive can be further divided into components related to the mean force output and those associated with fluctuations in the common input, often referred to as common noise. This noise is responsible for oscillations in the force signal (i.e., force variability) and reflects the stochastic nature of neural drive (Castronovo et al., 2015). The component of the common synaptic input that is functionally related to the motor output corresponds to the low-frequency components (approximately 5–10 Hz), commonly referred to as common drive (Negro et al., 2009).

Therefore, investigating neural control requires examining how common synaptic inputs to motoneurons are modulated. This can be achieved experimentally through the analysis of motor unit behaviour and the frequency content of neural signals, which are central to the methodologies employed in this thesis.

The analysis of the neural drive represents a core component in two of the experimental studies. The first is presented in chapter 6 and investigated the modulation of common input in the lower limb during motor learning, while chapter 7 extended this analysis to the upper limb by comparing isolated and synergistic actions. Together, these investigations provide a progressive understanding of how common input adapts to different tasks and muscles. These two chapters are an essential step that ultimately leads to the exploration of neural control in the shoulder.

2.4.2 Intrinsic factors

The intrinsic factors that influence force modulation are briefly described below. Although they are fundamental for understanding how movement execution occurs, these factors are not central for the experimental studies presented in the part three of this thesis.

Calcium concentration

When muscle contraction is not occurring, intracellular calcium is contained within the sarcoplasmic reticulum. The arrival of an action potential at the neuromuscular junction evokes the release of a molecule called acetylcholine (Tintignac et al., 2015). This allows for the depolarization of the muscle fibre membrane, the subsequent generation of a potential within the fibre that causing the release of calcium (Dulhunty et al., 2002). This process, create a chain of events following a single action potential, that is called a muscle twitch,

and there is a one-to-one relationship between the potential and the twitch (Katz & Miledi, 1965).

Muscle length

During a voluntary contraction at any force level, the force varies as a function of muscle length, which is determined by the degree of overlap between actin and myosin during contraction (Gordon et al., 1966). Indeed, a dependence exists between force and length, as described by Rassier and colleagues (Rassier et al., 1999). Briefly, muscles present three main different length conditions (shortened, optimal, lengthened). When the sarcomere is at a length of 2.0–2.20 μm , the overlap of actin and myosin filaments is optimal, allowing for the greatest number of cross-bridges and, consequently, the expression of maximal force.

Contraction velocity

The magnitude of force expression depends not only on the degree of overlap between thick and thin filaments but also on the velocity at which it occurs. Hill (1938) was the first to mathematically characterize this relationship. In this work, it was demonstrated that as the shortening velocity of a muscle increases, the produced force decreases hyperbolically (Hill, 1938).

Muscle stretch

Muscles have a property known as short-range stiffness, which characterizes the active muscle as a spring-like structure. This property is essential for joint support during movement and for the response to perturbations (Barrett et al., 2024; De Groote et al., 2017). When an active muscle undergoes a rapid stretch, if the stretch exceeds a certain threshold, these cross-bridges detach, leading to a drop in force known as yielding (Huyghues-Despointes et al., 2003).

Motor unit type

Historically, based on early animal studies, muscle fibres were first classified according to their colour into red and white fibres. Over time, more sophisticated classification systems have been developed. Among these, the most widely recognized methods focus on the histochemical, structural, and contractile properties of the fibres.

A landmark classification was proposed by Burke et al. (1973), in which fibres are categorized based on their mechanical response to sub-maximal stimuli. This type of

stimulation generates an unfused tetanus, to which motor units react with different tension. They distinguish fibres into three distinct groups. The first are classified as fast-fatigable (FF), characterized by fast-twitch contractions, phasic activity and a high content of glycolytic enzymes. This type of fibres allows for the expression of high force but only for short durations. The second group comprises fatigue-resistant (FR), which are resistant to fatigue and possess fast-twitch contraction properties. These types of fibres contain both glycolytic and oxidative enzymes, allowing for moderate force production and contraction duration. The final group consists of highly fatigue-resistant (Type S), which are characterized by slow-twitch contractions and consequently show a force production that, while reduced, is prolonged. These units are smaller, and utilize an oxidative metabolism (Burke et al., 1973; Dum & Kennedy, 1980; Kernell et al., 1983).

The study by Peter et al. (1972) further expanded this classification by integrating the biochemical properties of the fibres. Specifically, as FF fibres are characterized by the exclusive presence of glycolytic enzymes and metabolism, they were redefined as Fast-twitch Glycolytic. Conversely, Type S fibres, relying solely on oxidative pathways, are categorized as Slow-twitch Oxidative. Finally, FR fibres, which possess both glycolytic and oxidative components, are classified as Fast-twitch Oxidative-Glycolytic (Peter et al., 1972).

3. Electromyography

In this chapter, HD-sEMG is examined in detail, as it represents the primary methodology employed in the experimental studies included in chapters 6, 7 and 8.

First, the development of EMG is outlined, together with its main applications and the different available approaches. Subsequently, recommended procedures for accurate signal acquisition are discussed, along with potential factors that may affect signal quality.

The following sections focus on HD-sEMG signal pre-processing and decomposition, which form the methodological foundation of the studies included in this thesis. These approaches provide valuable insight into the mechanisms underlying neural input transmission and are essential for understanding how the raw electrical signal recorded at the skin surface is transformed into motor-unit discharge patterns, allowing indirect investigation of neural input transmission.

Finally, this chapter describes the parameters that can be extracted from the decomposed HD-sEMG signal and the methods used for their analysis. All procedures and methodological considerations presented here, from signal acquisition to decomposition and parameter extraction, were applied consistently across all experimental studies. The theoretical explanation is therefore intended to guide the reader through the entire pathway from manual signal recording to the analyses of the parameters related to neural control, which constitute the basis of the methods and results presented in part three.

3.1 Intramuscular and superficial EMG

The depolarization of muscle fibres generates local electric fields, which summate in both time and space to form the EMG signal (Plonsey, 1977). This signal can be recorded using electromyography through the application of electrodes either within the muscle (intramuscular EMG or iEMG) or over the skin surface (surface EMG or sEMG).

Historically, the first technique available was iEMG (Adrian et al., 1929; de Luca et al., 1982; Denier van der Gon et al., 1985; Howell et al., 1995; Kamen et al., 1992; Tax et al., 1989; Werner & Albers, 1995) (Figure 3.1 A). This technique involves the insertion of fine needles directly into the muscle (Rubin, 2019), allowing highly selective recordings of individual motor units. However, it samples only a limited number of motor units (LeFever et al., 1982; LeFever & De Luca, 1982). iEMG has been widely used in both research and

clinical contexts, particularly for diagnosing neuromuscular disorders (Katzberg & Abraham, 2021; O’gorman et al., 2018; Pitt, 2012; Rodriguez-Falces et al., 2025; Tanhehco, 2003; Wijntjes et al., 2024). It also provides valuable information about motor unit discharge properties, such as firing rate and variability, which are indicators of neural control.

With technological advances, sEMG was developed as a non-invasive alternative (Merletti & Parker, 2004). In this approach, electrodes are placed on the skin surface, resulting in signals that are attenuated and spatially filtered by the underlying subcutaneous tissues (Zalewska, 2009). While this reduces spatial selectivity, this technique provides a non-invasive overview of global muscle activation.

Until the 21st century, sEMG was used exclusively to estimate global signal features, such as amplitude or power spectral density, which provide only indirect estimates of neural activity (Merletti et al., 1995). These features must therefore be interpreted with caution, as they can be influenced by physiological and non-physiological characteristics. For example, Martinez-Valdes et al. (2018) have demonstrated that differences in the global EMG amplitude between the vastus medialis and vastus lateralis muscles cannot be considered reliable indicators of differences in neural drive, as they primarily reflect variations in motor unit action potentials (Martinez-Valdes et al., 2018).

The methodological limitations of traditional sEMG features have been largely overcome with the development (Holobar & Zazula, 2007) of HD-sEMG. HD-sEMG employs multi-channel electrode arrays to record signals from multiple locations across the muscle, providing a spatially distributed representation of muscle activation (Merletti & Muceli, 2019) (Figure 3.1 B). Moreover, through signal decomposition techniques, HD-sEMG enables the identification of individual motor unit discharge times (Farina et al., 2016; Gazzoni et al., 2004; Holobar et al., 2009; Holobar & Zazula, 2007; Negro et al., 2009a; Yeung et al., 2024). In contrast to earlier approaches, this method enables the separation of multiple overlapping signals, allowing the analysis of a large number of individual motor units (Merletti & Parker, 2004; Negro et al., 2016a).

HD-sEMG has proven to be a validated and reliable technique for the study of numerous parameters relevant for neural control investigations, such as conduction velocity, motor unit behaviour, central and peripheral fatigue, and neuromuscular adaptations under various conditions (Cabral et al., 2025; Cudicio et al., 2022; Drost et al., 2006; Farina & Negro, 2015; He et al., 2023; Imrani et al., 2023).

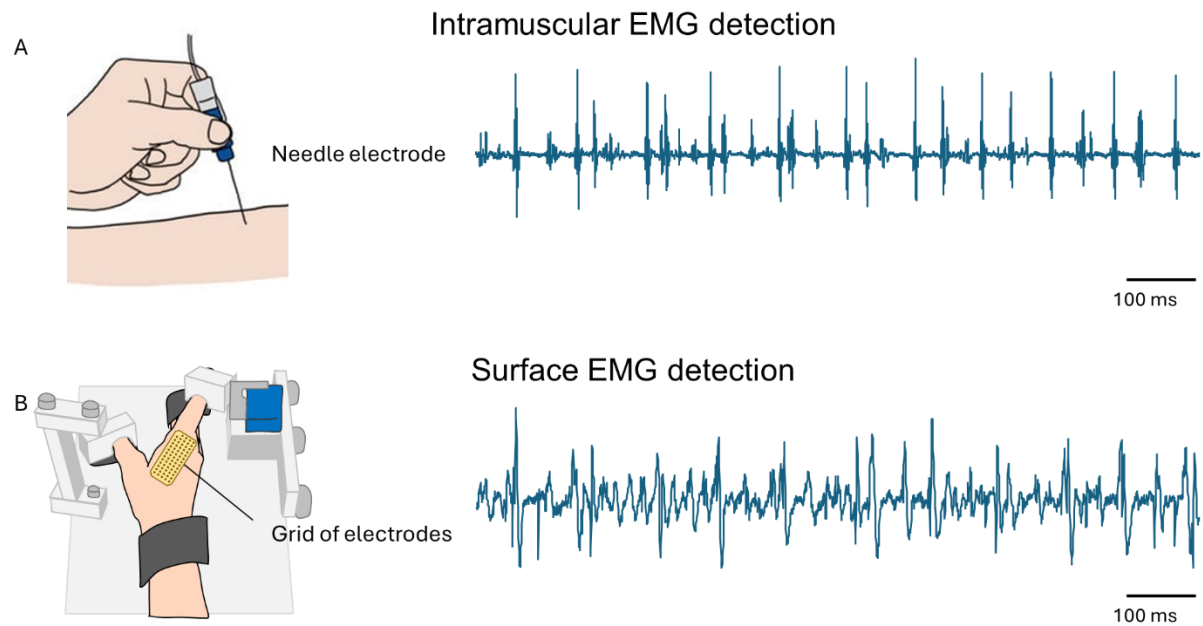


Figure 3.1. IEMG and HD-sEMG. Representation of the acquisition and visualization of the EMG signal using a needle electrode (A) and a grid of electrodes for surface EMG (B).

3.2 Motor unit analysis

This section describes the main steps for HD-sEMG acquisition, pre-processing, and decomposition, as well as the variables that can be extracted following decomposition. These procedures form the methodological basis of all experimental studies presented in this thesis.

3.2.1 HD-sEMG signal acquisition end pre-processing

It is crucial that the sEMG signal is properly acquired to allow the extraction of reliable neural information. sEMG signal quality is determined by the acquisition modality, instrumentation, and operator expertise in identifying and resolving technical issues (Merletti & Cerone, 2020). Extensive previous literature covered how to properly acquire sEMG signals (Barbero et al., 2012; Hermens et al., 2000; Kamen & Gabriel, 2009; Merletti & Farina, 2016; Denise. Mitchell, 2016).

The electromyographic signal is characterized by a very low amplitude, (approximately 1–2 mV) due to attenuation of biological tissues. Consequently, the acquired signal must be amplified and filtered (conditioning phase), followed by sampling and analogic-to-digital conversion (sampling phase) (Merletti & Cerone, 2020).

A key factor affecting sEMG quality is the electrode-skin interface. Skin impedance (i.e., resistance to signal conduction from the skin to the electrode) can be influenced by several factors, such as electrode type, conductive gel, hair, skin irregularities, and dead skin

cells. Therefore, proper skin preparation, typically involving cleaning using an abrasive paste and water, is essential to reduce impedance (Barbero et al., 2012).

Electrode characteristics also play a crucial role. Larger electrodes increase spatial averaging and attenuate high-frequency components. Smaller electrodes can reduce this attenuation effect but typically may increase impedance. Inter-electrode distance is another key factor, influencing both signal characteristics and spatial selectivity (Roeleveld et al., 1997).

Noise and artifacts that may affect the sEMG signal must also be considered. Common noise may arise from internal sources, such as ECG contamination, or external sources, such as electrical interference in the recording environment. Signal artifacts may result from subject movement or instrumentation-related issues (e.g., cable movement). Appropriate filtering and careful experimental setup are therefore essential (Merletti & Cerone, 2020).

Signal acquisition can be performed using monopolar or differential configurations. In monopolar configuration, the resulting signal is measured between each electrode and a reference electrode. In differential configuration, the resulting signal is obtained from the difference between two monopolar adjacent electrodes. While the monopolar configuration is more susceptible to external interference, it provides greater flexibility for signal processing as allows other configurations to be derived (Merletti et al., 2016).

After acquisition, the sEMG signal is typically band-pass filtered. For bioelectrical signals, typical cut-off frequencies are between 20 and 500 Hz (Merletti & Muceli, 2019). Visual inspection of the channels is recommended to remove noisy signals before selecting the portion of interest for analysis.

3.2.2 Motor unit decomposition and optimization

Advances in signal decomposition algorithms have enabled the use of HD-sEMG for studying the neural control of movement at motor unit level. In this thesis, the decomposition method proposed by Negro et al. (2016) is employed and, thus, this is the approach briefly described in this subsection.

The sEMG signal is generated by the algebraic summation of motor unit action potentials with different shapes and discharge timings (Day & Hulliger, 2001) (Figure 3.2 C-E). The resulting sEMG signal can be therefore described by a convolutive model. The

aim of the decomposition is to identify the individual sources and their discharge times (i.e., motor unit spike trains) from the composite signal (Figure 3.2 F). This is possible because motor unit action potential shapes are unique; consequently, when HD-sEMG electrode grids are positioned in regions where waveform differentiation is maximal, it becomes possible to discriminate between different motor units. (Del Vecchio et al., 2020).

The initial step of the decomposition method involves transforming the convolutive model into an instantaneous one by extending the observation matrix (i.e., HD-sEMG signals), allowing the application of blind source separation techniques. A whitening process is then applied to decorrelates the signals. An optimization procedure is subsequently performed to maximize signal sparsity and identify the individual motor unit sources. Finally, an orthogonalization procedure ensures that the same source is not extracted multiple times.

The quality of the identified motor units extracted by the decomposition algorithm is assessed using the Silhouette measure, a parameter that quantifies the degree to which each motor unit is separable from background noise. Typically, values greater than 0.90 indicates acceptable decomposition accuracy (Negro et al., 2016).

Following motor unit automatic decomposition, an expert operator must manually refine the results. The operator verifies the identified spike trains and correct potential errors. The motor unit filters are then recomputed to obtain an optimized estimate of motor unit discharge patterns (Holobar et al., 2014).

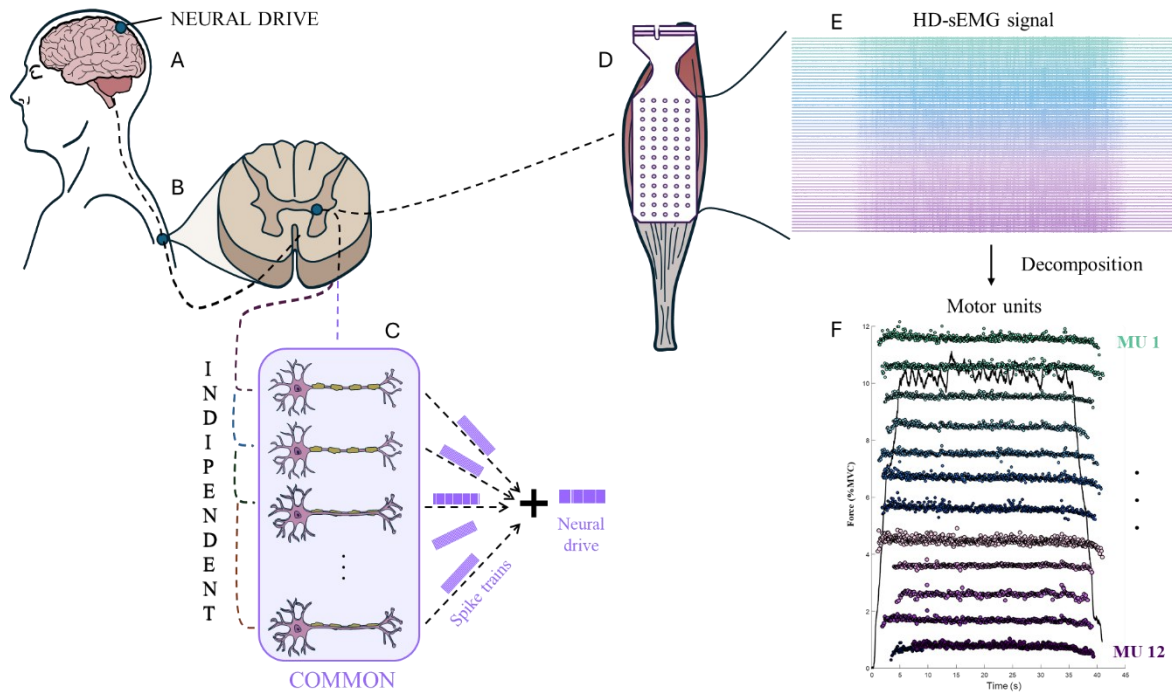


Figure 3.2. Pathway from the generation of the neural drive to its recording at the muscle. The neural drive is generated in the cerebral cortex (A) and travels through the motor cortex and brainstem to reach the alpha motoneurons (B). These motoneurons act as a linear filter, attenuating the independent components of the synaptic input and transmitting the common component, known as the effective neural drive (C), to the muscle as action potentials that leading contraction. Using HD-sEMG (E), the electrical activity can be recorded at the muscle surface through electrode arrays (D) and decomposed using blind-source-separation algorithms to identify the contribution of individual motor units to force generation (F). By analysing motor unit behaviour, it is possible to obtain indirect information about the neural drive to the muscle.

3.2.3 Motor unit matching

An innovative tool developed in recent years is motor unit matching or motor unit tracking (Gligorijević et al., 2015; Gooch & Harati, 1997; Li et al., 2024; Maathuis et al., 2008; Martinez-Valdes, Negro, et al., 2017; Valli et al., 2024; Yin et al., 2025). Indeed, it is now possible to identify the same motor unit and compare its discharge behaviour across different experimental tasks and recording sessions. This represents a significant technological advancement for studies comparing conditions, such as pre- and post-interventions in rehabilitation or strength training programs (Del Vecchio, Casolo, et al., 2019; Martinez-Valdes, Falla, et al., 2017; Valli et al., 2024; Vila-Chã et al., 2010), before and after motor learning (Cabral et al., 2024), during fatiguing contractions (Beck et al., 2005; Christie & Kamen, 2009), or monitoring pathological conditions that affect the transmission and translation of neural commands into movement (Gooch & Harati, 1997; Negro et al., 2020; Vecchio & Farina, 2019).

Two main approaches for motor unit matching have been employed in literature. The first is based on the blind source separation vector (or motor unit filter). In this method, the

separation vectors of motor units identified in one contraction are reapplied in the signals of another contraction (Rossato et al., 2022). After this type of tracking, it is then necessary to manually re-optimize the output spike train following the same cleaning procedure after the initial decomposition. The second approach is performing the correlation between motor unit action potentials, which is typically achieved by spike-triggered averaging techniques (Martinez-Valdes et al., 2017). The units are then considered to be the same if the average cross-correlation value is greater or equal than a threshold, commonly 0.87 (Martinez-Valdes et al., 2017).

Motor unit matching played a fundamental role in the experimental studies of this doctoral project, as it enabled the assessment of how the neural drive to the same motor units changed across different functional contexts. This approach was essential for comparing pre- and post-learning conditions (chapter 6), isolated versus synergistic tasks (chapter 7), and different levels of force (chapter 8), thereby providing a direct and robust evaluation of neural control modulation across tasks in different anatomical regions.

3.2.4 Motor unit discharge parameters

Several discharge parameters can be extracted from the decomposed HD-sEMG signal. The most common are the mean discharge rate and the coefficient of variation of the inter-spike interval. These parameters were the focus of the systematic review and meta-analysis presented in chapter 5, which provided reference values across different muscles of the human body and highlighted those that remain under investigated. Moreover, all experimental studies of this thesis analyse these variables, as they reflect key features of the neural drive sent to the motoneurons. Other variables frequently encountered in motor unit literature are the recruitment and de-recruitment thresholds. These variables are discussed in detail in these subsections.

Mean discharge rate

The discharge rate serves as an indicator of motoneuron discharge behaviour as a function of time and the force exerted during contraction (Farina et al., 2010). The mean discharge rate is analysed by initially identifying the firing times of each action potential (spike train) of the motor unit. The inter-spike interval is then calculated, and the instantaneous frequency of each interval is obtained as its reciprocal. The average of these

values is computed, and the resultant mean discharge rate is expressed in pulses per second (pps).

The study of this variable is one of the cornerstones of motor unit literature. Del Vecchio and colleagues (2019), for example, analysed neural drive adaptations expressed through changes in motor unit behaviour following four weeks of strength training. They demonstrated that increases in muscle force are mediated by increases in the neural drive sent to the motoneurons via increased rate coding (Del Vecchio et al., 2019).

Furthermore, the mean discharge rate can be used to analyse how the neuromuscular system responds to prolonged fatigue. Jensen, Pilegaard, and Sjøgaard (2000) investigated the response of the supraspinatus muscle to long-duration contractions at low-intensity and demonstrated that the central nervous system met sustained force demands primarily through recruitment, rather than by modulating the discharge frequency of already active motor units (Jensen et al., 2000).

Chou et al. (2013) analysed the mean discharge rate in lower limb muscles during isometric contractions at various intensities, comparing post-stroke individuals with healthy controls. The findings highlighted that, in this clinical population, abnormalities in the generation and transmission of the neural drive result in reduced force output. Furthermore, they showed that even at high effort levels, the ability to increase firing rates is severely impaired (Chou et al., 2013).

These examples in the literature demonstrates that analysing this parameter is highly informative for obtaining insights into the neural control of movement, as changes in motor unit discharge rates are linearly correlated with the synaptic inputs to alpha motoneurons (Negro et al., 2016a).

Coefficient of variation of the inter-spike interval

While the mean discharge rate represents the frequency at which motor units fire, the coefficient of variation of the inter-spike interval can be considered a standardized measure of the variability between consecutive discharges within motor unit spike trains (Enoka & Duchateau, 2017). It is calculated as the ratio between the standard deviation of the inter-spike intervals and their mean (Enoka et al., 2003). This parameter then serves as a reliable indicator for assessing the level of stability of the neural drive sent to the motoneurons. It is also a reliable estimator of neural adaptations to training and learning (Del Vecchio et al., 2019), as well as aging and neurological pathologies (Kang & Cauraugh, 2015). Aging, for

instance, often result in increased motor unit discharge variability, which, in turn, is correlated with greater fluctuations in exerted force (Vila-Chã et al., 2026).

Cabral and colleagues (2025) investigated neural drive adaptations following the learning of a new motor task through the repetition of an isometric motor task in different muscles. This study demonstrated that gradual learning is accompanied by increased neural drive stability, and that the coefficient of variation of the inter-spike interval is a suitable tool for assessing this (Cabral et al., 2025).

Lodha et al. (2010) showed that motor impairments following stroke are characterized by high variability and irregularity in force production, proportional to the severity of the pathology. The study further suggests that this variability is caused by an increase in motor unit discharge variability due to a more unstable neural drive (Lodha et al., 2010). Another example is that greater fluctuations in force in older individuals have been associated with increased motor unit discharge variability (Enoka, 2003).

As seen in these previous studies, the analysis of the coefficient of variation of the inter-spike interval is essential for gaining a broader perspective on neural drive adaptations across different tasks, as well as on neuromuscular degeneration in advanced life stages and following pathological conditions.

Recruitment and de-recruitment thresholds

Two additional fundamental parameters for understanding motor unit behaviour, and thus the transmission of neural drive, are the recruitment and de-recruitment thresholds. These variables refer to the percentage of maximal force at which a motor unit begins to fire (recruited) and ceases its activity (de-recruitment). Typically, these variables are calculated considering the first (or last) 3–5 spikes of the motor unit (Jesunathadas et al., 2010).

3.2.5 Evaluation of common synaptic input

The evaluation of common synaptic input represents a key step toward a comprehensive understanding of motor control within the neuromuscular system. Although the components of synaptic input functionally related to force output are primarily contained within low-frequency bands, the neural drive to motoneuron pool spans a broad spectrum of frequencies. These components can be investigated using both time- and frequency-domain analyses.

Initially, motor units were considered independent entities, and neural control was assessed using synchronization analyses between pairs of motoneurons. Under this framework, correlated discharge timings were interpreted as evidence of shared synaptic input (Semmler, 2002). More recent studies have shifted from individual motoneuron to population-based view, in which the motoneuron pool is considered as a whole. According to this framework, the central nervous system provides a common input to all motoneurons, which constitutes the main determinant of force production (De Luca & Erim, 1994). In this context, the motoneuron pool can be modelled as a linear system that attenuates independent inputs while preserving and transmitting the common component (Farina et al., 2014). This common component is referred to as the effective neural drive (Farina & Negro, 2015).

By summing the discharge timings of individual motor units, it is possible to construct the cumulative spike train (CST), which serves as an estimate of the effective synaptic input to the motoneuron pool (Negro et al., 2016). The CST can be analysed in both the time and frequency domains. Time-domain analysis involves cross-correlation measures, whereas frequency-domain analyses utilize coherence measures (Negro & Farina, 2012) (Figure 3.3). In this thesis, the focus is placed on frequency-domain analysis, as it allows for the quantification of linear coupling between CSTs across different frequency bands.

Coherence is commonly analysed within three frequency bands, each linked to a specific physiological mechanism. The delta band (1–5 Hz) reflects the low-frequency range directly related to voluntary force control (Farina et al., 2014; Negro et al., 2009). The alpha band (5–15 Hz) reflects physiological tremor from corticospinal and spinal pathways (Castronovo et al., 2015; Christakos et al., 2006). The beta band (15–35 Hz) is typically linked to cortical inputs and corticospinal interactions (Baker et al., 1997; Conway et al., 1995). Together, these bands provide insight into the contributions of both spinal and supraspinal centres to motor control (Negro & Farina, 2012).

From a computational perspective, coherence analysis requires converting motor unit discharge timings into binary spike trains (Negro & Farina, 2012). To quantify the effective common drive, an iterative randomization procedure is implemented (typically up to 100 iterations). During each iteration, the identified motor units are randomly divided into two equally sized groups (Castronovo et al., 2015). A CST is then generated for each group by summing the individual spike trains (Rossato et al., 2022) (Figure 3.3 D). Coherence is calculated between these two CSTs using Welch's periodogram, typically with a 1-second Hanning window without or with overlapping (e.g., 95% overlapping) (Gallet & Julien,

2011). These resulting coherence values are subsequently converted into Z-scores to normalize their distribution, and the area under the curve or average is calculated for the frequency bands of interest (Maillet et al., 2022).

Numerous studies in literature have employed this technique to investigate the effective neural drive to the muscle (Avrillon et al., 2021; Baker et al., 1997; Castronovo et al., 2015; Del Vecchio, Germer, et al., 2019; Farina et al., 2017; Free et al., 2023; Kilner et al., 1999; Negro et al., 2016b; Negro & Farina, 2012). For instance, Castronovo et al. (2015) have shown that increasing force requirements, as well as fatigue level, led to increased coherence within the delta and alpha bands, suggesting an increasing in common synaptic input accompanied by greater synaptic noise. Similarly, coherence analysis has been applied in pathological conditions. Farina et al. (2017) investigated motoneuron behaviour in muscles reinnervated through targeted muscle reinnervation following amputation. Their results showed that, although voluntary control was preserved, coherence values in delta and alpha bands is significantly lower compared to physiologically innervated muscles, indicating alterations in common synaptic inputs, particularly from sensory afferent pathways (Farina et al., 2017).

As demonstrated by these examples, the analysis of common synaptic input using CST coherence provides a fundamental tool for understanding how the nervous system adapts to different task demands and physiological conditions. In this thesis, coherence analysis represents a key methodological component for quantifying the modulation of common input across the different experimental contexts investigated in chapters 6 and 7.

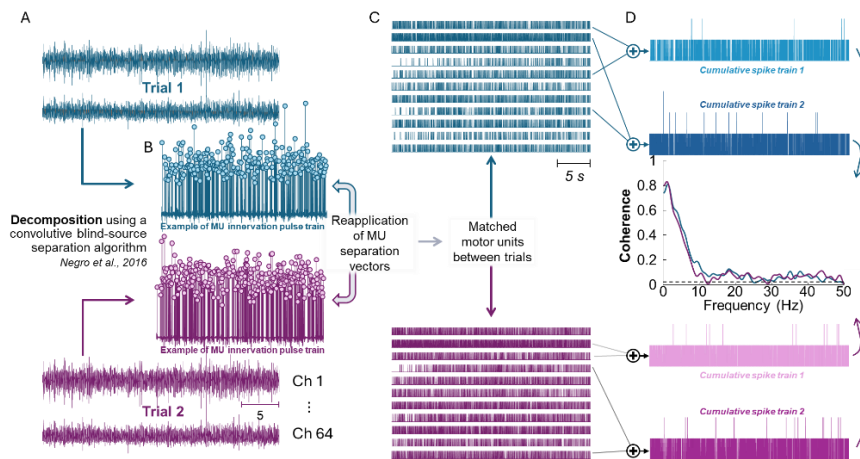


Figure 3.3 Evaluation of common synaptic input. Schematic representation of the methodological steps used to perform coherence analysis on CSTs to evaluate common synaptic input across different frequency bands. The global HD-sEMG signals recorded in two separate trials (A) are first decomposed into the constituent motor units (B), which are then matched across trials through the reapplication of motor unit separation vectors (C). The matched motor units are randomly divided into two groups, from which CSTs are generated. Coherence is subsequently computed between the two CSTs to quantify the strength of common synaptic input (D).

4. The shoulder complex

The shoulder joint complex is the focus of this chapter. Given its complexity, being composed of numerous muscles and articulations that must coordinate and work synergistically, it is essential to revisit its anatomy, physiology, and biomechanics. This understanding provides the opportunity to explain why the neuromuscular system adopts specific strategies to ensure proper movement.

This chapter focuses on the joint and muscle that were analysed in the last experimental study of this thesis (chapter 8): the glenohumeral joint and the deltoid muscle. As will be discussed in sub-section 4.2.2, the existing literature on deltoid motor unit behaviour is extremely limited and relies almost exclusively on invasive iEMG recordings. Consequently, the anatomical and biomechanical overview presented in this chapter is necessary to clarify why the shoulder represents a particularly challenging region for sEMG and why the deltoid has remained largely unexplored in the literature. Moreover, the territorial organisation of its motor units highlights the importance of characterising this muscle according to the spatial distribution of its compartments, which forms the basis and the rationale for the experimental investigation presented in chapter 8.

4.1 Joints

The shoulder complex represents proximal joint of the upper limb and play a crucial role in the majority of daily motor tasks. Consequently, it is also among the primary causes of musculoskeletal pain and is frequently subjected to disorders and injuries. (Bland et al., 1977).

From a research perspective, the shoulder constitutes a particularly challenging system due to its anatomical and functional complexity. It is the connection between the thoracic cage and the arm and comprised of five joints (Figure 4.1): scapulothoracic (e), sternoclavicular (a), acromioclavicular (b), subdeltoid (c), and glenohumeral (d) (Kapandji et al., 1987).

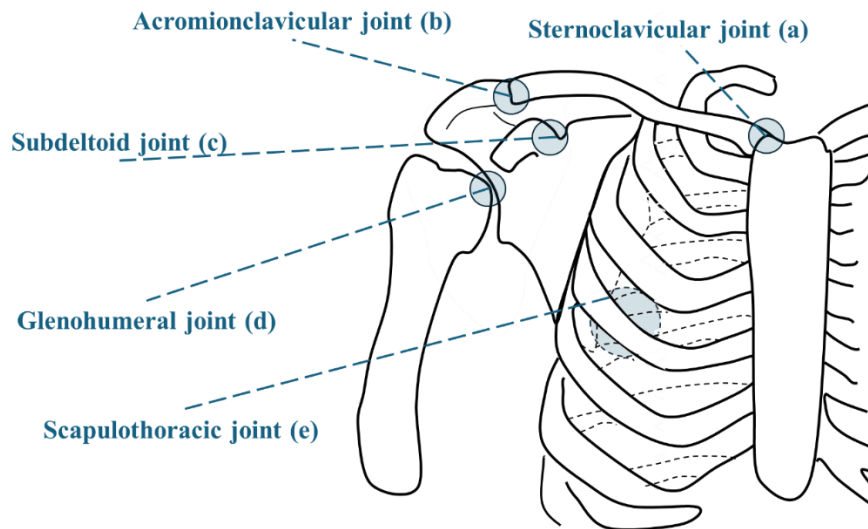


Figure 4.1. Anterior view of the shoulder complex. The figure highlights the five articulations constituting the complex: (a) Sternoclavicular joint, (b) Acromioclavicular joint, (c) Subdeltoid joint, (d) Glenohumeral joint, and (e) Scapulothoracic joint.

The latter, in particular, is a ball-and-socket joint that connects the humeral head to the glenoid cavity of the scapula. The glenoid cavity is approximately one-fourth the size of the humeral head, and the congruency of the articular surfaces is exclusively maintained by the glenoid labrum (Goldstein, 2004). However, ligamentous structures are also present, which aid in stabilizing the joint and limiting excessive movement (Burkart & Debski, 2002). This specific configuration allows a large range of motion, specifically movement across three axes with three degrees of freedom, making the joint particularly unstable and lax (Goldstein, 2004). For this reason, the muscles involved in generating movement perform a dual functional role, contributing not only to force production but also to joint stabilization. Consequently, precise intermuscular co-ordination is required to ensure optimal control of movement (Anders et al., 2004).

Furthermore, due to the presence of muscles of different types (mono- and multi-articular) that collaborate in the movement of the upper limb, the shoulder complex is capable of exerting forces in different directions (Veeger & van der Helm, 2007).

For these reasons, the shoulder can be considered the quintessential example of the perfect compromise between mobility and stability.

4.2 Muscles and Movements

As previously described, the shoulder is capable of performing a wide range of movements across three anatomical planes and axes (Figure 4.2).

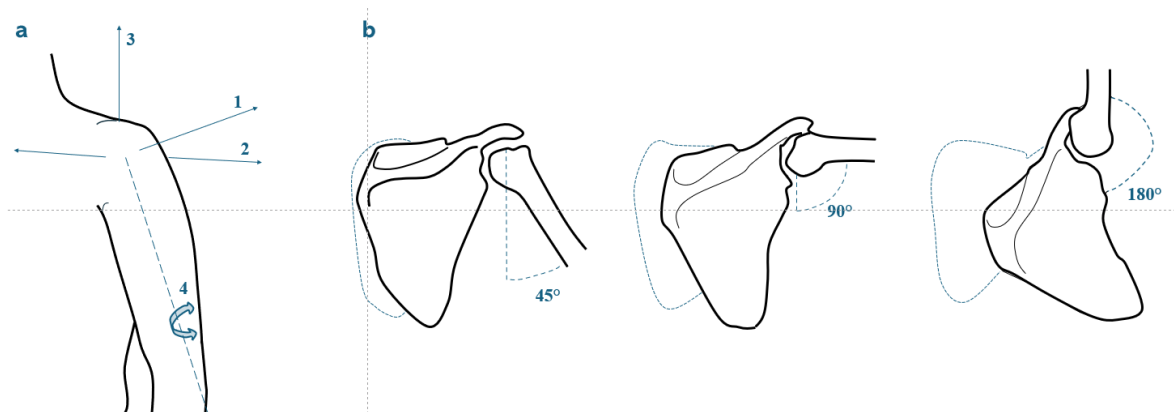


Figure 4.2. Movements of the shoulder. Representation of the movements of the shoulder complex in the anatomical planes (a). Specific movements occurring at the glenohumeral joint (b).

These movements include: flexion and extension (1), which occur in the sagittal plane and are controlled by the transverse axis, or which take place in the transverse plane and are controlled by the vertical axis; abduction and adduction (2), which are performed in the frontal plane and are governed by the sagittal axis; and internal and external rotation (4). The muscles involved in these shoulder movements are listed in the table below (Table 4.1).

Movement	Muscles
Flexion	Pectoralis major
	Deltoid
Extension	Latissimus dorsi
	Deltoid
Abduction	Deltoid
	Supraspinatus
Adduction	Pectoralis major
	Latissimus dorsi
	Teres major
	Subscapularis
Internal rotation	Pectoralis major
	Latissimus dorsi
	Teres major
	Subscapularis
External rotation	Infraspinatus
	Teres minor
	Deltoid

Table 4.1. Muscles involved in shoulder movements

4.3 The deltoid

This section will focus on the description of the deltoid muscle, as it is the primary muscle investigated in the studies included in this dissertation.

4.3.1 Anatomy and movements

The deltoid is a muscle located in the shoulder and accounts for approximately one fifth of its total muscle mass (Lee & An, 2002). Together with the rotator cuff, it is considered part of a synergistic unit that enables shoulder movement and stabilization (Hawkes et al., 2019). Specifically, the deltoid is a triangular muscle and represents the most superficial and largest muscle of the shoulder (Holzbaur et al., 2007). It envelops the shoulder at the level of the glenohumeral joint, and its shape gives the shoulder its characteristic contour (Moser et al., 2013).

The deltoid originates anteriorly mainly from the clavicle, specifically from the superior and anterior surface of the lateral third of the clavicle (Morgan et al., 2006), via the periosteum, and for approximately 25% from the acromion through tendinous and muscular fibres covering its superior and posterior surface (Kumar et al., 1997). In particular, the superior acromion contains tendinous fibres from which connective tissue septa arise; these insert into the central portion of the muscle and confer its characteristic multipennate structure. Due to the fishbone-like arrangement of the fibres, the length of this muscle portion is greater than that of the individual fibres, allowing greater force production (Peterson & Rayan, 2011). In the posterior region, the attachment of the coracoacromial ligament is present (Kumar et al., 1997). The lateral portion of the deltoid originates from the superior and lateral acromion, whereas the posterior portion originates from the inferior border of the scapular spine (Morgan et al., 2006). The muscle fibres converge inferiorly into a large tendon that connects to the brachial fascia and the lateral intermuscular septum and inserts onto the deltoid tuberosity of the humeral shaft on its lateral aspect, through multiple insertions forming an arch-shaped configuration (Rispoli et al., 2009; Standring, 2008).

The deltoid is highly vascularized, receiving blood supply mainly from the thoracoacromial artery (acromial and deltoid branches), the anterior and posterior circumflex humeral arteries, the subscapular arteries, and the brachial artery (deltoid branch) (Rocha et al., 2025).

Regarding innervation, it is innervated by the axillary nerve, originating from the posterior cord of the brachial plexus (Standring, 2008). This nerve arises from the fifth and sixth cervical nerve roots, courses along the anterior surface of the subscapularis, passes inferior to the glenohumeral joint through the quadrilateral space, and then divides into two branches: the posterior branch innervates the posterior deltoid, while the anterior branch supplies the medial and anterior portions of the muscle (Moser et al., 2013).

The deltoid is typically divided into three main portions: anterior, posterior, and lateral. The anterior and posterior portions are unipennate, whereas the lateral portion is multipennate (Morgan et al., 2006). These three portions act as primary movers for different movements due to fibre orientation (Neumann & Kelly, 2025) and the contribution of each portion to a given task is influenced by arm position (Lee & An, 2002). The anterior fibres act synergistically with the pectoralis major during shoulder flexion and internal rotation. The posterior fibres, which have a similar but longer orientation act in synergy with the latissimus dorsi and teres minor and are involved in external rotation and extension of the arm (Peterson & Rayan, 2011). Finally, the lateral fibres, which are capable of producing the greatest force due to their multipennate structure, are primarily responsible for shoulder abduction (Rasch & Burke, 1978; Smith & Lehmkuhl, 1996). Because of its architecture, which influences the physiological cross-sectional area, the anterior and posterior portions of the deltoid are more suited for producing faster movements, whereas the medial deltoid, characterized by a larger cross-sectional area, is specialized for force production and represents the most powerful component of the muscle (Peterson & Rayan, 2011).

Although the deltoid is most commonly divided into three portions, Moser et al. (2013) demonstrated that it is further subdivided by internal tendon bands. In total, nine bands were identified: six in the proximal region (one anterior, one posterior, and four medial) and three in the distal region, one for each muscle portion (Moser et al., 2013).

Other authors have similarly reported that the deltoid is subdivided by intramuscular tendons whose insertions form the three lines underlying the classic division of the muscle (Finni, 2006; Huijing, 2003; Klepps et al., 2004; Lieber & Fridén, 2001). These segments can be independently controlled by the central nervous system to modulate force output according to task demands (Campbell et al., 1973; Soderberg & Dostal, 1978).

Furthermore, motor units are distributed within specific regions of the muscle and can be independently activated (English et al., 1993), a property known as functional

differentiation (Paré et al., 1981). The deltoid is considered a prime candidate for this strategy, as it allows greater flexibility and efficient fine control of muscle activity during movement.

In his 1911 study, Fick divided the deltoid into seven muscular segments. Later, Wickham and Brown (1998) investigated the neural control mechanisms underlying the activation of individual segments during isometric abduction and adduction tasks, functionally distinguishing the deltoid segments. They confirmed the presence of seven functionally distinct segments, five of which, located superior to the joint's axis of rotation, were primarily responsible for abduction. Of these, two were anterior, three posteriors, one was multipennate and medially located, and another was positioned inferiorly, acting as a deep stabilizer. The remaining two segments were involved in rotation and adduction. The adduction segment could also be assisted by one of the posterior abduction segments, which is the last to activate during abduction, suggesting a stabilizing and movement-directing role. The force contribution of each segment can be modulated according to task demands, with the anterior segment typically initiating movement. Overall, the results of this study indicate that deltoid segments can be functionally classified as primary movers, synergists, stabilizers, and antagonists. These findings suggest that not only can segmental control be semi-independent, but that fundamental principles such as Henneman's size principle may apply at the level of individual muscle compartments rather than the whole muscle (Wickham & Brown, 1998).

Subsequent work by Brown et al. (2007) extended these findings to other muscles, including the pectoralis major and latissimus dorsi, across a wider range of movements. The authors confirmed that activation timing is related to the functional role and line of action of each segment and that this classification is task-dependent (Brown et al., 2007).

Furthermore, within the deltoid compartments, fibre type distribution is not uniform. The proportion of slow and fast fibres directly influences contractile properties and, consequently, functional role and neural control strategies (Johnson et al., 1973; Lieber, 2002; MacIntosh et al., 2006). Evidence suggests that the deltoid consists of approximately 42–61% slow fibres, with notable regional differences. The posterior portion has the highest proportion of fast fibres (>80%), whereas the anterior and medial portions show approximately 60–70% fast fibres (Fayet et al., 2001; Johnson et al., 1973; Singh et al., 2002).

Gorelick and Brown (2007) demonstrated that these portions differ in both contractile properties and architecture, with the medial head being primarily responsible for abduction and sustained arm elevation, and the anterior and posterior heads contributing to flexion and extension, respectively. The central nervous system exploits these differences to modulate muscle activity according to task requirements (Gorelick & Brown, 2007).

Overall, deltoid is the primary muscle involved in shoulder abduction and flexion, although studies report variable contributions, with reductions in shoulder force following axillary nerve block ranging from 25% to 80% (Colachis et al., 1969; Hawkes et al., 2019), or exceeding 70% (Gerber et al., 2007). These studies agree, however, that the maximal force that can be produced by the shoulder depends on the abduction angle. The deltoid is characterized by greater activation at larger moment arms, with the medial portion providing the largest contribution throughout the entire range of motion.

For example, the study by Alpert and colleagues (2000) showed that, following changes in load applied to the anterior and lateral deltoid, electromyographic activity increases and reaches a peak between 30° and 90° of abduction (Alpert et al., 2000).

Scholten, instead, analysed the involvement of the deltoid during abduction from a biomechanical perspective, considering how muscle mechanics influence movement. The authors stated that the execution of abduction is strongly influenced by the upward rotational action of the scapula. This action allows the deltoid to avoid excessive fibre shortening, which would otherwise compromise force production. Furthermore, the authors suggested that during the first 40° of abduction the supraspinatus may act together with the deltoid to produce abduction, whereas beyond 40° the deltoid becomes the primary abductor (Scholten et al., 1982).

Consequently, a thorough understanding of the anatomy and biomechanics of this muscle, together with the concept of functional differentiation, is of paramount importance. Shoulder pain has a prevalence ranging from approximately 4% to 26% of the population and represents a very common condition, with symptoms frequently localized in the deltoid muscle (Mitchell et al., 2005; Murphy & Carr, 2010; Pope et al., 1997). Therefore, the ability to plan and structure targeted rehabilitation interventions according to the specific pathology or type of impairment, by focusing on different muscle segments and tailoring exercises to specific joint angles, is fundamental for the effective management of these conditions.

4.3.2 Deltoid motor unit literature

From past to present, the literature includes only a very limited number of studies investigating motor unit discharge behaviour in the deltoid muscle.

The first study aimed at investigating motor unit discharge parameters dates back to 1982 and was conducted by de Luca. In this study, the authors experimentally compared two upper limb muscles, the first dorsal interosseous and the deltoid, using iEMG. Although these muscles share a similar muscle fibre composition, they differ substantially from both an anatomical and functional perspective. The first dorsal interosseous is a small muscle composed of approximately 120 motor units, each contributing substantially through discharge modulation to force control. In contrast, the deltoid is a very large muscle, composed of more than one thousand motor units and primarily devoted to force production; consequently, modulation of the discharge of individual motor units has a smaller impact on overall force output. The authors investigated these two muscles in healthy participants performing triangular isometric contractions at moderate-high (40% of maximal voluntary contraction) and high (80% of maximal voluntary contraction) force levels. The results showed that the two muscles adopt different strategies in response to increasing force demands. The first dorsal interosseous terminated recruitment early and relied primarily on discharge rate modulation of already recruited motor units to achieve higher force levels. In contrast, the deltoid relied on motor unit recruitment throughout the entire contraction, and its motor units tended to reduce their discharge rate during sustained contractions, which was significantly lower than that observed in the first dorsal interosseous at comparable force levels. This study represents a milestone in the deltoid literature, as it was the first to describe the neural control mechanisms underlying deltoid activation and how they adapt to different task demands (de Luca et al., 1982).

The second study available in the literature was conducted by Doherty and Stashuk (2003), who validated a decomposition algorithm and included the deltoid among the muscles used to establish normative values. Their results provide reference values for EMG signal amplitude parameters and motor unit discharge behaviour in the deltoid of healthy subjects, recorded using iEMG (Doherty & Stashuk, 2003).

The most recent study investigating motor units in the deltoid was conducted by Baslo and colleagues (2024), with the aim of verifying whether, as often reported, clinical and EMG abnormalities in amyotrophic lateral sclerosis are greater in distal than in proximal

muscles, by comparing healthy and pathological subjects. The deltoid was used as the proximal muscle and was assessed during both voluntary contractions and electrical stimulation. IEMG was employed to evaluate mean discharge rate and discharge variability. The authors reported that both mean discharge rate and its variability were higher in patients with Amyotrophic Lateral Sclerosis compared with healthy subjects. These findings suggest that the central nervous system may adopt compensatory mechanisms in response to motoneuron loss and disease-related degeneration (Alpaydin Baslo et al., 2024).

The limited number of studies on the deltoid highlights the lack of research on this muscle and how underexplored it remains in the literature. Moreover, all existing studies exclusively employed intramuscular EMG, an invasive technique that does not allow a representative assessment of the entire motor unit population within the muscle. Additionally, available studies focused solely on force production at moderate to high intensities, whereas most daily-life movements performed by the deltoid involve low to moderate force levels. Consequently, there is a clear need to further investigate the mechanisms underlying deltoid function and neural control under conditions that more closely resemble everyday tasks. Such studies should employ non-invasive techniques, in both healthy individuals to characterize normal neuromuscular function and in pathological populations, with the aim of developing effective, efficient, and targeted rehabilitation and re-education protocols. The final experimental study of this doctoral project (chapter 8) was designed precisely to address this gap, analysing deltoid motor unit behaviour at low and moderate force levels using a non-invasive HD-sEMG approach, with the aim of providing reference values for discharge parameters in healthy individuals and establishing a foundation for future investigations.

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Part 3: Experimental studies conducted

5 Study 1: Motor unit behaviour throughout force gradation.

Title: Motor unit discharge behaviour in human muscles throughout force gradation: a systematic-review and meta-analysis with meta-regression.

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Abstract: The analysis of motor unit (MU) discharge behaviour provides an effective way of assembling information about the generation and control of movement. In this systematic-review and meta-analysis we identified and summarized the literature investigating MU discharge rate and discharge rate variability (CoV-ISI) during voluntary isometric contractions at various force levels. Databases were searched up to January 7, 2025, and a total of 262 studies were included. The meta-means of MU discharge rate and CoV-ISI were estimated and compared across human muscles. The influence of contraction intensity on MU discharge behaviour was assessed through linear meta-regressions. At low-to-moderate forces (<60% MVC), the *first dorsal interosseous*, *biceps brachii* (BB) and *forearm extensors* (FE) had the highest discharge rate, while the *soleus* had the lowest. At high force levels (>60% MVC), the *tibialis anterior* (TA) had the highest mean discharge rate compared to all other muscles, with the *soleus* maintaining the lowest. Regarding CoV-ISI results at low forces (<30% MVC), the TA had the lowest CoV-ISI values, except in comparison to the *vastii*. Additionally, the *vastii* had lower CoV-ISI values than the FE, *gastrocnemius medialis*, and *soleus*. Contraction intensity was positively associated with the mean discharge rates in all muscles investigated, although some muscles showed steeper slopes than others. Similar results were observed for CoV-ISI meta-regressions, with muscle-specific differences in slope. These findings suggest potential variations in neural control strategies across muscles during force gradation, such as differences in the relative contribution of rate coding to facilitate increasing force demands.

Keywords: Neural control; rate coding; coefficient of variation of the inter-spike interval; HDsEMG; intramuscular electromyography

5.1. Introduction

Since the seminal work of Sherrington (Sherrington, 1906), the alpha motoneuron has been referred to as the final common pathway of the neuromuscular system, as it integrates the synaptic inputs generated from spinal and supraspinal pathways to generate muscle force. For this reason, analysing the discharge behaviour of motor units, consisting of the alpha motoneuron and all of the muscle fibres it innervates (Liddell & Sherrington, 1925; Sherrington, 1925), provides an effective way of assembling information about the generation and control of force production. From the pioneering works of Adrian and Bronk (Adrian & Bronk, 1929) and Denny-Brown's (Denny-Brown, 1929) use of concentric needle electrodes to the development of wire intramuscular (Basmajian & Stecko, 1962; Bigland & Lippold, 1954) and quadrifilar electrode (De Luca & Forrest, 1972; Mambrito & De Luca, 1984), the recordings of single motor units opened new perspectives in the physiological mechanisms underlying voluntary muscle control. Decades later, the advancements in high-density electromyography (HDEMG) techniques (Masuda et al., 1983, 1985; Merletti et al., 1999; Reucher et al., 1987) and motor unit decomposition algorithms (Chen & Zhou, 2016; Holobar & Zazula, 2007; Negro et al., 2016; Ning et al., 2015) have led to recording large numbers of concurrently active motor units during voluntary contractions. Together, the use of the multi-channel thin-filament intramuscular electrodes (Negro et al., 2016; Muceli et al., 2015) and HDEMG surface grid electrodes combined with the advancements in motor unit decomposition algorithms (M. Chen & Zhou, 2016; Holobar & Zazula, 2007; Negro, Muceli, et al., 2016b; Ning et al., 2015), has given rise to a new line of research questions aimed to characterise motor unit discharge behaviour under a variety of conditions. Considering the result of muscle contraction is the generation of force, it is fundamental to understand the relation between motor unit control strategies and the force generation capacity of the muscle throughout force gradation (Bigland & Lippold, 1954; Monster & Chan, 1977; Milner-Brown et al., 1973; Person & Kudina, 1972).

The nervous system controls muscle force over most of the operating range of a muscle by concurrently modulating the motor unit discharge rate (rate coding) and the number of active motor units (recruitment) (Enoka & Duchateau, 2017). However, the relative contributions of rate coding and recruitment vary across the force operating range and appear to differ between muscles (Boccia et al., 2024; Del Vecchio et al., 2019; Škarabot et al., 2023). Experimental evidence indicates that during gradual increases in isometric force, which is an indicator of increased net excitatory synaptic input and may result from

increases in persistent inward currents (PICs) (Fuglevand et al., 1993; Heckman et al., 2008; Heckman & Binder, 1991), motor unit discharge rates initially increase with force and then stabilize (i.e., saturation stage) (Bigland & Lippold, 1954; Bracchi et al., 1966; De Luca & Contessa, 2015; Monster & Chan, 1977b). Discharge rate saturation is likely attributable to intrinsic mechanisms of the motoneurons (Cushing et al., 2005; Fuglevand et al., 2015; Heckman et al., 2008), and the progressive involvement of inhibitory or excitatory inputs as contraction intensity increases (Elias & Kohn, 2013). The inhibitory and excitatory drive increases to the point where the dendrites saturate and can no longer respond to the synaptic drive. For these reasons, while the mechanisms underlying force gradation are well-established in the literature, the effect of contraction intensity on motor unit discharge behaviour across different human muscles remains unclear.

Most human studies have been limited to the investigation of a small fraction of motor units over a narrow force range due to technical limitations. As such, this has constrained our understanding of how the modulation of rate coding influences force gradation across its entire range. Additionally, comparing motor unit discharge behaviour across human muscles has been complicated by the broad range of methods applied across many studies, including variations in the methods used to record motor units, contraction intensities, and muscles being examined. Therefore, a systematic review and meta-analysis to pool the available data across many studies provides a more robust investigation of motor unit behaviour in various human muscles. Although a number of excellent reviews have addressed anatomical and contractile properties of motor units (Duchateau & Enoka, 2002; Heckman & Enoka, 2012) how motor unit activity controls force production (Enoka & Duchateau, 2017), the effect of aging on motor unit discharge rates (Orssatto et al., 2022), the effect of resistance training on motor unit behaviour (Herda, 2022), and sex differences in motor unit behaviour (Lulic-Kuryllo & Inglis, 2022), no previous study has systematically reviewed and synthesized findings on motor unit discharge behaviour across human muscles using a meta-analytic approach, particularly with a focus on the effects in individual muscles throughout the full spectrum of force gradation.

The primary aim of this systematic-review and meta-analysis was to identify and summarize the findings from studies investigating motor unit discharge behaviour during voluntary isometric contractions at various force levels. Specifically, we pooled data from studies published up to January 7, 2025, to estimate and compare the meta-means of motor unit discharge rate and discharge rate variability in a wide variety of human muscles. As a

secondary aim, we conducted linear meta-regressions to assess whether the effect of contraction intensity on motor unit discharge behaviour differed between muscles. Lastly, this review discusses shortcomings in this area of study and where advancements are needed to propel the field of electrophysiology forward.

5.2. Methods

This systematic review was conducted according to guidelines set forth by the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA statement 2020) (Page et al., 2021). The PRISMA checklist is provided in **supplementary file 0**. As our systematic review involved a meta-analysis of means rather than a meta-analysis of between-group or within-group differences (which is more common), certain PRISMA criteria were not applicable or required modification. For instance, we did not assess the risk of bias, as the primary tools available (e.g., ROBINS-I) are designed for studies of interventions. Consequently, we also did not evaluate the certainty of evidence. These deviations are explicitly marked as “N/A” in the PRISMA checklist.

5.2.1. Eligibility criteria

The eligibility criteria for study inclusion were delineated using the PICOS framework (P: Population, I: Intervention, C: Comparator, O: Outcomes, and S: Study design). However, given the present review is a meta-analysis of means (i.e., estimating the overall mean of motor unit discharge behaviour), the 'Comparator' category was not applicable.

5.2.1.1. Population

Studies where healthy adults between the ages of 18 and 65 years old and without current or a history of neural or musculoskeletal disorders were included.

5.2.1.2. Intervention

Studies included in this review investigated motor unit discharge behaviour during isometric contractions in human muscles. Only studies assessing isometric voluntary sustained contractions were included; thus, studies focusing on evoked, automatic (e.g., postural) or dynamic contractions were not considered in this review. Studies where muscle contraction was not isometrically sustained around a target force (e.g., following a triangular

profile or explosive contractions), were also excluded. There were no restrictions regarding the muscle or muscle group investigated.

The measurement tools considered for the assessment of motor unit discharge behaviour were surface or intramuscular electromyographic myoelectric recording electrodes and complimentary decomposition software; therefore, studies that did not incorporate electromyographic recordings were excluded from this review. Additionally, only studies that estimated motor unit discharge behaviour were included.

5.2.1.3. Outcomes

The broad outcome domain of interest in this review was the assessment of motor unit discharge behaviour in human muscles. The specific outcome domains included motor unit discharge rate and motor unit discharge rate variability (i.e., coefficient of variation of the inter-spike interval; CoV-ISI). CoV-ISI values were considered only if relative values were provided. Another outcome of interest was the contraction intensities at which the motor unit discharge was assessed. Only studies that directly provided the contraction intensity (or at least an approximation of the intensity) relative to participant's maximal voluntary contraction (% MVC) were included. If a study assessed multiple contraction intensities, it was included only if the values were reported separately for each intensity.

5.2.1.4. Study design

Considering our main review question (meta-analysis of means), the types of studies included were observational studies, either cross-sectional or longitudinal, where there was no intentional manipulation of the variables of interest. However, studies in which participants received an intervention (e.g., exercise, pain, and fatigue) were only included if baseline or control measures were available. Additionally, only peer-reviewed studies published in English were included.

5.2.2. Information sources and search strategy

Studies published up to June 27, 2023, were initially identified through a search conducted by one reviewer (HVC). This search was subsequently updated on May 9, 2024, and again on January 7, 2025, by the same reviewer. The electronic databases utilised were MEDLINE (Ovid interface), PubMed, and Web of Science (Clarivate Analytics). Searching in databases may not retrieve all relevant studies, as reports may be missing or inadequately indexed due to insufficient detail in titles and abstracts (Higgins et al, 2019). To complement

the database searches, two reviewers (JGI and HVC) performed hand-searches of reference lists from selected studies and relevant reviews published in key journals (e.g., The Journal of Physiology, Journal of Electromyography and Kinesiology, Journal of Neurophysiology, and Journal of Applied Physiology).

The search strategy was designed by three reviewers (JGI, HVC, and CC), and the primary concepts were centred on the outcomes and measurement tools considered, which were structured as follows:

(“MOTOR UNIT OUTCOME”) AND (“ELECTROMYOGRAPHY
TECHNIQUE”).

“Motor unit outcome” identified the terms typically employed in studies assessing motor unit discharge (e.g., “discharge rate” or “firing rate”). “Electromyography technique” encompassed the terms commonly used to describe the measurement tools used to evaluate motor unit activity (e.g., “intramuscular electromyography” or “high-density surface electromyography”). The search strategies employed in each database are detailed in **supplementary file 1**.

5.2.3. Selection and data extraction processes

All search results were retrieved from databases and imported by one reviewer (HVC) into EndNote version 20 (Clarivate analytics), which facilitated the identification and removal of duplicates. Subsequently, the remaining citations and abstracts were imported into Rayyan (Ouzzani et al., 2016), where the initial screening process (title and abstract) was independently conducted by two reviewers (JGI and CC). The measure of agreement between the two reviewers was 0.94 (Cohen's kappa statistic), indicating almost perfect agreement (Landis & Koch, 1977). The disagreements were resolved through discussion, and a third reviewer (HVC) was consulted for arbitration when necessary. Given the substantial number of eligible reports for the second stage of the screening (full text), this process was undertaken by three reviewers (JGI, CC, and AB), with each reviewer assessing one-third of the eligible reports.

Like the full-text screening, the data extraction process was conducted by three reviewers (JGI, CC, and AB). A customised excel spreadsheet was used for data extraction, and all extracted data were subsequently cross-checked for accuracy by two reviewers independently (JGI and HVC). In cases where multiple reports of the same study were

identified, they were merged (Moher et al., 2009). The extracted data included participants characteristics (e.g., sample size and age), intervention details (e.g., muscle assessed and measurement tool), task contraction intensity, and summary statistics (means and standard deviation) of the outcomes of interest (i.e., mean discharge rate and CoV-ISI). If a study provided inter-spike intervals (ms), the data was transformed into mean discharge rate (pps) (i.e., the multiplicative inverse of the inter-spike interval) and CoV-ISI (i.e., the standard deviation of the inter-spike interval divided by its mean). When the study results were solely presented in graphical form, WebPlotDigitizer software version 4.6 (Rohatgi, 2015) (retrieved from <http://arohatgi.info/WebPlotDigitizer>) was employed to extract the data from the figures.

5.2.4. Data synthesis and meta-analysis

All analyses were conducted in R (version 4.4.2; R Foundation for Statistical Computing, Vienna, Austria) using the RStudio environment (version 2024.12.0+467). Meta-analysis and moderator (meta-regression) analysis were performed using the *metafor* package (Viechtbauer, 2010).

The primary aim of this systematic review was to estimate and compare motor unit discharge rate and discharge rate variability across human muscles. To achieve this, we combined data from individual studies in a multilevel meta-analysis of means. Specifically, we employed a two-level hierarchical model using the restricted maximum likelihood (REML) method. This approach was chosen because the included studies often reported multiple estimates for the same muscle (e.g., different contraction intensities within the same study). The two-level meta-analytic model accounts for the clustering of multiple estimates within the same group of participants (Assink & Wibbelink, 2016; Cheung, 2014, Viechtbauer, 2010) and has been previously applied in meta-analyses of similar data (Orssatto et al., 2022). Meta-analytic models were conducted separately for the three different force ranges (low < 30% MVC; moderate 30-60% MVC; and high > 60% MVC) with ‘muscle’ included as a moderator to assess differences in motor unit discharge behaviour between muscle groups within each force range. Importantly, only muscles with at least five estimates within a specific force range were included in the analysis.

Results from the meta-analyses are presented graphically using orchard plots (*orchard* package (Nakagawa et al., 2023)). These plots illustrate the estimated meta-analytic means for each level of the moderator (muscle), depicted as central circles (the

"trunk"), with confidence intervals represented by thick horizontal lines (the "branches") and prediction intervals by thin horizontal lines (the "twigs"). Individual estimates are shown as transparent circles, with their size proportional to precision ($1/\text{standard error}$), meaning larger circles indicate more precise estimates. Importantly, the estimated meta-analytic means are derived from the two-level hierarchical model, meaning they do not necessarily coincide with any single data point but rather represent the model-based estimate that accounts for individual estimates variance as well as clustering within studies. This explains why, in some cases (e.g., **Figure 5.3**: *vastus medialis*, *abductor digiti minimi*, *gastrocnemius medialis*, and *soleus* at low intensity), the meta-analytic mean may appear outside the range of individual estimates. Finally, in cases where the standard deviation of an estimate was not reported in the study, missing standard deviations were imputed separately for each muscle using the coefficient of variation from all complete cases, following the method proposed by Bracken (Bracken, 1992).

To explore whether the experimental method (surface or intramuscular electromyography) influenced reported motor unit discharge behaviour, we conducted an additional analysis using the experimental method as a factor in the two-hierarchical meta-analytic model. This analysis was restricted to the low force range (0–30%), as it included the largest number of muscles, estimates, and methodological variations.

The secondary aim of this review was to determine whether contraction intensity influenced motor unit discharge rate or discharge rate variability to a different degree between muscles. For this purpose, we conducted a linear meta-regression using a two-level hierarchical model. The interaction between ‘contraction intensity’ and ‘muscle’ as moderator was included as the moderator to assess whether the slope of the relationship between contraction intensity and motor unit discharge behaviour varied among muscle groups. Meta-regression analyses were performed only for muscles with at least ten estimates, ensuring sufficient data for robust statistical analysis. Results are presented graphically using bubble plots (**Figures 5.4-6**), which display the regression slope for each muscle and the relative weight of individual estimates based on their precision (i.e., $1/\text{standard error}$). Pairwise comparisons between muscles for both the meta-analyses and meta-regressions were performed using the *emmeans* package (Lenth et al., 2019) with Tukey’s adjustment for multiple comparisons.

All individual estimates extracted from each study (i.e., sample size; contraction intensity; means, and 95% confidence intervals of motor unit discharge behaviour) are

provided at <https://doi.org/10.6084/m9.figshare.27325089> and as supplementary material, separated by muscle. The estimates of mean discharge rate (**supplementary file 3**) and CoV-
ISI (**supplementary file 4**) are provided for all muscles, including those not included in the meta-analyses.

5.3. Results

5.3.1. Study selection

The PRISMA flow diagram for the studies' selection process is presented in **Figure 5.1**. From the initial 16,096 records retrieved through database searches, and after duplicates were removed, 9,919 records underwent title and abstract screening. Following the removal of 9,185 (including 24 that were not retrievable), 736 records (707 from databases and 29 from hand-searching) proceeded to the full-text screening. After further excluding 456 reports (440 from databases and 16 from hand-searching) due to the lack of population, outcome, or interventions of interest, and after collating multiple reports of the same study (**supplementary file 2**), a total of 262 studies were included in the review.

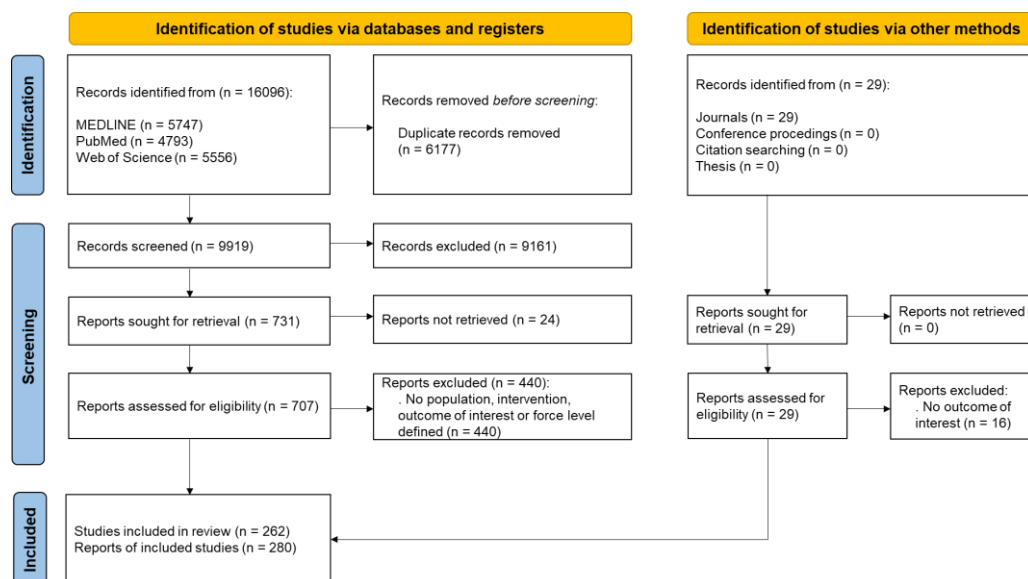


Figure 5.1: PRISMA flow diagram. PRISMA, preferred reporting items for systematic reviews and meta-analyses.

5.3.2. Study characteristics

The 262 studies included a total of 3,547 participants with ages ranging from 18 to 65 years old (11 studies did not report age), average height from 152 ± 6 to 184 ± 7.1 cm (138 studies did not report height), and average mass from 57.9 ± 10.6 to 96.8 ± 15.7 kg

(132 studies did not report mass). Intramuscular electromyography was used in 123 studies, surface electromyography in 132 studies, and 7 studies utilised both measurement techniques. The muscles assessed in the studies included (in order of prevalence): *tibialis anterior* (n = 66), *vastus lateralis* (n = 59), *biceps brachii* (n = 40), *first dorsal interosseous* (n = 39), *vastus medialis* (n = 28), *soleus* (n = 18), *triceps brachii* (n = 13), *gastrocnemius medialis* (n = 12), *gastrocnemius lateralis* (n = 10), *abductor digiti minimi* (n = 9), *forearm extensors* (n = 8), *forearm flexors* (n = 8), *thenar* (n = 5), *biceps femoris* (n = 4), *adductor pollicis* (n = 4), *trapezius* (n = 4), *brachioradialis* (n = 3), *semitendinosus* (n = 3), *masseter* (n = 2), *abductor hallucis* (n = 2), *anconeus* (n = 2), *rectus femoris* (n = 2), *peroneus* (n = 1), *pectoralis major* (n = 1), *semispinalis cervicis* (n = 1), *brachialis* (n = 1), *dorsal interosseus* (n = 1), *erector spinae* (n = 1), *extensor hallucis longus* (n = 1), *occipitofrontalis* (n = 1), and *sternocleidomastoid* (n = 1). Additionally, several contraction intensities were assessed in the studies, including lower than 10% MVC (n = 49), 10% MVC (n = 115), 11-19.9% MVC (n = 24), 20% MVC (n = 90), 21-29.9% MVC (n = 51), 30% MVC (n = 70), 35% MVC (n = 7), 40% MVC (n = 33), 50% MVC (n = 77), 60% MVC (n = 23), 70% MVC (n = 30), 75% MVC (n = 19), 80% MVC (n = 11), 90% MVC (n = 4), and 100% MVC (n = 43).

5.3.3. Results of syntheses

5.3.3.1. Overall pooled effect (meta-means)

The pooled mean discharge rates, along with 95% confidence interval (CI) and 95% prediction intervals for each muscle, are shown in **Figure 5.2**, separated by force ranges (low, moderate, and high). Additionally, **Table 5.1** provides all meta-estimates of the mean discharge rates for the muscles included within each force range. Overall, there was a significant effect of ‘muscle’ on the pooled mean discharge rate across all force ranges (test of moderators; $p < 0.001$ for all). Detailed contrasts, including estimated mean differences in mean discharge rate between muscles and corresponding p -values, are provided in **supplementary file 5**.

At the low force range (< 30% MVC; **Figure 5.2A**), the motor units of the *first dorsal interosseous*, *biceps brachii*, and *forearm extensors* presented the highest mean discharge rates compared to other muscles ($p < 0.007$ for all), with no significant differences between these three muscles ($p > 0.11$ for all). In contrast, *soleus* motor units had the lowest mean discharge rates compared to all other muscles ($p < 0.001$ for all) except for the *biceps femoris*

($p = 0.731$) and *forearm flexors* ($p = 0.294$). Additionally, the *abductor digiti minimi* showed higher mean discharge rates than the *triceps surae* ($p < 0.001$ for all) and *vastus lateralis* ($p = 0.012$). The *triceps brachii* also showed significantly greater mean discharge rates than both the *vastii* ($p < 0.001$ for both) and the *gastrocnemii* ($p < 0.001$ for both). Furthermore, *tibialis anterior* motor units presented higher discharge rates compared to the *gastrocnemii* ($p < 0.001$ for both). Lastly, the *gastrocnemius medialis* had higher mean discharge rates than the *vastus medialis* ($p = 0.002$).

At the moderate force range (30-60% MVC; **Figure 5.2B**), motor units in the *first dorsal interosseous* exhibited the highest mean discharge rates compared to all other muscles ($p < 0.003$ for all), except for the *biceps brachii* ($p = 0.965$). In contrast, *soleus* motor units showed significantly lower mean discharge rates than those of other muscles ($p < 0.049$), except for the *vastii* ($p > 0.250$ for both). Additionally, *biceps brachii* and *vastus medialis* motor units had significantly higher discharge rates than those in the *vastus lateralis* ($p < 0.010$ for both). The *biceps brachii* also showed higher discharge rates than the *gastrocnemius medialis* ($p = 0.003$).

At the high force range ($> 60\%$ MVC; **Figure 5.2C**), motor units in the *tibialis anterior* showed the highest mean discharge rates compared to all other muscles ($p < 0.028$ for all). Similar to the pattern observed at moderate force levels, *soleus* motor units displayed significantly lower mean discharge rates than all other muscles ($p < 0.003$), except for the *vastii* ($p > 0.450$ for both). Additionally, the *vastus medialis* showed higher discharge rates than the *vastus lateralis* ($p = 0.008$). Moreover, both the *biceps brachii* and *triceps brachii* motor units presented higher discharge rates than the *vastii* ($p < 0.025$ for all). The *first dorsal interosseous* also showed greater discharge rates than the *vastii* ($p < 0.034$ for both).

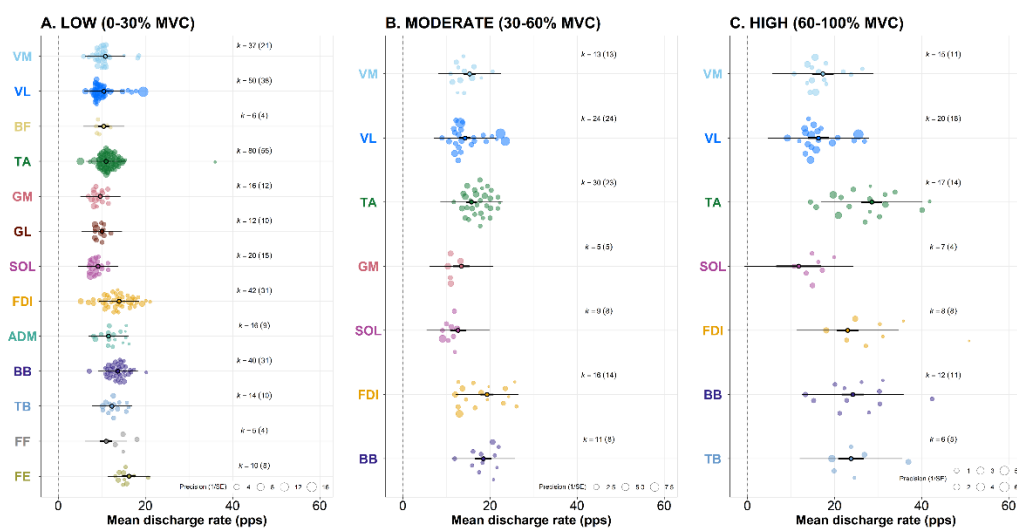


Figure 5.2: Orchard plots displaying the meta-analysis of means for motor unit discharge rates across different human muscles (y-axis), separated by low (A), moderate (B) and high (C) force ranges. Each circle represents individual estimates. The central circle (trunk), ticker horizontal line (branch), and thinner horizontal line (twig) represent the meta-mean estimate, 95% confidence level interval, and prediction interval, respectively. For each muscle, *k* indicates the total number of estimates with the total number of studies in brackets. Muscle abbreviations: VM, vastus medialis; VL, vastus lateralis; BF, biceps femoris; TA, tibialis anterior; GM, gastrocnemius medialis; GL, gastrocnemius lateralis; SOL, soleus; FDI, first dorsal interosseous; ADM, abductor digiti minimi; BB, biceps brachii; TB, triceps brachii; FF, forearm flexors; FE, forearm extensors.

MUSCLE	Low (< 30% MVC)			Moderate (30-60% MVC)			High (> 60% MVC)		
	Meta mean	95% LCI	95% UCI	Meta mean	95% LCI	95% UCI	Meta mean	95% LCI	95% UCI
Forearm Extensors	16.22	14.62	17.82	-	-	-	-	-	-
Forearm Flexors	10.95	9.6	12.3	-	-	-	-	-	-
Abductor Digiti Minimi	11.53	11.01	12.06	-	-	-	-	-	-
Biceps Brachii	13.67	13.22	14.11	18.49	16.58	20.41	24.2	21.63	26.77
Biceps Femoris	10.4	9.14	11.65	-	-	-	-	-	-
First Dorsal Interosseous	13.94	13.4	14.47	19.33	17.83	20.82	23.01	20.46	25.57
Gastrocnemius Lateralis	10.02	9.5	10.54	-	-	-	-	-	-
Gastrocnemius Medialis	9.64	9.15	10.13	13.44	11.61	15.28	-	-	-
Soleus	9.1	8.62	9.58	12.67	10.84	14.49	11.78	6.6	16.96
Tibialis Anterior	10.95	10.55	11.36	15.74	14.47	17.01	28.57	26.19	30.95
Triceps Brachii	12.31	11.75	12.86	-	-	-	23.78	20.81	26.75
Vastus Lateralis	10.48	9.98	10.98	14.35	12.96	15.62	16.31	13.85	18.78
Vastus Medialis	10.78	10.28	11.29	15.4	13.96	16.72	17.33	14.85	19.82

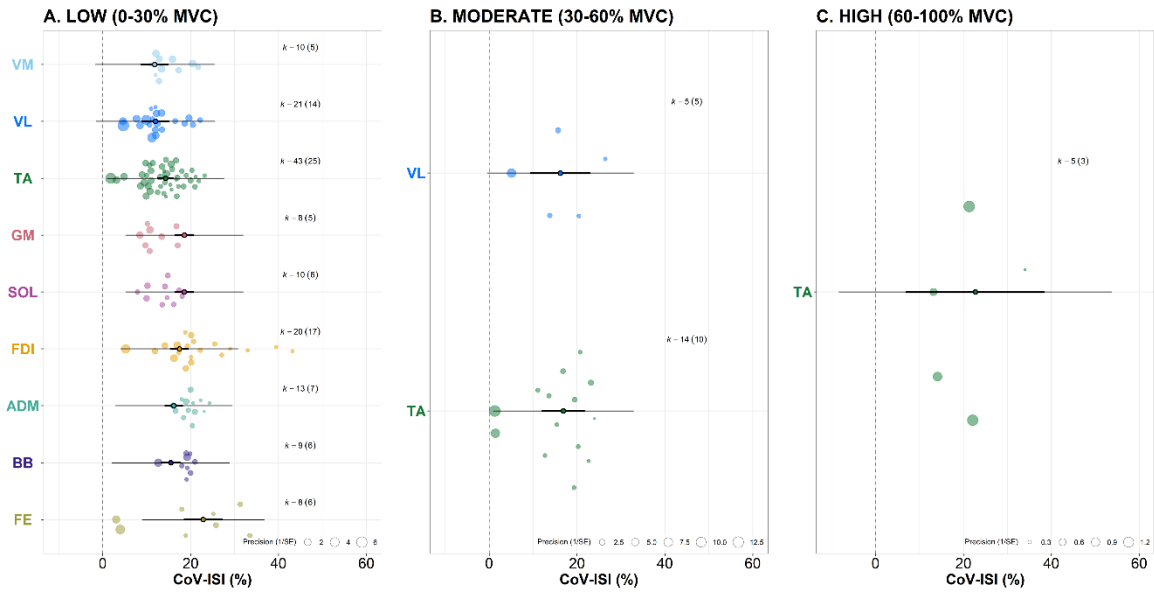
LCI, lower confidence interval; UCI, upper confidence interval

Table 5.2: Meta-estimates of mean discharge rate (pps)

The pooled CoV-ISI values, along with 95% CI, are presented in **Figure 5.3** and **Table 5.2**, separated by force ranges (low, moderate, and high). At high force ranges (**Figure 5.3C**), the only muscle with at least five estimates was the *tibialis anterior*, making it not feasible to perform comparisons between muscles.

At the low force range (< 30% MVC; **Figure 5.3A**), there was a significant effect of ‘muscle’ on the pooled CoV-ISI (test of moderators; $p < 0.001$). *Tibialis anterior* motor units had significantly lower CoV-ISI values compared to other muscles ($p < 0.02$ for all), except for the *vastii* ($p > 0.890$ for both) and *biceps brachii* ($p = 0.791$). Additionally, *vastii* motor units exhibited lower CoV-ISI values compared to the *forearm extensors* ($p < 0.003$ for both), *gastrocnemius medialis* ($p < 0.020$ for both) and *soleus* ($p < 0.020$ for both). No other significant differences were found between muscles. Detailed contrasts, including estimated mean differences in CoV-ISI between muscles and corresponding p -values, are provided in **supplementary file 5**.

At the moderate force range (30-60% MVC; **Figure 5.3B**), the *tibialis anterior* and the *vastus lateralis* were the only muscles with at least five estimates. No significant differences were found in the CoV-ISI between these muscles ($p = 0.864$).



To assess whether the experimental method (surface vs. intramuscular electromyography) influenced motor unit discharge behaviour, we conducted a moderator analysis using estimates from the low force range (0–30% MVC). No significant differences were found between methods for mean discharge rate (test of moderators; $p < 0.985$) or CoV-ISI (test of moderators; $p < 0.329$).

5.3.3.2. Effect of contraction intensity on motor unit discharge behaviour

To investigate whether the slope of the relationship between contraction intensity and motor unit discharge behaviour varied across muscle groups, we performed linear meta-regressions including all muscles with at least ten estimates. For the mean discharge rate meta-regressions, the slopes were significant for all muscles analysed ($p < 0.001$ for all), indicating that higher contraction intensities were consistently associated with increased mean discharge rates (**Figures 5.4 and 5.5**). However, the slopes differed significantly between muscles (test of moderators; $p < 0.001$), ranging from 0.069 for the *soleus* to 0.199 for the *forearm extensors*. Detailed statistical results, including estimated slopes for each muscle, are provided in **supplementary file 6**.

The *soleus* exhibited the lowest slope compared to the other muscles ($p < 0.022$ for all). Excluding the comparison between the *vastus lateralis* and *gastrocnemius medialis* ($p = 0.755$), the *vastus lateralis* presented significantly lower slopes than all other muscles ($p < 0.047$ for all). Similarly, the *vastus medialis* and *gastrocnemius medialis* showed lower slopes compared to most other muscles, aside from the *forearm extensors* ($p > 0.097$ for both) and *biceps femoris* ($p > 0.058$ for both), with no significant differences between them ($p = 1.000$). The *gastrocnemius lateralis* also displayed significantly lower slopes than most other muscles, except for the *forearm extensors* ($p = 0.314$), *biceps femoris* ($p = 0.296$), and *vastus medialis* ($p = 0.134$). In contrast, the *biceps brachii* and *triceps brachii* exhibited the steepest slopes across muscles ($p < 0.001$ for all), except when compared to the *biceps femoris* ($p = 1.000$ for both), *forearm extensors* ($p = 1.000$ for both) and *abductor digiti minimi* ($p > 0.778$ for both), with no significant differences between them ($p = 0.367$).

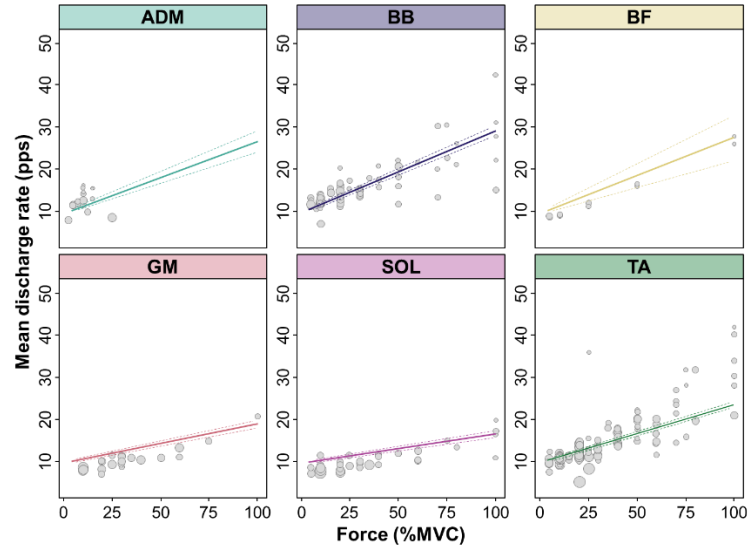


Figure 5.4: Bubble plots displaying the meta-regressions for the effect of force contraction intensity (% maximal voluntary contraction; MVC; x-axis) on motor unit discharge rates (y-axis) across different human muscles. Muscle abbreviations: ADM, abductor digiti minimi; BB, biceps brachii; BF, biceps femoris; SOL, soleus; TA, tibialis anterior.

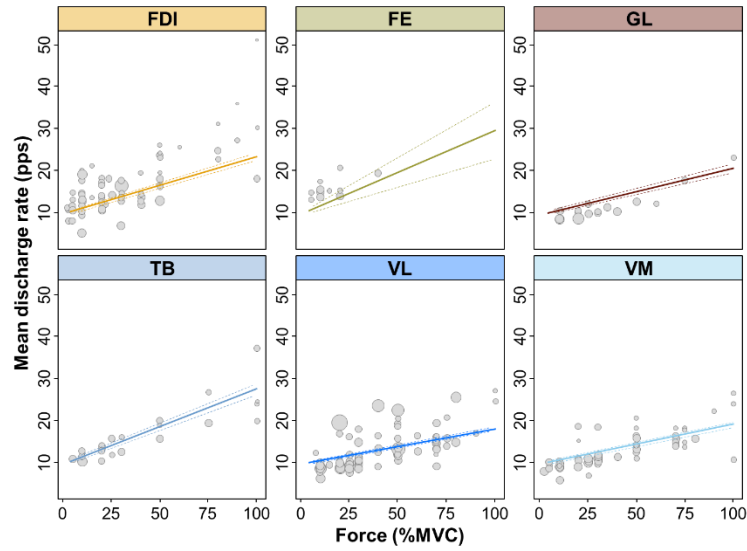


Figure 5.5: Bubble plots displaying the meta-regressions for the effect of force contraction intensity (% maximal voluntary contraction; MVC; x-axis) on motor unit discharge rates (y-axis) across different human muscles. Muscle abbreviations: FDI, first dorsal interosseous; FE, forearm extensors; GL, gastrocnemius lateralis; TB, triceps brachii; VL, vastus lateralis; VM, vastus medialis.

Regarding the CoV-ISI meta-regressions, the slopes were significant for all analysed muscles ($p < 0.030$ for all), indicating that higher contraction intensities were consistently associated with increased CoV-ISI (**Figure 5.6**). The slopes also differed significantly between muscles (test of moderators; $p < 0.001$), ranging from 0.013 for the *tibialis anterior* to 0.217 for the *abductor digiti minimi* (**supplementary file 6**).

Excluding comparisons with the *biceps brachii* ($p > 0.190$ for both), the *first dorsal interosseous* and *abductor digiti minimi* showed the steepest slopes compared to other muscles ($p < 0.016$ for all), with no significant difference between them ($p = 1.000$). In contrast, the *soleus* and *tibialis anterior* exhibited the lowest slopes relative to all other muscles, except for the *biceps brachii* ($p > 0.565$ for both), with no significant differences between them ($p = 0.831$).

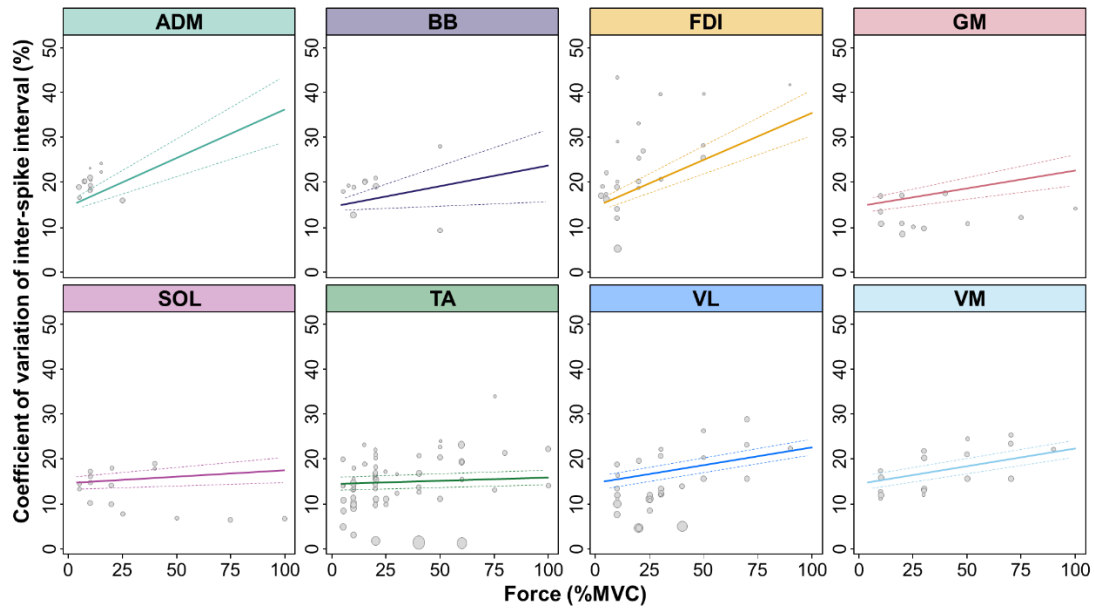


Figure 5.6: Bubble plots displaying the meta-regressions for the effect of force contraction intensity (% maximal voluntary contraction; MVC; x-axis) on the coefficient of variation of the inter-spike interval (CoV-ISI; y-axis) across different human muscles. Muscle abbreviations: ADM, abductor digiti minimi; BB, biceps brachii; FDI, first dorsal interosseous; GM, gastrocnemius medialis; SOL, soleus; TA, tibialis anterior; VL, vastus lateralis; VM, vastus medialis.

5.4. Discussion

5.4.1. Main findings

This systematic review and meta-analysis aimed to estimate and compare motor unit discharge behaviour across human muscles, separated into low, moderate, and high force ranges. We summarized the findings of studies published up to January 7, 2025, investigating motor unit discharge rate and discharge rate variability (CoV-ISI) during voluntary isometric contractions at various force levels. The main findings were that at low-to-moderate forces ($< 60\%$ MVC) the *first dorsal interosseous*, *biceps brachii*, and *forearm extensors* had the highest discharge rates, while the *soleus* had the lowest. At high force levels ($> 60\%$ MVC), the *tibialis anterior* was reported to have the highest mean discharge

rate compared to all other muscles, with the *soleus* again showing the lowest. Regarding the CoV-ISI main findings, due to a lack of studies investigating more than the *tibialis anterior* and *vastus lateralis* at moderate-to-high force ranges, main comparisons were possible in the low force range only. The *tibialis anterior* had the lowest CoV-ISI compared to all other muscles, except for the *biceps brachii* and the *vastii*. Additionally, the *vastii* had lower CoV-ISI than the *forearm extensors*, *gastrocnemius medialis*, and *soleus*.

We also explored whether the effect of contraction intensity on motor unit discharge behaviour varied across different muscles using meta-regressions. Our main findings indicate that contraction intensity was positively associated with the mean discharge rates in all muscles investigated. The *biceps brachii* and *triceps brachii* presented the steepest slopes, while the *soleus* and *vastus lateralis* showed the lowest slopes. Contraction intensity was also positively associated with the CoV-ISI in all muscles investigated. The *first dorsal interosseous* and *abductor digiti minimi* exhibited the steepest slopes, while the *tibialis anterior* and *soleus* presented the lowest slopes.

5.4.2. Motor unit rate coding

Minimum discharge rates of human motor units during voluntary contractions have been reported to discharge between 5 and 7 pps (Kudina & Alexeeva, 1992; Spiegel et al., 1996; Van Cutsem et al., 1997), which was corroborated by the individual estimates extracted in this meta-analysis (minimum value of 5.07 pps; see **supplementary file 3**). As contraction intensity increases, discharge rates progressively rise, as visually observed in the meta-means results (note the rightward shift in the x-axis from **Figure 5.2A** to **5.2C**), indicating an overall mean discharge rate of approximately 12 pps, 16 pps, and 21 pps at low, moderate and high force ranges, respectively (**Table 5.1**). In all muscles investigated, this positive association between force and mean discharge rate was statistically confirmed through meta-regressions, revealing that an increase of 1% MVC in contraction intensity was associated with an increase in the mean discharge rate ranging from 0.07 to 0.20 pps (see angular coefficients in **supplementary file 5.6**). These increases in motor unit discharge rate throughout force gradation can be explained by the fact that, after the motor units are recruited, rate coding is required to continue increasing muscle tension to achieve maximum force (Kamen & Du, 1999). Consequently, this mechanism becomes responsible for generating force beyond the muscle recruitment range (de Luca et al., 1982; Hatze, 1977; Milner-Brown et al., 1973). Thus, differences in motor unit discharge rate slopes across force

gradation between muscles could be explained by variations in the relative contribution of rate coding and recruitment to facilitate increased force demands. Muscles with steeper slopes, such as the *triceps brachii*, may rely on discharge rate modulation more than motor unit recruitment at lower force levels compared to the *soleus* and *vastii* muscles, which have lower slopes and subsequently require continuous recruitment to achieve higher force levels (**Figures 5.4 and 5.5**). However, it is important to note that, unlike studies that investigated force gradation through ramp contractions (a progressive increase in force during a single contraction), this systematic review pooled results from studies investigating isometric contractions performed at different force levels. Therefore, the rate coding mechanisms observed here are distinct from those seen during ramp contractions, which depend on the rate of torque development and should not be directly compared to those contractions.

Modulation of the motor unit discharge rate is a complex mechanism that depends on several factors (Kamen & Du, 1999; Henneman et al., 1965). In fact, motor unit discharge behaviour is correlated with: (1) synaptic drive (Kamen & Du, 1999), (2) membrane resistance and rheobase current (Fleshman et al., 1981), (3) motoneuron activation history (Inglis et al., 2011), (4) motoneuron size (Henneman et al., 1965), (5) function (ter Haar Romeny et al., 1982), or (6) the characteristics of the active population of motor units (Fleshman et al., 1981). The observed differences in individual muscle's motor unit discharge rate behaviour may, therefore, result from variations in some or all of these correlated factors. Specifically, the function, anatomy, and specific tasks performed by the muscles appear to be key determinants of the mean discharge rate (de Luca et al., 1982). In this meta-analysis, upper-body muscles, such as the *first dorsal interosseous* and *forearm extensors*, presented greater discharge rates compared to the muscles of the lower-body, such as the *triceps surae* (**Figure 5.2**). This difference in discharge rate likely reflects the increased need for upper-body muscles (included in this review) to produce smooth tension development, as these muscles are used more in fine motor control (Milner-Brown et al., 1973). A higher motor unit discharge rate implies a greater degree of motor unit twitch fusion (Nordstrom et al., 1989), resulting in smoother increases in tension development. Additionally, similar to other sampling processes, higher motor unit discharge rates allow synaptic inputs to be more accurately represented (Lazar & Pnevmatikakis, 2008), which may be relevant for achieving precise motor control in the upper limbs. Furthermore, as elegantly demonstrated by Bigland-Ritchie and colleagues (Bigland-Ritchie et al., 1983) on the force-frequency curve, even small increases (1-2 pps) in the steepest portion of the force-

frequency curve can significantly increase the force output of a muscle. In contrast, muscles like the *triceps surae* do not require higher discharge rates as they are primarily responsible for providing long-duration ankle joint stability during stance and walking tasks (Honeine et al., 2013). Previous research has shown that the discharge pattern of *gastrocnemius medialis* motor units is highly intermittent during standing, and that this muscle relies more on recruitment than on discharge rate to stabilize posture (Vieira et al., 2012). Moreover, the *soleus*, whose anatomical function is to maintain balance and posture, primarily requires sustained low-level contractions rather than rapid, powerful ones (Gollnick et al., 1974), which explains its lower discharge rate. Other factors may account for the differences observed between upper- and lower-body muscles, such as the characteristics of the descending tracts innervating these muscle groups such as differences in activation thresholds (Brouwer & Ashby, 1990).

Interestingly, the motor unit discharge rate of muscle groups that either work together to create torque around a joint (synergists) or stabilize the forces around a joint (agonist-antagonist relationship) can differ significantly. Synergist muscles appear to have similar discharge behaviours in human muscles at low force levels, as seen with no differences between the *gastrocnemii* or between the *vastii* muscles. However, as the force increases to moderate-to-high ranges, the *vastus medialis* discharges at significantly higher rates than the *vastus lateralis* (~15% greater). This increased discharge rate in the *vastus medialis* compared to the *lateralis* may be associated with differences in the mechanics of these muscles and their roles during knee extension (Gallina & Vieira, 2015; Lin et al., 2004). At higher force levels, it is possible that the *vastus medialis* must discharge at greater frequencies to compensate for the mechanical advantage of the *vastus lateralis* in force production (de Souza et al., 2018), otherwise, asymmetry in tension development could destabilized the knee joint, potentially leading to injury through improper patella tracking. Additionally, the fibres of the *vastus medialis* are oriented more obliquely to the femur from the proximal to the distal region (Blazevich et al., 2006; Lin et al., 2004), which may reduce its ability to transmit extension tension to the patella as part of its contribution to the medial pull during knee extension (Grob et al., 2018).

Regarding the agonist-antagonist relationship, in low force range, the motor unit discharge behaviour, based on the meta-means of the muscle groups with enough estimates, indicated that the discharge rate of the *tibialis anterior* was moderately higher than the *triceps surae* (~12 %), the *biceps brachii* was higher compared to the *triceps brachii* (~10%),

and the *forearm extensors* were higher than the *forearm flexors* (~32%) (see estimated mean differences and corresponding *p*-values in **supplementary file 5**). However, this was not the case for the *vastii* and *biceps femoris* relationship (~2%). Interestingly, when the force was increased to high levels, the significant differences between the *biceps brachii* and *triceps brachii* disappeared (~1.7%). The differences seen in the low force range may be a result of the amount of slack or pre-tension in the musculotendinous unit. At lower force levels, depending on the joint angle, either the agonist or antagonist muscle may have a certain amount of slack or tension in the muscle tendon unit, which allows a lower discharge rate to efficiently transfer the tension along the tendons line of action to produce torque about the joint (Inglis et al., 2017). However, at higher forces, the amount of pre-tension would be greater during the steady portion of the force output, which was the focus of this meta-analysis. As a result, opposing muscles working to stabilize a joint would be similarly advantaged in maintaining joint stability, thereby not requiring significantly different discharge rates to maintain the desired force. It should be noted that these findings need to be taken with caution. The use of broad force ranges (30-40%) may skew results, as we did not compare individual force levels of individual studies but a pooled meta mean of the low, moderate, and high force ranges. Additionally, one cannot discount the importance of task-dependent reciprocal inhibition in joint stability and the modulation of the motor unit discharge rate, even during isometric contractions. As Sherrington observed (Sherrington, 1913), the excitatory activity of the agonist muscle requires a corresponding relaxation of the antagonist muscle for optimal function. Furthermore, if reciprocal inhibition were to collapse, as is the case in upper motoneuron disorders (Hirabayashi et al., 2019) and in aging (Baudry et al., 2009; Hortobágyi & DeVita, 2006), this would result in excessive co-contraction of the opposing muscles, leading to little movement (Hirabayashi et al., 2019). Kagamikara and colleagues reported that reciprocal inhibition was present at low (5 and 15% MVC) but seemed to dissipate at higher forces (> 30%MVC), which may be an optimization mechanism at lower forces allowing for a smoother force production without interruption of competing muscle groups (Yanagisawa et al., 1976). Moreover, reciprocal inhibition may be responsible for the asymmetry of motor unit discharge profiles (modulation), leading to a smoother production of forces at low levels than symmetry at higher forces, which results in greater variability in force production. The reduction in reciprocal inhibition is evident during tonic isometric contractions at higher force levels when reciprocal inhibition is saturated and greater antagonist muscle activation is seen, despite the contractions being

isometric where co-contraction is lower. Additionally, the decrease in reciprocal inhibition at higher forces may be due to the importance of joint stability in injury avoidance from excessive force production from one muscle group over the other (Nielsen & Kagamihara, 1992).

5.4.2. Motor unit discharge rate variability (CoV-ISI)

Based on the meta-estimates results of the CoV-ISI, human motor units discharge within a narrow range of variability (i.e., relatively low CoV-ISI values), with minimal variation across muscles. As shown in **Table 5.2**, the CoV-ISI meta means ranged from 11 to 23%. Several factors may account for this narrow operational range of variability during isometric voluntary contractions. Firstly, the low discharge rate variability in motor units is believed to optimize force steadiness, particularly at low-to-moderate force levels (Inglis & Gabriel, 2021; Negro et al., 2009; Negro et al., 2016a; Negro & Farina, 2011). The variability in motor unit discharge rate is a result of both common synaptic inputs and synaptic noise, which accounts for approximately 70% of the variability (Farina & Negro, 2015; Negro et al., 2009; Negro et al., 2016a). Notably, both simulated and experimental data have consistently demonstrated that low-frequency oscillations of synaptic inputs shared across the motoneuron pool are the primary determinant of force production (Negro et al., 2009; Negro, Yavuz, et al., 2016b; Negro & Farina, 2011). Secondly, common synaptic inputs reduce discharge variability by coherently controlling motor unit behaviour (Bremner et al., 1991; Farina et al., 2014b; Farmer et al., 1993; Negro & Farina, 2011; Nordstrom et al., 1989), which further contributes to stable force production (Negro & Farina, 2012). Finally, motor unit behaviour studies often set a cutoff for motor unit discharge variability, therefore including only motor units with CoV-ISI below 20-30% (Stashuk, 2001), which would artificially compress these observed results leading to the variability falling within this range.

Interestingly, the upper-body muscles overall showed greater variability in motor unit discharge rate than the lower-body muscles. This increased variability may be related to greater synaptic noise to the muscles of the upper- compared to the lower-body (Negro et al., 2009). This hypothesis aligns with the meta-regression results of the CoV-ISI (**Figure 5.6**), where the *first dorsal interosseous* and the *abductor digiti minimi* showed significantly larger variations in CoV-ISI throughout force gradation (i.e., steepest slopes) compared to other muscles. Furthermore, as previously discussed, greater motor unit discharge rates were

reported in the upper- compared to lower-body muscles. Given that an increased number of discharges has been associated with greater synaptic noise (Castronovo et al, 2015; Farina & Negro, 2015), it would be expected that higher motor unit discharge rates would correspond with greater CoV-ISI.

5.4.3. Limitations and future directions

Despite the relevance of the current findings, several limitations must be acknowledged. First, it must be acknowledged that the findings of the current review are restricted to sustained isometric contractions and may not necessarily be applicable to what may occur during rapid and/or dynamic contractions (e.g., concentric or eccentric actions). Second, the number of studies investigating muscles beyond the *tibialis anterior*, *vastii*, *first dorsal interosseous*, and *biceps brachii* is limited. This lack of data restricted the statistical power of some analyses in this review. For instance, in the moderate-to-high force ranges, we were only able to calculate the CoV-ISI meta means for the *tibialis anterior* and *vastus lateralis* (**Figure 5.3**), as these were the only muscles with at least five reported estimates. Muscles in other regions, such as the shoulder complex, remain significantly understudied. Third, the included studies in this review often focused on specific force levels, primarily isometric contractions at 10%, 20%, 30%, and 50% of MVC, limiting our understanding of motor unit discharge behaviour across the full force gradation process. Fourth, it is now widely recognized that motor unit data exhibit hierarchical dependencies, as multiple motor units recorded from the same muscle within a participant are not statistically independent (Tenan et al., 2024). Historically, analyses often treated each motor unit as an independent observation, which we now understand may introduce bias. Recent studies have begun addressing this issue using linear mixed models to account for within-subject clustering, and we recommend that future studies adopt these models to improve the accuracy of their analyses. Finally, several studies lacked detailed reporting of all relevant variables, often omitting numerical descriptions of results, which further limited the scope of our analyses. To strengthen future research, the literature would benefit from more investigations across a wider range of muscles, force levels, and more comprehensive reporting of results.

Of the studies identified as part of the search strategy, 132 were from HDEMG surface data. Of the 132 studies, 91 utilized grids with more than five electrodes, and 41 used four or five electrodes and focused on populations between the ages of 18-35 where the majority of the participants were healthy young males with a limited physiological range

(Lulic-Kuryllo & Inglis, 2022). Some limitations have been reported in these studies that utilized this new and evolving technology. Some of the highlighted limitations from the investigated literature suggest that the HDEMG surface grid electrode results may be influenced by: (1) a bias towards more superficial motor units, limited volume detection, cross-talk and signal selectivity (Boccia et al., 2019; Casolo et al., 2021; Chen et al., 2017; Divjak et al., 2018; Hassan et al., 2019, 2021; He et al., 2017; Holobar et al., 2010; Lulic-Kuryllo et al., 2021; Maillet et al., 2022; Nishikawa et al., 2017; Park et al., 2016; Piitulainen et al., 2013; Thompson et al., 2022); (2) the superposition of discharges leading to potential cancellation, overlap or misidentification of unique motor units (Aoyama & Kohno, 2022; Casolo et al., 2021; Chen et al., 2023; Holobar et al., 2009; Mallette et al., 2021; Martinez-Valdes et al., 2020; McManus et al., 2019; Škarabot et al., 2022); (3) force level, with low force outputs from simple tasks (i.e. slow trapezoidal) while avoiding rapid changes in force output for favourable motor unit identification accuracy (Barrera-Curiel et al., 2019; Boccia et al., 2019; Cudicio et al., 2022; Del Vecchio et al., 2022; Dimmick et al., 2022; Fernandes et al., 2023; Hassan et al., 2019; Lecce et al., 2023; Lulic-Kuryllo et al., 2021; Mackay et al., 2023; Martinez-Valdes et al., 2018; Škarabot et al., 2022; Valli et al., 2024); (4) a preference towards the identification of later recruited and larger amplitude motor units (Aoyama & Kohno, 2022; Dimmick et al., 2022; Divjak et al., 2016; Farina et al., 2005; Holobar & Zazula, 2004; Martinez-Valdes et al., 2020, 2021; McKeown et al., 2019; Orssatto et al., 2021; Valli et al., 2023); (5) the influence of the thickness of sub-cutaneous tissue on motor unit separability (Chalchat et al., 2019; Gerdle et al., 2008; Jenz et al., 2023; Lulic-Kuryllo et al., 2021; Park et al., 2016; Stock et al., 2012; Watanabe et al., 2018) and (6) the ability to track changes in MU shape, particularly following interventions (i.e., muscle swelling and fatigue) (Del Vecchio et al., 2019; Hassan et al., 2019; Holobar et al., 2010; Lulic-Kuryllo et al., 2021; Miller et al., 2019, 2020; Piitulainen et al., 2013; Rossato et al., 2022; Škarabot et al., 2022; Valli et al., 2024)

Although considered the gold standard, intramuscular recordings are not free of challenges. Beyond the obvious potential discomfort associated with electrode insertion and placement, limitations include the small number of motor units that can be detected due to volume conduction and difficulties in distinguishing far-field motor units (i.e., high degree of selectivity of the intramuscular electrode) (Merletti & Farina, 2009). Accurate electrode placement can also be difficult, both in terms of positioning and depth, as it is possible to position the electrode in an area with no or low activity (e.g., due to muscle

compartmentalization). Consequently, there is often the need to reposition the needle to locate an area with higher activity, potentially leading to a recording bias based on the electrodes pick-up volume (Stalberg, 1980). Additionally, the movement of the needle, especially during force variations, can lead to difficulty during decomposition due to changes in the motor unit shape (Stashuk, 2001) and increased complexity of the interference pattern (Merletti & Farina, 2009). However, HDEMG does provide a robust alternative to record the myoelectric signal, namely by having the ability to record a greater number of motor units within the active motor unit pool (without needle relocation) and the ability to track single motor units across trials and over days (assuming no significant changes in muscle physiology or architecture) (Del Vecchio et al., 2019; Dick et al., 2023; Farina et al., 2016; Martinez-Valdes et al., 2023).

Underrepresented populations also remain a challenge in this field. Studies on these populations are considerably fewer than those conducted on healthy individuals, and the literature would benefit from more research focused on (1) persons with disability (Chen et al., 2023); (2) older adults and persons following bed rest with possible muscle atrophy or low quality muscle (i.e., fibromyalgia) or those who have potentially undergone motor unit remodelling (i.e. ALS) (Divjak et al., 2016; Gerdle et al., 2008; Hassan et al., 2021); (3) females (Lulic-Kuryllo & Inglis, 2022; Taylor et al., 2022), (4) populations with different muscle dimensions (Chalchat et al., 2019) or (5) to understand planned rehabilitation for disease, injury or athletic training (Piitulainen et al., 2013). Future directions should prioritize technological advancements, including the development of novel, potentially population-specific, grid electrodes and optimization of supporting decomposition algorithms. Additionally, the study of motor unit discharge behaviour will likely require innovative electrode configurations and a hybrid approach combining both surface and intramuscular recordings to fully capture the complexity of motor unit discharge behaviour across diverse populations and force levels.

5.5. Conclusions

In this systematic review, we identified 262 studies that reported motor unit discharge behaviour during isometric steady state contractions at various force levels across a variety of human muscles and muscle groups. Overall, motor unit discharge rate increased with the intensity of the force output regardless of muscle or muscle group, and contraction intensity was also positively associated with the variability in the motor unit discharge rate in all

muscles. These findings augment the importance of motor unit discharge behaviour in joint stability throughout force gradation. Additionally, this review highlighted the importance of motor unit discharge rate in force production, which continues to increase in many muscles above 50 to 90% MVC in order to achieve maximal outputs.

The individual muscle analysis revealed that smaller muscles, such as the *first dorsal interosseous* and *forearm extensors* have greater discharge rates at lower forces compared to the *triceps surae*. Additionally, the analysis revealed the muscles of the upper-body discharge at greater frequencies than those of the lower-body. As for the variability of the motor unit discharge rate, the analysis revealed that human muscles operate within a narrow range (low CoV-ISI) which may be necessary for joint stability and optimization of force stability, particularly during low to moderate force tasks. Interestingly, it was the muscles of the upper-body that had greater discharge variability compared to those of the lower-body. This, again, may be task dependent on the muscles that had enough estimates to be compared, such as the *tibialis anterior*, *soleus*, and *first dorsal interosseus*.

These findings only reflect those of an isometric contraction and may not represent dynamic or force varying contractions. Additionally, they may not necessarily represent the neural control of force output in females as most of the motor unit data comes from young, healthy males (Lulic-Kuryllo et al., 2021b). It must be emphasized that our systematic-review also revealed that there is much more work to be done to increase our understanding of the complexities of motor unit behaviour, specifically in the understudied muscle groups, such as the deltoid and pectoralis. It is also important that we continue to advance the technology utilized to study motor unit behaviour to estimate the changes in behaviour through electrophysiology better. This field of study has come a long way yet remains in its infancy, with many technological and physiological advancements on the horizon that must be considered as part of future investigations.

SUPPLEMENTAL MATERIAL

SupplementaryFile0_PRISMA_checklist: doi.org/10.6084/m9.figshare.27325089.

SupplementaryFile1_SearchStrategy: doi.org/10.6084/m9.figshare.27325089.

SupplementaryFile2_CollatedPapers: doi.org/10.6084/m9.figshare.27325089.

SupplementaryFile3_IndividualEstimates_MDR_R1:
doi.org/10.6084/m9.figshare.27325089.

SupplementaryFile4_IndividualEstimates_CoVISI_R1:

doi.org/10.6084/m9.figshare.27325089.

SupplementaryFile5_PairwiseComparisonsMuscles_R1:

doi.org/10.6084/m9.figshare.27325089.

SupplementaryFile6_MetaRegressions_R1: doi.org/10.6084/m9.figshare.27325089.

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6 Study 2: Motor skill learning.

Title: Differential changes in the effective neural drive following new motor skill acquisition between vastus lateralis and medialis

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Abstract: *Purpose:* To investigate whether short-term acquisition of a new motor task is mediated by changes in common synaptic inputs to motor neurons within and between synergistic muscles. *Methods:* Twenty-one participants performed 15 repetitions of a complex force-matching task at 10% of maximal voluntary contraction. Two trials were selected for analysis, the one with the highest force-target error (pre-learning) and the one with the lowest (post-learning). High-density surface electromyograms recorded from vastus medialis (VM) and vastus lateralis (VL) were decomposed into motor unit spike trains, and units were tracked between trials. Motor unit discharge behavior and common synaptic oscillations across the delta, alpha, and beta bands were calculated and compared between pre- and post-learning. *Results:* Force-target matching improved across trials, accompanied by a significant decrease in discharge rate variability in VL ($p < 0.001$), while the mean discharge rate remained similar ($p > 0.14$). The area under the curve in delta band coherence decreased for VM ($p = 0.01$), VL ($p < 0.001$) and VM-VL motor units ($p < 0.001$). In contrast, alpha band decreased for VL ($p < 0.001$), but not for VM ($p = 0.41$). Notably, reductions in alpha band correlated significantly with performance improvements only in VL ($R = 0.77$) but not VM. *Conclusion:* The acquisition of a new motor task is mediated by modulations in common synaptic inputs to motor units, leading to improved force control. Our findings suggest muscle-specific associations between these changes in common synaptic inputs and short-term motor learning, particularly in the alpha band.

Keywords: short-term skill acquisition, force control, HDsEMG, discharge patterns, synaptic input.

6.1 Introduction

The acquisition of a new motor skill task is defined as the process by which movements become progressively more accurate through practice and interaction with the environment (Willingham, 1998; Cheung et al., 2020; Doyon et al., 2003). This incremental process begins with a rapid initial phase, during which task repetition within a single session induces specific changes in movement control, ultimately enabling precise execution of the new motor skill (Doyon and Benali, 2005). These early changes in movement control reflect neural plasticity at both cortical and subcortical levels, ensuring the coordinated activation of synergistic muscles required for the intended movement (Dayan and Cohen, 2011; Ungerleider et al., 2002; Ziemann et al., 2004; Stefan et al., 2006; Aizenstein et al., 2004; Hikosaka et al., 2002; Doyon et al., 2002; Floyer-Lea and Matthews, 2004; Cheung et al., 2020). For instance, the early learning phase is dominated by cerebellar activity, with peak activation of the primary motor cortex linked with error correction (Lohse et al. 2014). Thus, research on functional and neural circuitry adaptations at the supraspinal level has significantly advanced our understanding of motor skill learning mechanisms.

Spinal mechanics underlying voluntary movement generation have also been explored in the context of motor skill learning (Ely et al., 2022; Kinany et al., 2023; Vahdat et al., 2015; Landelle et al., 2021). Neural inputs transmitted from spinal and supraspinal sources to spinal motor neurons consist of independent synaptic inputs, as well as synaptic inputs shared across the entire pool (Negro and Farina, 2012; Castronovo et al., 2018; Enoka and Farina, 2021; Farina and Negro, 2015). Due to the spread of shared inputs, the motor neuron pool functions as a selective linear filter, canceling out in the neural drive input components that are not common to all motor neurons. As a result, the cumulative spike train of action potentials received by the muscle (Farina and Negro, 2015), is primarily determined by the common synaptic inputs (Hug et al., 2023; Negro et al., 2016; Thompson et al., 2018; Farina et al., 2014; Negro et al., 2009). Therefore, investigating changes in the neural drive provides direct information on alterations in the strength of common synaptic inputs to alpha motor neurons. Building on this concept, a recent study investigated how the acquisition of a force-matching task influences the low-frequency oscillations of shared synaptic inputs to alpha motor neurons (Cabral et al., 2024), which are key determinants of force generation and variability (Enoka and Farina, 2021; Hug et al., 2023; Farina and Negro, 2015, Negro et al., 2009). The findings revealed that the short-term acquisition of the task was associated with reductions in physiological tremor oscillations within the shared synaptic inputs, with

these reductions correlating with improvements in performance. Additionally, compelling evidence suggested that learning a new motor task may modulate presynaptic inhibition by reducing H-reflex excitability, potentially facilitating motor sequence consolidation within spinal circuits (Lungu et al., 2010; Giboin et al., 2020; Perez et al., 2005). However, previous research has primarily focused on individual muscles controlling a single joint. How shared synaptic inputs are transmitted across synergistic muscles and modulated during skill acquisition remains unclear.

Therefore, the aim of this study was to investigate whether the short-term acquisition of a motor task is mediated by changes in the common synaptic inputs to motor neurons of synergistic muscle. We investigated this issue in the vastus lateralis (VL) and vastus medialis (VM) muscles, which act synergistically with the rest of the quadriceps group during knee extension. This synergistic pair is particularly relevant because VM and VL are believed to receive a high proportion of shared synaptic input during voluntary isometric contractions (Laine et al., 2015). However, they are anatomically and functionally distinct. Due to differences in muscle architecture (Flandry and Hommel, 2019; Blazevich et al., 2006), the VL has a greater potential for force generation (Lin et al., 2004; Lieber et al., 2000). Therefore, we consider it relevant to investigate whether these architectural and functional differences would lead to distinct modulation of common synaptic inputs during the short-term acquisition of a motor task. We hypothesized that the acquisition of a new skill training task would be similar across synergistic muscles and that the effects of neural drive oscillations on the overall force output would mainly depend on the different anatomical conformation of the muscles, the mechanical constraints, and the functions they perform.

6.2 Methods

6.2.1 Participants

Twenty one healthy individuals (15 males and 6 females, age: 29 ± 6 years; height: 178 ± 11 cm; weight: 73 ± 18 kg) were included in this study. All participants were right-leg dominant and were free from neuromuscular pathologies or conditions affecting the lower limb. This study was conducted following the approval of the local ethical committee (number NP5665) and in accordance with the latest Declaration of Helsinki. Before the beginning of experiments, all participants provided written informed consent.

6.2.2 Experimental protocol

A single experimental session was performed, lasting approximately 90 minutes. Participants were seated in an isokinetic dynamometer (HUMACNORM Extremity System, CSMi Solutions, Stoughton, MA) with hip and knee joints positioned at 90° of flexion (0° being fully extended) (**Fig. 6.1a**). The ankle was secured three centimeters above the malleolus to assess isometric knee extension force.

First, participants performed three five-second maximal voluntary contractions (MVCs). To ensure that contractions were not influenced by fatigue, a three-minute rest period was provided between each MVC (Inglis and Gabriel, 2020). The maximal force across the three MVCs was used to calculate the force level for the subsequent submaximal contractions (%MVC). Following three minutes of rest, participants performed 15 trials of a complex force-matching task at 10% MVC. The complex force-matching task consisted of a ramp-up phase at a rate of 10% MVC/s, a 30s phase of a stochastic force oscillation, and a ramp-down phase at a rate of 10% MVC/s. During the 30s stochastic force phase, subjects tracked a randomly generated signal with a frequency content below 1.5 Hz (-3 dB low pass frequency), which oscillated above and below 10% MVC (gray thick line on the top of **Fig. 6.1a**). The same trajectory was used throughout all trials and for all the participants. This protocol was based on prior studies (Knight and Kamen, 2004; Cabral et al., 2024), which demonstrated clear performance improvement in a similar protocol, quantified through a reduction in the root-mean-square error (RMSE) between the force and target signals, across the 15 repetitions. Visual feedback of the force output was displayed on a monitor along with the target force trace (**Fig. 6.1a**). At the end of the protocol, two additional MVCs were performed to verify that there was no decline in maximal force production, as an indication of fatigue. No differences were found between the MVCs recorded at the beginning and the end of the protocol (before: 354.25 ± 160.85 kg; after: 380.01 ± 230.24 kg; Wilcoxon signed-rank test; $P = 0.24$).

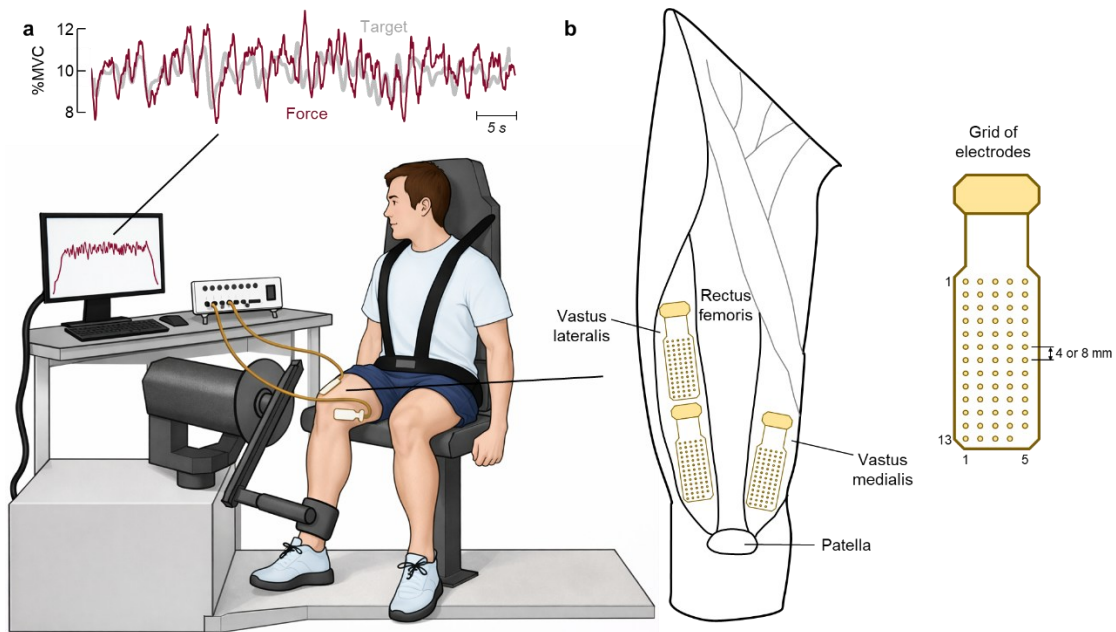


Fig. 6.1 Experimental Setup (a) Participant's position in the isokinetic dynamometer for the recording of the isometric knee extension task. Participants were seated on the dynamometer chair with the hip and knee joints positioned at 90° of flexion (0° being fully extended). The learning task consisted of following a randomly generated target signal, which oscillated above and below 10% of maximal voluntary contraction (gray thick line on the top panel). Visual feedback of the force (dark red line on the top panel) was displayed together with the target. (b) Schematic representation of the placement of electrode grids over the vastus lateralis and vastus medialis muscles. Three grids of 64 electrodes arranged in 13 rows x 5 columns were used

6.2.3 Data collection

During all 15 trials, the myoelectric signals from vastus medialis (VM) and vastus lateralis (VL) were recorded using high-density surface electromyography (HDsEMG). Specifically, three grids of 64 electrodes arranged in 13 rows x 5 columns (1 mm diameter, 8 mm inter-electrode distance, GR08MM1305, OT Bioelettronica, Turin, Italy) were placed over the distal VM region and the distal and proximal VL regions (**Fig. 6.1b**). The position of the grids was determined following Barbero et al. (2012). Following a preliminary analysis that revealed a very low number of extracted motor units from the VM muscle (see Results section), for the last 6 participants, three grids of 64 electrodes with smaller inter-electrode distance (4 mm) were applied over the VM, as recent evidence suggests that a larger number of close-spaced electrodes may provide a greater motor units yield (Caillet et al., 2023). Reference electrodes (WS1.1, OT Bioelettronica, Turin, Italy) were positioned on the right patella and the right ankle. Prior to electrode placement, the skin was shaved and mildly abraded (EVERI, Spes Medica, Genova, Italy) and cleaned with water to minimize skin impedance. Double-sided adhesive foam (FOA08MM1305 and FOA04MM1305, OT

Bioelettronica, Turin, Italy) was used to attach each matrix to the skin. A thin layer of conductive paste (AC cream, OT Bioelettronica, Turin, Italy) was spread over the electrodes to ensure electrode-skin contact. The myoelectric signals were acquired in a monopolar configuration with a sampling frequency of 2048 Hz using a 16-bit amplifier (10-500 Hz bandwidth; Quattrocento, OT Bioelettronica, Turin, Italy).

6.2.4 Data Analysis

The analyses were conducted using customized MATLAB scripts (R2023b).

6.2.4.1 Force. The force signals were initially low-pass filtered using a third-order Butterworth filter with a cutoff frequency of 15 Hz. Subsequently, during the 30s force oscillation phase, the RMSE, and the cross-correlation between the target profile and the force were computed for all 15 trials. To evaluate improvements in task performance, as a measure of the short-term force-matching skill acquisition, the RMSE between the produced force and the target was calculated. Then, two out of the 15 trials were selected for analysis based on the RMSE values of each participant: the one with the highest (pre-learning) and lowest (post-learning) RMSE values between force output and target traces (Fig. 2a). The trials selection was based on a recent paper from our group (Cabral et al., 2024) showing that, when using the same protocol, there were no statistical differences in force-target RMSE values within both the initial two and the last two trials. Then, to maximize the number of matched motor units that could be reliably tracked before and after skill acquisition, we kept only one initial trial and one final trial. Specifically, the trials selected represented the highest (pre-learning) and lowest (post-learning) RMSE between force output and the target trace (**Fig. 6.2a**). To assess changes in force steadiness with the force-matching skill acquisition, the coefficient of variation of the force was calculated as the ratio of the standard deviation to the mean value during the 30-s force oscillation phase (Laidlaw et al., 2000). Finally, to examine whether participants temporally shifted their force output to match the target skill better, the time lag at which the cross-correlation with the target peaked was compared between pre- and post-learning trials.

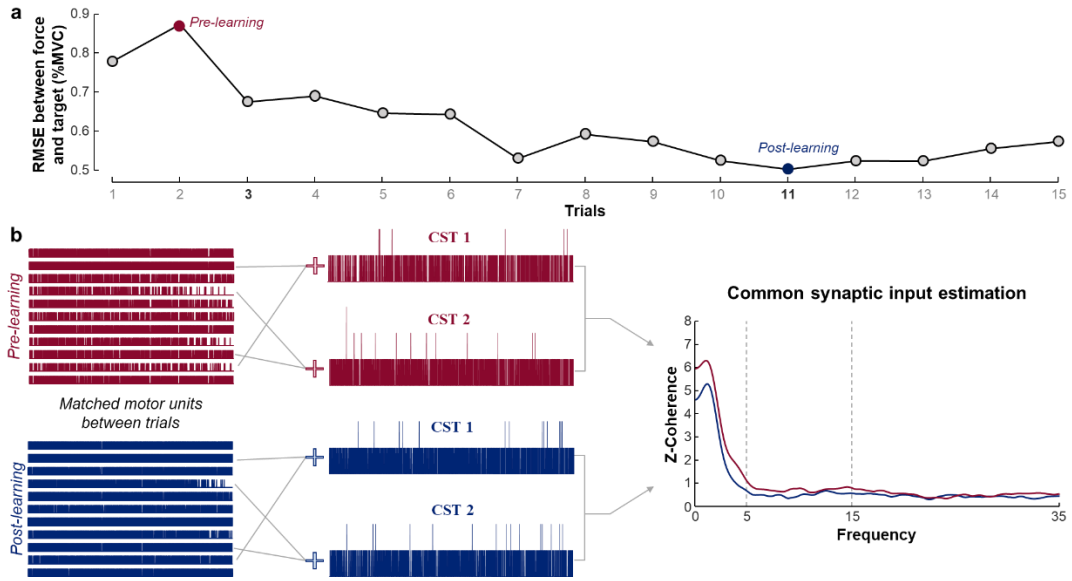


Fig. 6.2 Common synaptic input estimation (a) Representative case of the root-mean-square error (RMSE) between force and target across the 15 trials of the learning task. Two trials were selected for analysis: the trial with the highest RMSE value (pre-learning; red circle) and the trial with the lowest RMSE value (post-learning; blue circle). (b) Motor units were decomposed and tracked between the pre-learning (red) and post-learning (blue) trials. Common synaptic oscillations were estimated through coherence analysis between two equally sized cumulative spike trains obtained by summing the discharge times of randomly selected pairs of motor units

6.2.4.2 Motor unit decomposition. HDsEMG signals were initially bandpass filtered using a third order Butterworth filter with cut-off frequencies between 20 and 500 Hz. Subsequently, signals were visually inspected by an expert operator, and noisy channels resulting from electrode contact issues or artifacts were excluded. The signals during the middle 30s of the target (oscillatory region) were then fully decomposed using a convolutive blind source separation algorithm (Negro et al., 2016). This method allows for the automatic extraction of motor unit spike trains from the HDsEMG signals. The decomposition was performed independently on each electrode grid. Only motor units with a silhouette (SIL) of at least 0.87 were considered for further analyses. The SIL is a measure of accuracy of the decomposition that assesses the separability between identified motor unit spikes and noise (Negro et al., 2016). The same expert operator visually inspected all the identified motor units. Any missed or misidentified pulses (inter-spike intervals < 20 ms or > 250 ms (Negro et al., 2009)) were manually added or removed based on the changes in the innervation pulse trains and SIL values of motor units. After the manual optimization of the extracted motor units, the occurrence of the same motor unit being recorded by different grids placed on the same muscle (e.g., proximal and distal VL regions) was assessed. In cases, duplicated units were identified across grids (i.e., motor units that shared more than 30% of their discharge

times), the motor unit with the highest coefficient of variation of the inter-spike interval value was excluded for the subsequent analyses.

6.2.4.3 Motor unit tracking. Following the decomposition, the identified motor units were tracked between the pre- and post-learning trials by reapplying the motor unit separation vectors from the pre-learning to the post-learning trial and vice-versa (Oliveira and Negro, 2021; Cabral et al., 2024). This procedure was performed separately for each electrode grid. For subjects who did not have at least four units tracked between trials ($n = 8$), the signals were further inspected to track the motor units based on the similarity of their shape (i.e., motor unit template matching method) (Martinez-Valdes et al., 2017). For this analysis, only motor units with a cross-correlation between their action potentials greater than 0.8 were considered (Martinez-Valdes et al., 2017). For all tracked motor units between trials, the mean discharge rate and the coefficient of variation of the inter-spike interval were calculated.

6.2.4.4 Common synaptic input within and between muscles. To assess changes in common synaptic inputs to the motor neuron pool following the short-term acquisition of a new motor skill task, coherence analysis was performed between motor unit spike trains (Castronovo et al., 2015; Negro and Farina, 2012). Because of the summation process when converting synaptic inputs to the neural drive to the muscle, the motor neuron pool tends to cancel the noncorrelated components of the synaptic inputs so that only correlated components (i.e., common synaptic inputs) remain in the ensemble of the output spike trains (Farina et al., 2014; Dideriksen et al., 2012; Negro et al., 2009). In other words, the motor neuron pool acts as an effective linear filter (Farina and Negro, 2015) and, thus, linear methods such as coherence analysis can be used to estimate the strength of the common synaptic inputs (Negro and Farina, 2012). This analysis measures the linear coupling between two signals in the frequency domain, where zero indicates no coupling and one perfect coupling at a given frequency. Both within-muscle (VM and VL separately) and between-muscle (pooled VM-VL) coherence were performed. Changes in common synaptic oscillations were estimated separately for the delta (1-5 Hz), alpha (5-15 Hz), and beta (15-35 Hz) bands. The selection of these specific frequency bandwidths was driven by their physiological relevance in motor control. The delta band in motor unit coherence is thought to reflect oscillations associated with the voluntary control of force, as it contains the frequency range of the force output (Farina and Negro, 2015). The oscillations within the alpha band are usually attributed to physiological tremor within the Ia afferent feedback loop

(Christakos et al., 2006; Conway et al., 1995a) and can be viewed as common noise inputs that limit force accuracy. Finally, the beta band oscillations are believed to be of cortical origin, as significant corticomuscular coherence has been reported within this bandwidth (Baker et al., 1997; Conway et al., 1995b). Coherence analysis in these three frequency bands therefore provides information about common synaptic inputs to the alpha motoneuron pool arising from supraspinal and spinal pathways (Negro and Farina, 2012). Specifically, for this study, the discharge times of motor units were converted into binary sequences (i.e., motor unit spike trains), and two equally sized cumulative spike trains (CSTs) were obtained by summing the binary sequences of two randomly selected motor units from the matched units (**Fig. 6.2b**) (Negro and Farina, 2012; Rossato et al., 2022; Maillet et al., 2022; Castronovo et al., 2015). This process was repeated for up to 100 iterations and the average coherence was calculated for further analysis. For all iterations, coherence was calculated between the two detrended CSTs using Welch's periodogram with a Hanning window of one second and an overlap of 95% (**Fig. 6.2b**). The averaged coherence values were converted into standard z-scores as suggested by Gallet and Julien (2011). Only z-coherence values greater than the bias, which was determined as the mean z-coherence between 250 and 500 Hz (Castronovo et al., 2015), were considered. To assess changes in the common synaptic inputs across different frequency bandwidths, the area under the curve was calculated for delta, alpha, and beta bands. Subsequently, the ratio of the area under the curve (post/pre) was calculated and subtracted by one. In this way, positive ratio values indicate an increase in coherence with the short-term skill acquisition, while negative values indicate a decrease.

6.2.5 Coupling between force/neural drive and target oscillations. To assess whether there were changes in the linear coupling between the oscillations in force and the target oscillations, as well as between oscillations in the neural drive (i.e., CSTs) and the target oscillations, coherence analysis between these signals was performed. This analysis was carried out similarly to the coherence between motor unit spike trains. However, as delta band oscillations contain the frequency range of the force output and, thus, are associated with voluntary force control, only the delta band was considered for this analysis. For both force-target and CST-target coupling, an increase in the coupling indicates an enhancement in the representation of common synaptic input oscillations relevant to optimal force control in the intended motor task.

6.2.5 Statistical analysis

Statistical analyses were performed in R (v. 4.3, R Core Team, 2023) using the RStudio environment (v. 2023.03.1). Non-parametric tests were used considering the data was non-normally distributed (Kolmogorov-Smirnov test; $P < 0.001$ for all). The Wilcoxon signed-rank test was performed to compare the force-target RMSE, the force-target cross-correlation values and the force-target cross-correlation lags between trials. The Wilcoxon signed-rank test was also used to compare the coefficient of variation of force between the pre-learning and post-learning trials.

Linear mixed models (LMMs) were employed to compare the mean motor unit discharge rate and the coefficient of variation of the inter-spike interval between pre- and post-learning. LMMs were implemented with the *lmerTest* package (Kuznetsova et al., 2017) using the Kenward-Roger method to approximate the degrees of freedom and estimate P -values. For this analysis, random-intercept models were used, considering time (pre-learning and post-learning), as a fixed effects and participant as a random effect. The marginal means with 95% confidence level intervals were estimated using the *emmeans* package (Lenth, 2016). To compare z-coherence values between pre- and post-learning trials, changes in the area under the curve ratio (post/pre) were assessed using the one-sample Wilcoxon test (null hypothesis $\mu_0 = 0$), separately for each frequency band. To determine whether the changes in the area under the curve of the z-coherence were associated with the number of motor units, Pearson's correlations were performed between the number of matched motor units and the area under the curve ratios, separately for the delta and alpha bands. For this analysis, the VM and VL results were pooled together. To evaluate changes in the area under the curve ratio of the force-target z-coherence and CST-target z-coherence, the one-sample Wilcoxon test was used.

Separately for each muscle, repeated measures correlations were performed between the force-target RMSE and the area under the curve ratio within the delta and alpha bands to test whether the observed reductions in delta and alpha band coherence (see *Results*) were correlated with performance improvements. These analyses were performed using *rmcorr* package with fixed slopes (Bakdash and Marusich, 2017). Considering the limited number of participants, bootstrapping was used in order to report the 95% confidence interval and obtain bias-corrected intervals. A total of 1,000 iterations were performed, each using 75% of the sample size (10 participants). To compare the effect of muscle on the relationship

between z-coherence–RMSE in the delta and alpha frequency bands, a LMM was performed with RMSE as the dependent variable and subject as a random effect. The area under the curve (delta or alpha band), muscle (VM or VL), and their interaction were included as fixed effects. For all the variables, statistical significance was set at an alpha of 0.05. All individual data of motor unit discharge times for both the VM and VL acquired in the pre- and post-learning trials are available at <https://doi.org/10.6084/m9.figshare.28847492>.

6.3 Results

6.3.1 Changes in force oscillations between pre- and post-learning

To assess improvements in force-matching between pre- and post-learning trials, the RMSE and cross-correlation values between the force output and the target trace were calculated. **Fig. 6.3a** shows the force traces of a representative participant during the pre-learning (red) and post-learning (blue) trials. It can be visually observed that there is a greater overlap between the force and the target (gray line) in the post-learning compared to the pre-learning, indicating improvements in performance.

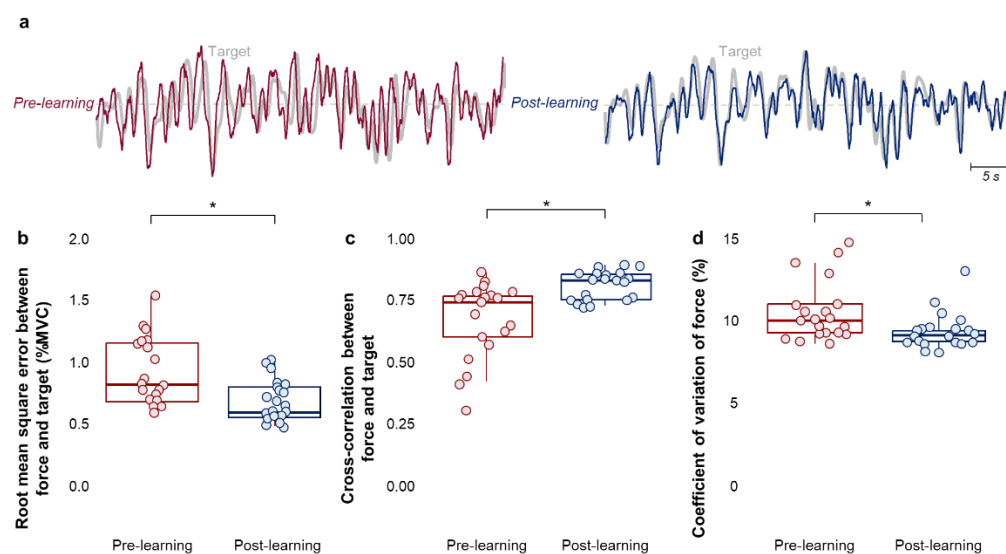


Fig. 6.3 Force-target matching results (a) Representative case of the force during the pre-learning (red) and post-learning (blue) trials. The target is shown in gray. (b) Group results of the root-mean-square error between the force and the target. (c) Group results of the cross-correlation between the force and the target. (d) Group results of the coefficient of variation of force. Circles identify individual participants. Horizontal traces, boxes, and whiskers denote the median value, interquartile interval, and distribution range. * $p < 0.05$

These observations were confirmed by the group results. There were statistically significant decreases in RMSE (**Fig. 6.3b**; $p < 0.001$) and increases in the cross-correlation (**Fig. 6.3c**; $p < 0.0001$) between the force and the target when comparing post-learning with

pre-learning trials. However, the cross-correlation lags did not significantly change between the pre- and post-acquisition trials ($p = 0.65$). Moreover, to assess changes in force steadiness, the coefficient of variation of the force was calculated and compared between trials. As shown in **Fig. 6.3d**, there was a significant decrease in the coefficient of variation of force ($p < 0.001$), which indicates an increase in force steadiness with force-matching skill acquisition.

6.3.2 Motor unit discharge behaviour

A total of 324 motor units were identified from the VM (164 pre-learning; 160 post-learning), with an average ($\pm$ standard deviation) of 7 ± 4 motor units per participant in the pre-learning (6 ± 3 , 3 ± 2 and 3 ± 2 in the three grids over the VM) and 7 ± 4 motor units in the post-learning (5 ± 3 , 4 ± 2 and 4 ± 2 in the three grids over the VM). When comparing the number of motor units identified after changing the matrix configuration in the VM with the number identified initially, they were not significantly different (first matrix configuration: 6 ± 3 matched motor units per participant; second matrix configuration: 6 ± 3 matched motor units per participant; $P = 0.83$). In the VL, 927 motor units were identified (455 pre-learning; 472 post-learning), with an average of 21 ± 10 (14 ± 9 and 7 ± 3 in the two grids over the VL) and 21 ± 11 (15 ± 9 and 8 ± 3 in the two grids over the VL) motor units per participant in the pre- and post-learning trials, respectively. These motor units were subsequently matched between pre- and post-learning trials, leading to an average of 6 ± 2 matched motor units for the VM (5 ± 2 , 3 ± 1 and 3 ± 1 in the three grids over the VM) and 12 ± 8 for the VL (12 ± 8 and 5 ± 2 in the two grids over the VL). The average ($\pm$ standard deviation) SIL values of the cleaned, matched motor units were 0.93 ± 0.04 for the VM and 0.95 ± 0.04 for the VL.

To assess changes in motor unit discharge behavior following the short-term acquisition of the force-matching skill task, the mean discharge rate and coefficient of variation of the inter-spike interval of tracked motor units were calculated. For both VM and VL, there were no significant changes in mean discharge rate between pre- and post-learning (VM: from 8.38 [7.86, 8.89] pps to 8.00 [7.46, 8.54] pps, $p = 0.14$; VL: from 8.27 [7.77, 8.77] pps to 8.18 [7.67, 8.68] pps, $p = 0.44$). In contrast, the coefficient of variation of the inter-spike interval significantly decreased between trials for the VL only (from 40.9 [36.3, 45.5] % to 26.4 [21.8, 31.0] %, $p < 0.0001$), but not for the VM (from 38.9 [32.2, 45.7] % to 32.3 [25.3, 39.4] %, $p = 0.08$).

6.3.3 Common synaptic input oscillations

To evaluate alterations in common synaptic inputs, the area under the curve ratio (post/pre) was calculated for the delta (1–5 Hz), alpha (5–15 Hz), and beta (15–35 Hz) bands. While delta band oscillations in motor unit coherence are linked with changes in voluntary force control, changes in alpha band are commonly associated with physiological tremor oscillations that hampers force control (Christakos et al., 2006). Finally, alterations in beta band are connected to changes in corticomuscular coupling (Baker et al., 1997). Only participants with at least four tracked motor units between trials were included in the study (see Methods); thus, 13, 19 and 20 participants were included for VM, VL and VM-VL coherence, respectively. In both muscles, significant reductions were observed in the area under the curve within the delta band ($p = 0.005$ for VM; $p < 0.001$ for VL). In the alpha band, a significant decrease was observed only in VL ($p < 0.001$), but not VM ($p = 0.41$). In the beta band, neither muscle showed significant differences between pre- and post-learning ($p = 0.79$ for VM; $p = 0.57$ for VL) (**Fig. 6.4a** and **6.4b**). Similar to the results in VL, significant VM-VL coherence decreases were observed in the delta and alpha bands with the force-matching skill acquisition ($p < 0.01$ for both), but not for the beta band ($p = 0.06$; **Fig. 6.4c**). To assess if observed changes in common synaptic oscillations were associated with the number of included motor units, we performed a Pearson's correlation analysis between the area under the curve ratio (post/pre) and the number of matched motor units. For both the delta ($R = -0.06$, $p = 0.76$) and the alpha bands ($R = -0.17$, $p = 0.36$), the number of motor units was not significantly correlated with changes in the area under the curve.

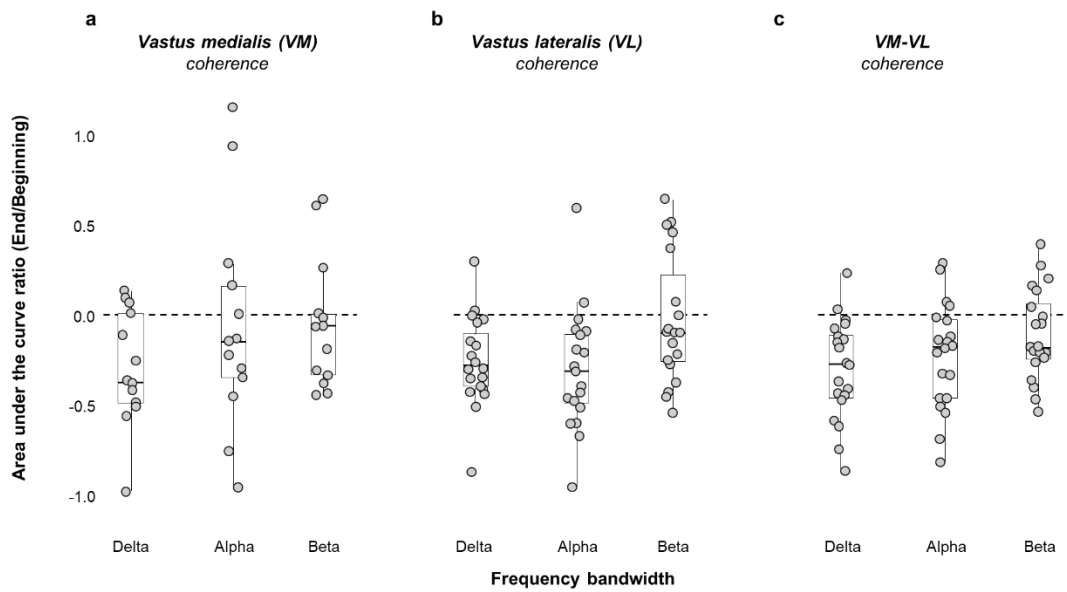


Fig. 6.4 Motor unit coherence results Group results of the area under the curve ratio of coherence for VM (a), VL (b), and pooled VM-VL muscles (c). Three frequency bandwidths were analyzed: delta (1-5 Hz), alpha (5-15 Hz), and beta (15-35 Hz). Median value, interquartile range, and distribution range are denoted by the horizontal traces, boxes, and whiskers, respectively. Circles represent individual participants

To investigate whether reductions in motor unit z-coherence within the delta and alpha bands were associated with improvements in the force-matching task, we applied a repeated measures correlation. For both bands, a significant positive correlation was observed between the force-target RMSE and the z-coherence in the VL (delta: $R = 0.52$ [0.47, 0.66]; **Fig. 6.5a**; alpha: $R = 0.77$ [0.66, 0.82]; **Fig. 6.5c**). In contrast, for the VM, only the correlation between the force-target RMSE and the z-coherence in the delta band was significant (delta: $R = 0.59$ [0.21, 0.85]; **Fig. 6.5b**; alpha: $R = 0.12$ [-0.23, 0.39]; **Fig. 6.5d**). LMM analysis revealed a significant interaction between muscle and alpha-band z-coherence ($p < 0.01$), indicating stronger within-subject associations between z-coherence and force-target RMSE in VL compared with VM. In contrast, there was no significant muscle and z-coherence interaction for delta-band ($p = 0.37$).

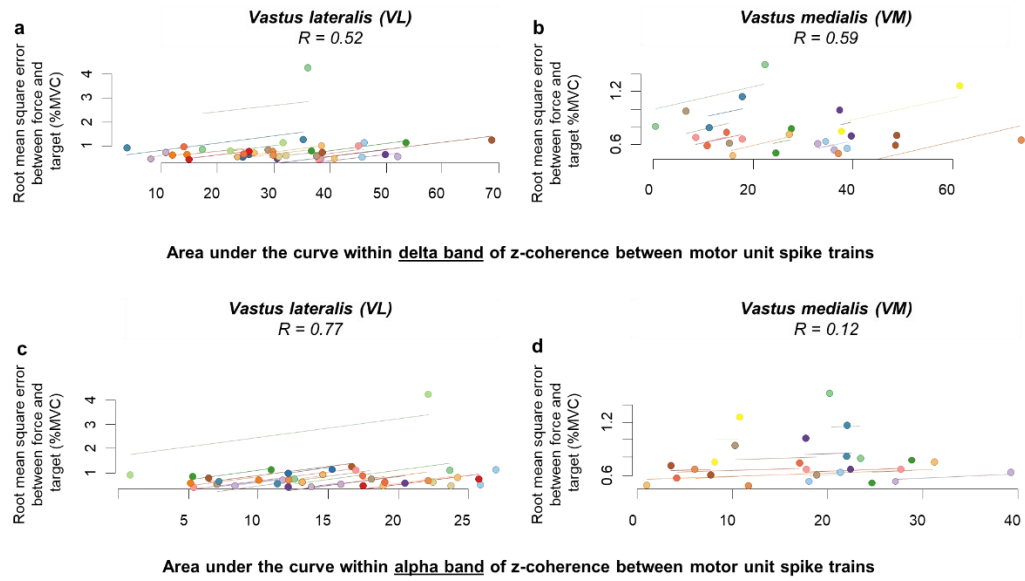


Fig. 6.5 Correlation results Repeated measures correlation between the force-target root-mean-square error and the area under the curve of motor unit z-coherence within the delta band (a and b), and the alpha band (c and d). The left panel shows the results for the vastus lateralis muscle and the right panel for the vastus medialis muscle

6.3.4 Linear coupling between force/neural drive and target oscillations.

Changes in the linear coupling between the force/neural drive oscillations and the target trace oscillations were investigated using coherence analysis between these signals within the delta band. **Fig. 6.6** shows the pooled z-coherence (i.e., average across participants) between the force and target, and between the neural drive (i.e., CST) and the target for both VM and VL. For both force-target z-coherence ($p < 0.001$; **Fig. 6.6a**) and VL CST-target z-coherence ($p = 0.03$; **Fig. 6.6b**), there was a significant increase in the area under the curve between pre- and post-learning trials. In contrast, the z-coherence between the VM CST and target did not significantly change between trials ($p = 0.53$; **Fig. 6.6c**).

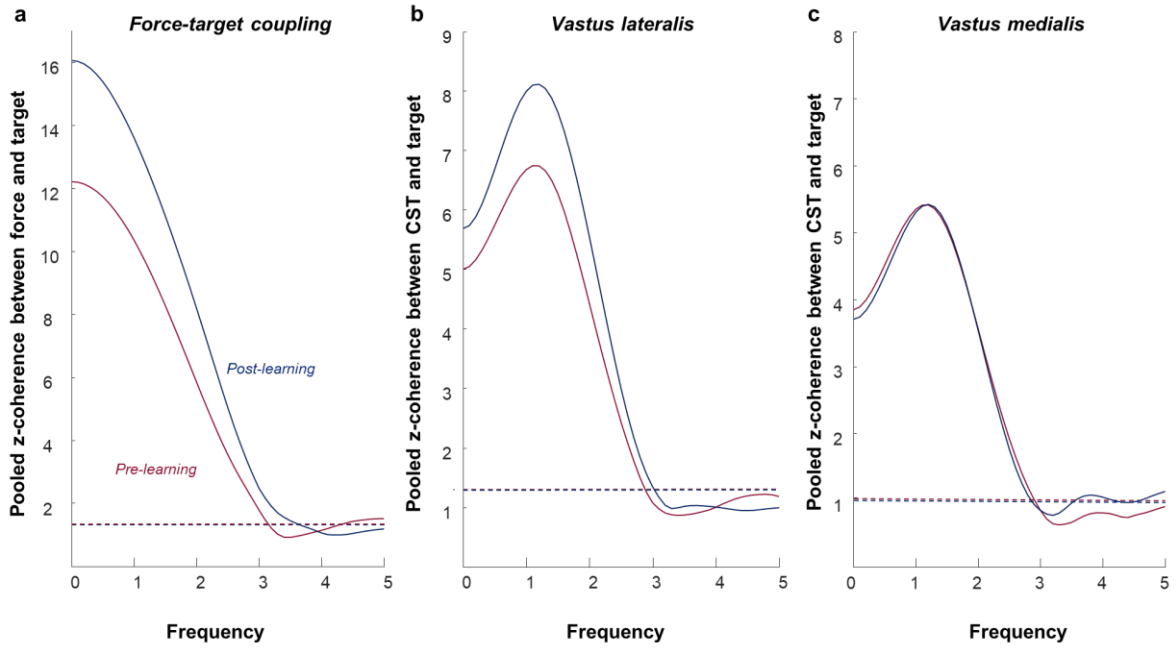


Fig. 6.6 Coherence results between force/neural drive and target oscillations The pooled z-coherence profiles between force and target (a) and cumulative spike train (CST) and target (b and c) are shown. The CST-target coherence analysis was performed separately for the vastus lateralis (b) and vastus medialis (c) muscles. The red and blue lines denote the pre-learning and post-learning trials, respectively

6.4 Discussion

This study investigated changes in the common synaptic input to synergistic muscles (VM and VL) during the short-term acquisition of a novel isometric motor task. Our main findings demonstrated that force-matching performance improved with skill acquisition. These improvements were accompanied by a reduction in common synaptic oscillations to spinal motor neurons in the delta coherence band, both within and between synergistic muscles, and in the alpha band for the VL and between synergistic muscles. Interestingly, reductions in alpha band oscillations were positively correlated with performance improvements, but only for the VL. As discussed below, these results suggest muscle dependent associations between changes in neural drive oscillations and short-term motor acquisition, underscoring the importance of considering the functional role of synergistic muscle.

The neural drive received by the muscle (i.e., the cumulative spike train of alpha motor neuron action potentials) is low-pass filtered by the contractile properties of active motor units at approximately 12 Hz (Windhorst, 2012). Consequently, low-frequency oscillations in the neural drive (i.e., delta and alpha bands of motor unit coherence) play a

determinant role in force control (Farina and Negro, 2015; Hug et al., 2023). Therefore, we investigated whether short-term acquisition of a new force-matching task is mediated by changes in the effective neural drive to synergistic muscles, by assessing coherent oscillations in motor unit spike trains of VM and VL. Our results revealed that short-term skill acquisition was followed by reductions in alpha band coherence both in VL and between vastii motor units (**Fig. 6.4**). Considering alpha band (physiological tremor) oscillations are considered an involuntary noise component of the common synaptic inputs that negatively influence force accuracy (McAuley and Marsden, 2000; Yavuz et al., 2015; Watanabe et al., 2018; Erimaki et al., 1999), their reduction during skill acquisition likely represents a neural mechanism to enhance force precision (Laine et al., 2014; Henderson et al., 2022; Williams et al., 2010; Koželj and Baker, 2014). These findings support previous studies that demonstrated a strong link between reduced physiological tremor oscillations and improved force control (Novak and Newell, 2017; Christakos et al., 2006; Williams et al., 2010; Koželj & Baker, 2014; Laine et al., 2016). Although the precise mechanisms underlying alpha band reduction during short-term acquisition remain unclear, spinal interneurons phase-cancelling descending alpha oscillations have been proposed as a plausible mechanism (Cabral et al., 2024). Additionally, learning a new motor task has been shown to modulate presynaptic inhibition by reducing H-reflex excitability (Lungu et al., 2010; Giboin et al., 2020; Perez et al., 2005), which could also explain alpha band reductions. Finally, the reduction in alpha-band oscillations may be attributed to supraspinal mechanisms, particularly to attentional processes (Adrian and Matthews 1934; Pollen and Trachtenberg, 1972; Hanslmayr et al., 2011; Klimesch, 2012). Cortical alpha suppression has been considered a marker of enhanced processing of visual feedback (Bacigalupo and Luck, 2022) and may be associated with observed alpha band reductions in motor unit coherence after short-term skill acquisition.

Interestingly, nonetheless similar results in coherence were observed between VL and pooled VM and VL motor units (see **Fig. 6.4**), only reductions in delta-band coherence were positively correlated with performance improvements in both muscles (**Fig. 6.5a**; **Fig. 6.5b**). In contrast, reductions in alpha-band coherence were positively correlated with performance improvements only in VL (**Fig. 6.5c**), but not VM (**Fig. 6.5d**). These differences in the correlations were confirmed by a linear mixed-effects model, which revealed a significant interaction between muscle and z-coherence on the alpha band–RMSE relationship, with the VL showing stronger associations than VM. Given that VM and VL

are synergistic muscles that share most of their synaptic inputs (~75-95%; Laine et al., 2015), this finding was unexpected. However, architectural differences between these muscles may explain this discrepancy. Although both VM and VL are part of the same muscle group (i.e., quadriceps), their muscle architecture differs significantly (de Souza et al., 2018; Lieber and Fridén, 2000; Ward et al., 2009; Lefebvre et al., 2006). The VL has a larger cross-sectional area, with muscle fibers arranged more transversely from the distal to the proximal region (Blazevich et al., 2006). These anatomical features provide the VL with a mechanical advantage for force production, while the VM primarily contributes to knee joint stabilization and force transmission to the tendon (De Souza et al., 2018; Lin et al., 2004; Lefebvre et al., 2006; Farahmand et al., 1998; Lieber and Fridén, 2000). Thus, the positive correlation between alpha band reductions and performance improvements only in VL might suggest a mechanical advantage to produce force, which facilitates more efficient transduction of neural inputs into force. This interpretation was further supported by the CST-target coupling analysis, which revealed a significant increase in coupling between pre- and post-learning trials for the VL (**Fig. 6.6b**), but not for the VM (**Fig. 6.6c**). Therefore, while synergistic muscles such as the vastii share a significant portion of their synaptic inputs (Laine et al., 2015), their motor output is influenced by their distinct contractile properties and force-generating capacity. A further explanation for the different behaviours between the two synergistic muscles could be attributed to the specific joint angle (90° of flexion). At this angle, the VL is closer to its optimal length and is mechanically more effective, which could explain why the most adaptations and correlations occurred in this head. Conversely, the VM is mechanically more effective at lower flexion angles due to its role in patellar stabilization (Abelleyra Lastoria et al., 2023; Panagiotopoulos et al., 2006). Additionally, at 90° of knee flexion, the contribution of the rectus femoris is minimal during extension, and as suggest in some studies, the vastus intermedius typically shows activation patterns similar to the vastii (de Oliveira et al. 2018). Furthermore, during learning, the coordination of multiple muscle heads during skill acquisition is non-deterministic and the maintaining of the same target torque can be achieved through multiple combinations of individual muscle force contributions (Bersani et al., 2023). Therefore, evaluate these neural control strategies across multiple joint angles, that modify the contributes of the four head of the quadriceps components, could clarify better the muscle-specific adaptations when investigating neural drive changes during motor short-term acquisition.

Our findings also revealed a decrease in common synaptic oscillations within the delta band following skill short-term acquisition, which plays a critical role in optimal force production and task execution accuracy (Negro et al., 2009). A plausible explanation for the delta band reductions observed in this study relates to the motor strategy adopted by participants during the short-term acquisition of the force-matching isometric knee extension task. In both pre- and post-learning trials, participants followed the target similarly in time, as confirmed by the absence of significant differences in force-target cross-correlation lags (see *Results*). Thus, improvements in force-matching accuracy were achieved by decreases in force amplitude oscillations, which was confirmed by significant decreases in the coefficient of variation of force. This reduction in force variability, together with the increased coupling between force and feedback across trials (**Fig. 6.6a**), explains the delta band coherence reductions.

A final consideration concerns alterations in motor unit discharge behaviour during task short-term acquisition. Unlike previous studies (Knight and Kamen, 2004; Patten and Kamen, 2000; Cabral, et al., 2024), no significant changes in mean discharge rate between pre- and post-learning trials were observed. This suggests that mean discharge rate alone may not explain the observed changes in common synaptic oscillations. Instead, the reduction in motor unit discharge variability between trials in VL, quantified by the coefficient of variation of the inter-spike interval, contributed to the observed changes in common synaptic oscillations. This aligns with previous findings demonstrating a correlation between reductions in common synaptic oscillations and decreases in the coefficient of variation of the inter-spike interval (Heckman et al., 2009). Consistent with these findings, numerous studies have suggested that improvements in force steadiness are primarily driven by modulation in the common drive to motor neurons, rather than changes in motor unit discharge rate (Semmler et al., 2006; Dideriksen et al., 2012; Bilodeau, 2000; Tracy et al., 2004; Marmon et al., 2011; Farina and Negro, 2015)

There are a few limitations of this study that we would like to acknowledge. Although the results of this study suggest that short-term acquisition is associated with modulations in the shared synaptic inputs to alpha motor neurons, we acknowledge that due to the chosen protocol, these findings may not be generalized to overall motor learning. Existing literature highlights that retention and/or transfer tests must be conducted to refer to motor learning, as these allow for the evaluation of persistence beyond the within-session practice (Kim et al., 2021; Lage et al., 2024; Chung et al., 2021; Hung et al., 2021). Since such tests were not

performed in this study, the results can only be interpreted as reflecting short-term acquisition. Moreover, the present study does not include a control condition to assess whether the observed changes in neural drive cannot be solely attributable to the repetition of the motor task independent of short-term acquisition. However, in a recent study of our group (Cabral et al., 2024), we showed that the repetition of fifteen steady isometric contractions (control condition) led to an increase in alpha band coherence, which is the opposite of what was observed during the short-term skill acquisition. These findings rule out the possibility that the observed changes in alpha band are only attributable to motor task repetition independent of acquisition. Furthermore, despite the absence of a correlation between the number of motor units and the coherence values, the significantly lower motor unit yield, particularly in VM, may have partly affected the robustness of the results. Thus, between-muscle differences should be interpreted with caution and future studies with larger samples should further investigate muscle-specific synergistic mechanisms underlying motor learning.

Finally, although previous literature has highlighted that motor unit behavior differs between sexes (Lulic-Kuryllo and Inglis, 2022), it was not possible to perform statistical comparisons between sexes in this study. This limitation is mainly due to the difficulty in decomposing and tracking motor units in females, which has already been reported in the literature (Taylor et al., 2022).

In conclusion, this study demonstrated that, similar to individual muscles, the acquisition of a new motor task is mediated by modulations in the common synaptic inputs to motor units, leading to improved force control and short-term motor acquisition. Notably, our findings suggest muscle-dependent associations between alpha band oscillations and improvement in force-target error, highlighting the importance of assessing muscle-specific adaptations in motor short-term acquisition.

6.5 References

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7 Study 3: Synergistic control during hand prehension task

Title: Adaptations in common synaptic inputs to spinal motor neurons during grasping versus a less functional hand task

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Abstract: Previous evidence suggests that shared synaptic inputs across spinal motor neurons play a key role in coordinating multiple muscles during hand movements, reducing control complexity. In this study, we investigated how the nervous system modulates these common synaptic inputs during a functionally relevant grip (grasping) compared to less functionally relevant hand tasks. Seventeen participants performed three different tasks: simultaneous four-finger flexion without thumb involvement (four-finger flexion), thumb flexion, and simultaneous flexion of both fingers and thumb (grasping). For each task, subjects sustained isometric contractions at 5% and 15% of maximal voluntary contraction, while high-density surface electromyograms (HDsEMG) were recorded from the extrinsic flexor muscles of the hand. Motor unit spike trains were decomposed from HDsEMG and tracked across tasks, and their mean discharge rate was calculated. Coherence between motor units was quantified within the delta, alpha, and beta bands to estimate common synaptic oscillations. At both force levels, the mean discharge rate decreased during grasping compared to four-finger flexion but increased during grasping compared to thumb flexion. Additionally, the area under the curve of coherence within the alpha band decreased by ~20% during grasping compared to the four-finger flexion task, with no significant delta or beta bands changes. These reductions in alpha band coherence were reflected in force oscillations, showing decreased force-neural drive coupling within the alpha band and increased force steadiness during grasping compared to four-finger flexion. Our findings suggest that a functionally relevant and frequently used grip involves distinct neural control mechanisms that ultimately enhance force control.

Keywords: Hand; neural control; motor unit; low-dimensional control

7.1 Introduction

The human hand is capable of performing a wide range of movements with remarkable versatility, enabling complex interactions with the environment (Napier, 1956). While humans possess some degree of finger individualization, most hand movements rely on the coordinated flexion of multiple fingers, particularly for grasping, a fundamental action in daily activities (Young, 2003; Almécija et al., 2015; Schieber & Santello, 2004). The anatomical specialization of the human hand, such as a fully opposable thumb, larger intrinsic muscles, and the ability to arch the palm of the hand, supports the performance of prehensile movements, allowing for efficient object manipulation (Napier, 1956; Napier, 1960; Napier, 1965; Marzke, 1997; Susman, 1979; Marzke & Shackley, 1986). Furthermore, the functional relevance of grasping is evolutionarily evident from early development, as newborns display an instinctive grasping reflex that later transitions into voluntary grasping, reinforcing its role as a fundamental component of human motor behaviour (Dennis, 1943; Twitchell, 1965). Taken together, these findings highlight that while humans exhibit some degree of independent finger control, most everyday manual tasks, from tool use to object manipulation, rely on the coordinated flexion of multiple digits, making grasping one of the most functionally relevant hand movements.

Precision and dexterity during grasping require a finely coordinated interplay of the forces produced by the multiple muscles controlling the fingers (Landsmeer, 1963; Long et al., 1970). Rather than individually controlling each extrinsic hand muscle, the central nervous system synergistically coordinates them, resulting in the required hand movement, which reduces the degrees of freedom and, consequently, simplifies control (for review, see (for review, see Santello et al., 2013). This modular control of fingers and hand movements, referred to as “hand synergies”, has been extensively documented through different methodologies, including kinematic data (Soechting & Flanders, 1997; Santello et al., 1998; Santello et al., 2002), force measurements (Li et al., 1998; Reilly & Hammond, 2000; Santello & Soechting, 2000), and muscle activation recordings (Maier & Hepp-Reymond, 1995; Weiss & Flanders, 2004; Tanzarella et al., 2021). These studies demonstrate that a small set of low-dimensional components can account for a wide range of hand movements. This has also been observed at the neuronal level, where synaptic inputs largely shared across spinal motor neurons, particularly in extrinsic hand muscles (Hockensmith et al., 2005; Winges & Santello, 2004), are thought to underlie this modular control (Tanzarella et al., 2021; Hockensmith et al., 2005; Winges & Santello, 2004; Keen & Fuglevand, 2004;

Johnston et al., 2005). For example, Hockensmith, Lowell (2005) reported a high degree of commonality in motor unit discharge times between two extrinsic hand muscles acting on the thumb and index finger. Interestingly, this between-muscle commonality was as pronounced as that observed in motor units within individual muscles. These findings align with recent evidence which reported that motor unit discharge rates decoded from several extrinsic hand muscles during a grasping task can be largely explained by a single low-dimensional component derived using principal component analysis (Tanzarella et al., 2021). However, it remains unclear whether the nervous system modulates these common synaptic inputs across different hand movements, particularly when comparing tasks that differ in functional relevance (Tanzarella et al., 2021).. For instance, grasping is a frequently used and highly practiced action in activities of daily living, while the simultaneous flexion of all four fingers without involvement of the thumb is less functionally relevant. Given recent results showing that task familiarity through practice (i.e., learning) is associated with reductions in alpha-band oscillations in common synaptic input to motor neurons (Cabral et al., 2024), we hypothesized that grasping would exhibit reduced alpha-band coherent oscillations and improved force steadiness compared to a less commonly performed hand action.

In this study, we sought to assess the common synaptic oscillations to spinal motor neurons of the extrinsic finger flexor muscles during three tasks: simultaneous flexion of the metacarpophalangeal joints of four fingers without involvement of the thumb (hereafter referred to as four-finger flexion), thumb flexion, and simultaneous flexion of both fingers and thumb (hereafter referred to as grasping). Specifically, we explored whether the common synaptic inputs differ between a functionally relevant and frequently used grip (grasping) and less functionally relevant hand actions. To achieve this, we decomposed motor unit spike trains from high-density surface electromyograms (HDsEMG) recorded from the extrinsic finger flexors and tracked motor units across tasks. We then used coherence analysis to estimate the shared synaptic oscillations in the delta, alpha, and beta bands. Our results revealed a reduction in alpha band oscillations during grasping compared to four-finger flexion. This reduction in alpha band oscillations was reflected in force oscillations, revealing decreased force-neural drive coupling within the alpha band and increased force steadiness during grasping. These findings suggest that a more natural, functionally relevant grip involves specific neural control mechanisms to enhance force control.

7.2 Methods

7.2.1 Participants

Seventeen healthy individuals (5 females; mean $\pm$ standard deviation: age 30 ± 4 years) with no history of upper limb injuries volunteered to participate in this study. Before starting the experiments, all participants provided written informed consent. The experimental procedures received approval from the local ethics committee at the University of Brescia (code NP5665) and were conducted in accordance with the latest version of the Declaration of Helsinki.

7.2.2 Experimental protocol

The study comprised a single experimental session lasting approximately 1.5 hrs. Participants were seated comfortably in a chair with their right forearm supported on a custom-built device which was secured to the testing table (**Figure 7.1A**). The elbow was flexed at 45° (0° being the anatomical position), and the wrist was positioned neutrally (i.e., midway between full supination and full pronation). Both the forearm and wrist were secured to the device with Velcro straps. The four fingers were aligned with the forearm (i.e., pointing forward), and the thumb was held in a mid-flexed position. The distal segments of the thumb and fingers were fixed to adjustable supports attached to the load cells (thumb: SM-100 N; fingers: SM-500 N, Interface, Arizona, USA) to record the isometric forces produced by simultaneous flexion of the four fingers and thumb, either separately or simultaneously (**Figure 7.1A**). The chosen wrist position was adopted to ensure standardization across participants and to provide a comfortable posture during the task.

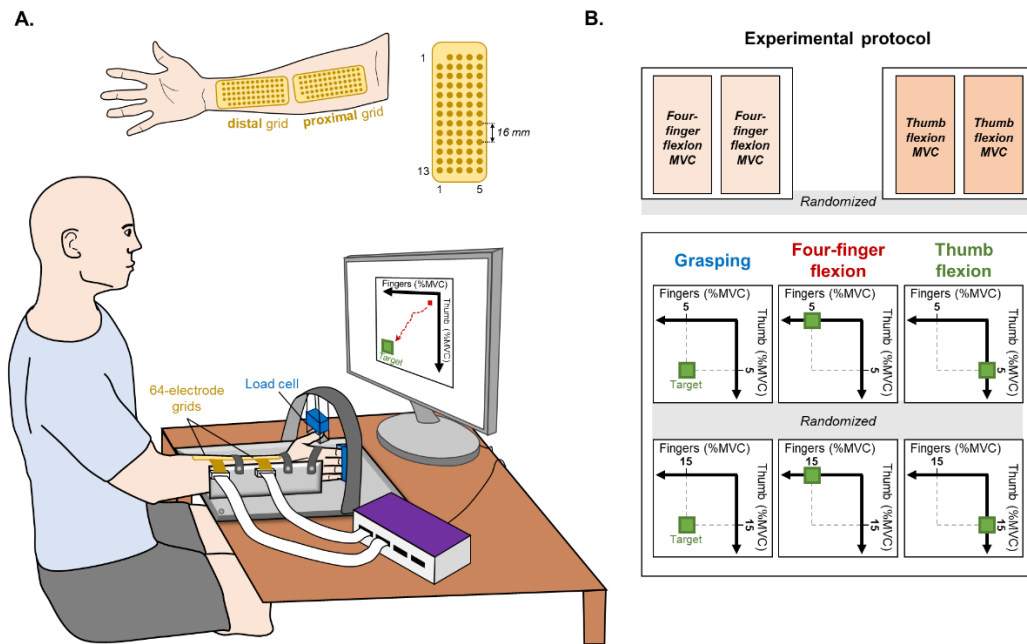


Figure 7.1: Experimental setup and protocol. A, positioning of participants to perform the experimental task. The distal segments of the fingers and the thumb were fixed to adjustable supports attached to load cells to measure isometric forces produced by simultaneous flexion of the four fingers and thumb, either separately or simultaneously. Two high-density grids of 64 electrodes each were used to record the muscle activity from the extrinsic flexor muscles of the hand (top panel). B, participants performed maximal voluntary isometric contractions (MVC) of four-finger and thumb flexion (top), followed by isometric force-matching contractions (bottom) involving three different tasks: simultaneous flexion of the four fingers without involvement of the thumb (i.e., four-finger flexion), thumb flexion, and simultaneous flexion of both fingers and thumb (i.e., grasping). For each movement, two force levels were performed, 5% and 15% MVC. Visual feedback was provided as a two-dimensional coordinate plane, where the x- and y-axis corresponded to the flexion forces of the four-fingers and thumb, respectively, and a green square indicated the target.

Initially, participants performed maximal voluntary isometric contractions (MVCs) for 3 s, with a 2 min interval of rest in between. Two MVCs of finger flexion and two MVCs of thumb flexion were performed in a randomized order (top panel of **Figure 7.1B**). For each action, the highest value from the two MVCs was considered the maximal isometric force and used as a reference for subsequent submaximal contractions. Subsequently, participants were familiarized with the tasks by performing simultaneous flexion of the four fingers and thumb at 5% MVC, either separately or simultaneously, for approximately 10 s. This familiarization period was repeated for each task (maximum of three times) to ensure that participants could maintain the target force level and correctly perform isolated four-finger or thumb flexion. Following the familiarization period, participants were instructed to perform six isometric force-matching contractions involving three different tasks: simultaneous flexion of the four fingers without involvement of the thumb (i.e., four-finger flexion), thumb flexion, and simultaneous flexion of all fingers with the thumb (i.e.,

grasping). For each task, participants performed two steady isometric contractions at two target forces: 5% and 15% MVC. Visual feedback of the produced forces was provided as a moving red square in a two-dimensional coordinate plane, where the x- and y-axis corresponded to the forces of four-finger flexion and thumb flexion, respectively, and a green square indicated the target (bottom panel of **Figure 7.1B**). Participants were required to sustain the force at the target level for 50 s within a tolerance of $\pm 5\%$, indicated by the borders of the green square. During the individual movements (four-finger or thumb flexion), the force acquired by the uninvolved load cell was recorded but not displayed on the monitor. The computer monitor displaying the target and produced forces was positioned ~ 60 cm in front of the participant.

7.2.3 Data collection

For all tasks, HDsEMG were recorded in monopolar mode from the extrinsic flexor muscles of the hand using two grids of 64 electrodes each (GR08MM1305; 8 mm inter-electrode distance; OT Bioelettronica, Turin, Italy; **Figure 7.1A**). To cover as much of the surface area of the muscles as possible, the electrodes were positioned from proximal to distal over the volar surface of the forearm (**Figure 7.1A**). We refer to the recorded muscles as the extrinsic flexor muscles of the hand, acknowledging the difficulty of precisely identifying individual forearm muscles using non-invasive HDsEMG due to the anatomical proximity of superficial and deep muscle layers. However, it is likely that HDsEMG signals were recorded predominantly from the flexor digitorum superficialis during the four-finger flexion task and from the flexor pollicis longus during the thumb flexion task, as these are the primary extrinsic flexors involved in these respective movements. The supplemental figure S1 (<https://doi.org/10.6084/m9.figshare.28923704>) shows examples of motor unit action potential shapes from decomposed motor units for each task, indicating that their spatial distributions align well with the approximate anatomical regions of the involved muscles. Before electrode placement, the skin was shaved and mildly abraded (EVERI, Spes Medica, Genova, Italy), and the surface was cleaned with water. The electrode grids were then fixed to the skin using a double-sided adhesive foam, and a conductive paste (AC cream, Spes Medica, Genova, Italy) was used to fill the wells in the foam to ensure optimal electrode-skin contact. A strap electrode dampened with water was used as a reference electrode and positioned on the involved wrist. HDsEMG were sampled at 2048 Hz using a 16-bit amplifier (10-500 Hz bandwidth; Quattrocento, OT Bioelettronica, Turin, Italy).

Force output from the load cell was amplified by a variable factor (MISO II, OT Bioelettronica) and sampled synchronously with HDsEMG.

7.2.4 Data analysis

Data were analyzed offline using MATLAB custom-written scripts (version 2022b, The MathWorks, Natick, MA, USA).

7.2.4.1 Identification and tracking of motor units. The monopolar HDsEMG were bandpass filtered between 20 and 500 Hz using a 3rd order Butterworth filter. The filtered signals were then visually inspected, and channels with artifacts or contact problems were excluded. Subsequently, the HDsEMG from each electrode grid were decomposed separately into motor unit spike trains using a convolutive blind-source separation algorithm (**Figure 7.2A**) (Negro et al., 2016). This algorithm has been validated previously (Negro et al., 2016). and widely applied in the literature to assess single motor unit activity in upper limb muscles (Cabral et al., 2024; Kapelner et al., 2018; Maillet et al., 2022). Missing or misidentified motor unit pulses were iteratively edited by an expert operator, followed by the re-estimation of motor unit spike trains (Martinez-Valdes et al., 2017; Hug et al., 2021). After motor unit editing, the motor units were tracked between four-finger flexion and grasping tasks, as well as between thumb flexion and grasping tasks, by reapplying the motor unit separation vectors from one contraction to the other, and vice-versa (Cabral et al., 2024b; Oliveira & Negro, 2021; Rossato et al., 2022). The motor unit separation vectors are distinct for each individual motor unit and serve as spatio-temporal matched filters to estimate motor unit spike trains. Consequently, the same motor units were analyzed across four-finger flexion and grasping tasks, as well as between thumb flexion and grasping tasks. **Figure 7.2B** shows examples of the innervation pulse trains (i.e., motor unit discharge times) and the two-dimensional representations of the motor unit action potentials of motor units tracked between tasks. For each matched motor unit, the instantaneous discharge rate was obtained as the multiplicative inverse of the inter-spike intervals, and the average value over the 50-s steady force was calculated (i.e., mean discharge rate) and retained for further analysis. It is important to note that common units from both the proximal and distal electrode grids were identified, and the one with the highest coefficient of variation of the inter-spike interval was removed from the analysis. Motor unit spike trains were considered to be generated by the same motor unit if they had at least 30% of common discharge times (Negro et al., 2016).

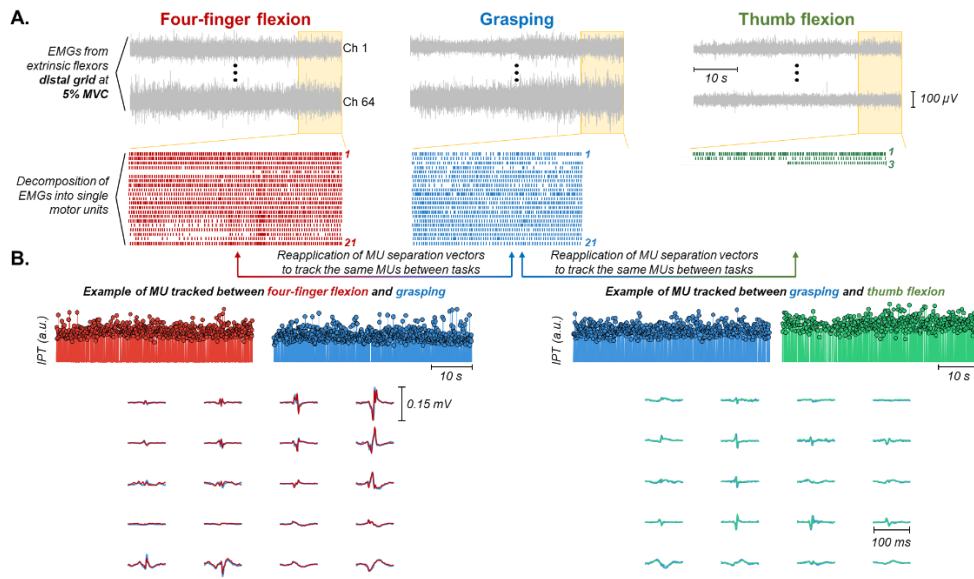


Figure 7.2: Data analysis. A, high-density surface electromyograms acquired during four-finger flexion (red), thumb flexion (green), and grasping (blue) were decomposed into motor unit spike trains using a convolutive blind source separation algorithm (29). Raster plots of decomposed motor units for each task are shown. Note that, for visualization purposes, the raster plots are shown for the last 10 s of the task (yellow boxes). B, motor units were tracked between tasks by reapplying the motor unit separation vectors from one contraction to the other, and vice-versa, so the same motor units were analyzed across four-finger flexion and grasping, as well as between thumb flexion and grasping. Examples of the innervation pulse trains (i.e., motor unit discharge times) and the two-dimensional representations on twenty selected single-differential channels of the motor units tracked between tasks are shown.

7.2.4.2 Estimates of common synaptic oscillations. Coherence analysis between motor unit spike trains was used to estimate common synaptic oscillations to spinal motor neurons (Cabral et al., 2024a; Castronovo et al., 2015; Negro & Farina, 2012; Cabral et al., 2024b). 28, 36, 37, 38). Due to the low number of matched motor units between thumb flexion and grasping tasks (see *Results*), only matched motor units between four-finger flexion and grasping were included in this analysis. Specifically, two equally-sized cumulative spike trains (CSTs), obtained as the sum of binary discharge times from randomly selected motor units, were used to calculate the coherence. Each CST comprised half of the total number of matched motor units, with this process repeated up to 100 random permutations (Cabral et al., 2024b). Only participants with at least four matched motor units were included in the coherence analysis (Cabral et al., 2024a). For each interaction, coherence was estimated between the two detrended CSTs using Welch's periodogram with 95% overlapping Hanning windows of 1 s. This analysis was performed over a 30-s time window during the steady contraction, which was selected to maximize the number of active motor units (Maillet et al., 2022).

The obtained coherence values were averaged across interactions (i.e., pooled coherence) and z-transformed as previously described (Gallet & Julien, 2011). Only values higher than the bias, defined as the mean value of z-scores between 250 and 500 Hz (Maillet et al., 2022; Castronovo et al., 2015), were considered for further analysis. These steps reduce the dependency of coherence estimates on motor unit yield and allow comparisons across participants with different numbers of matched units. To assess changes between four-finger flexion and grasping tasks, the area under the curve of the z-coherence was calculated separately for the delta (1-5 Hz), alpha (5-15 Hz) and beta (15-35 Hz) bands. Then, the area under the curve ratio (grasping/four-finger flexion) was computed and 1 was subtracted (Cabral et al., 2024a). Thus, ratio values higher than and lower than 0 indicated, respectively, increases and decreases in z-coherence during grasping compared with the four-finger flexion task.

7.2.4.3 Coupling between neural drive and force oscillations. Force signals were initially low-pass filtered using a 3rd order Butterworth filter with a cutoff frequency of 15 Hz. To assess potential changes in the coupling between neural drive and force oscillations when comparing four-finger flexion and grasping tasks, coherence was calculated between the neural drive and force signals (Cabral et al., 2024b; Negro et al., 2009). The neural drive to the extrinsic flexor muscles of the hand was estimated as the CST of all identified motor units (Thompson et al., 2018). Similar to the analysis of common synaptic inputs, the area under the curve ratio (grasping/four-finger flexion) of z-coherence was calculated to evaluate changes between four-finger flexion and grasping tasks, but focusing only on the frequency bandwidths relevant for force production (i.e., delta and alpha bands; (Cabral et al., 2024b)). Additionally, to quantify alterations in the force steadiness between tasks, we measured the coefficient of variation of force. For this analysis, we used the same 50-s sustained contraction interval used for HDsEMG decomposition.

7.2.5 Statistical analysis

All statistical analyses were conducted in R (version 4.3.0) within the RStudio environment (version 2023.03.1).

Linear mixed-effect models (LMM) were used to compare the mean discharge rate between four-finger or thumb flexion and grasping tasks, separately for each force level (5% and 15% MVC), as we did not have a prior hypothesis that potential differences in motor unit control between tasks would depend on contraction intensity. Additionally, because we

tracked motor units between tasks but not across force levels, the inclusion of force as a factor in a unified model would be inconsistent with the tracking approach used. The LMM statistical approach accounts for the hierarchical nature of motor unit data, recognizing the non-independence of observations (Tenen et al., 2014), and allows for the inclusion of all matched motor units rather than a single mean value for each participant. Random intercept models were used with ‘task’ as the fixed effect and ‘participant’ as the random effect. LMMs were implemented using the *lmerTest* package (Kuznetsova et al., 2017) with the Kenward-Roger method employed to approximate the degrees of freedom and estimate the *P*-values. The *emmeans* package was applied to determine estimated marginal means and their differences with 95% confidence intervals (Lenth et al., 2019).

To compare estimates of common synaptic inputs (i.e., z-coherence between motor unit spike trains) and CST-force coupling between four-finger flexion and grasping tasks, changes in the area under the curve ratio (grasping/four-finger flexion) were statistically tested using one-sample Wilcoxon signed-rank test (null hypothesis; $\mu_0 = 0$), separately for each frequency bandwidth. To evaluate whether the task-related changes in alpha-band coherence were associated with changes in motor unit mean discharge rate (see Results), Pearson’s correlation analyses were conducted separately for each force level. For this analysis, the average mean discharge rate across motor units was calculated for each participant, and a change ratio between tasks (grasping/four-finger flexion) was computed. Additionally, to compare the coefficient of variation of force between four-finger flexion and grasping tasks, Wilcoxon signed-rank tests were used separately for each force level.

The effect sizes, along with their 95% confidence intervals, are reported for all the results. For the results of motor unit mean discharge rate, effect sizes were calculated using the *eff_size* function in R, which estimates Cohen’s *d* directly from the outputs of the *emmeans* package. For the area under the curve ratio results, effect sizes were calculated using Cohen’s *d_s* as reported in Jané, Xiao (45). For the coefficient of variation of force results, effect sizes were calculated using Cohen’s *d_z* as reported in Jané, Xiao (2024).

For the results of motor unit mean discharge rate, the values in the text are reported as mean with 95% confidence intervals. All the other values are reported as median/interquartile ranges unless indicated otherwise. All individual data on matched motor unit discharge times are available at <https://doi.org/10.6084/m9.figshare.28923704>.

7.3 Results

7.3.1 Motor unit identification and mean discharge rate

During the individual movement tasks (four-finger or thumb flexion), the average force produced by the uninvolved digit was calculated and normalized to the corresponding MVC peak. At 5% MVC, the average thumb flexion force during the four-finger flexion task was $0.17 \pm 0.21\%$ MVC, and the average four-finger flexion force during thumb flexion was $0.24 \pm 0.33\%$ MVC. At 15% MVC, these values were $0.24 \pm 0.19\%$ MVC for thumb flexion during four-finger flexion, and $0.37 \pm 0.40\%$ MVC for four-finger flexion during thumb flexion. These results confirm that the force generated by the uninvolved digit remained negligible during each task. To compare changes in the mean discharge rate of motor units between four-finger or thumb flexion and grasping tasks, we decomposed HDsEMG from the extrinsic flexor muscles of the hand. For 5% MVC, 20 ± 8 motor units were identified per participant during four-finger flexion, 12 ± 9 during thumb flexion, and 19 ± 7 during the grasping task. For 15% MVC, 22 ± 8 motor units per participant during four-finger flexion, 12 ± 9 during thumb flexion, and 22 ± 8 during grasping were identified. Then, the identified motor units between tasks were tracked to ensure the same units were analyzed. For 5% MVC, 9 ± 6 units were matched between four-finger flexion and grasping tasks, and 2 ± 2 units between thumb flexion and grasping tasks. For 15% MVC, these numbers were 10 ± 8 between four-finger flexion and grasping, and 2 ± 4 between thumb flexion and grasping.

Significant reductions in motor unit mean discharge rate were observed between four-finger flexion and grasping tasks for both force levels. For 5% MVC, the mean discharge rate changed from 12.7 [11.8, 13.5] to 12.0 [11.1, 12.8] pps (LMM, DoF = 286.4, $F = 9.15$, $P = 0.003$, Cohen's $d = -0.35$ [-0.59, -0.11]), and for 15% MVC, from 14.5 [13.4, 15.5] to 13.6 [12.5, 14.6] pps (LMM, DoF = 311.2, $F = 18.64$, $P < 0.001$, Cohen's $d = -0.47$ [-0.72, -0.24]). Conversely, the mean discharge rate was significantly higher at thumb flexion compared to grasping task for both 5% MVC (from 11.5 [10.3, 12.7] to 13.2 [12, 14.4] pps; LMM, DoF = 60.36, $F = 14.38$, $P < 0.001$, Cohen's $d = 0.89$ [0.36, 1.43]) and 15% MVC (from 12.6 [11.6, 13.6] to 13.6 [12.5, 14.6] pps; LMM, DoF = 62.84, $F = 4.16$, $P = 0.045$, Cohen's $d = 0.48$ [0.04, 1]) force levels.

7.3.2 Estimates of common synaptic inputs

To investigate changes in common synaptic inputs, we calculated the z-coherence between motor unit spike trains. The ratio of the area under the curve was then quantified separately for the delta (1-5 Hz), alpha (5-15 Hz) and beta (15-35 Hz) bands. For this analysis, only matched motor units between four-finger flexion and grasping tasks were utilized, as very few units were matched between thumb flexion and grasping. Additionally, only participants with at least four matched motor units were included. Consequently, coherence analysis was performed for 12 and 11 participants for 5% and 15% MVC, respectively. **Figure 7.4** displays the pooled z-coherence for all participants and the area under the curve ratio results. For both 5% MVC (top panel) and 15% MVC (bottom panel) force levels, the area under the curve was lower in the alpha band (yellow area) for the grasping task (blue) compared to the four-finger flexion task (red). For 5% MVC, a significant median reduction of ~15% was observed in the area under the curve within the alpha band (**Figure 7.3A**; one-sample Wilcoxon signed-rank test, $V = 7$, $\text{DoF} = 11$, $P = 0.009$, Cohen's $d_s = -0.81$ [-1.45, -0.14]), but no significant changes were found for the delta (one-sample Wilcoxon signed-rank test, $V = 46$, $\text{DoF} = 11$, $P = 0.622$, Cohen's $d_s = 0.20$ [-0.38, 0.77]) or beta bands (one-sample Wilcoxon signed-rank test, $V = 39$, $\text{DoF} = 11$, $P = 1.000$, Cohen's $d_s = 0.01$ [-0.56, 0.58]). Similarly, for 15% MVC, the area under the curve within the alpha band for the grasping task was significantly lower compared to the four-finger flexion task by a median of ~22% (**Figure 7.3B**; one-sample Wilcoxon signed-rank test, $V = 9$, $\text{DoF} = 10$, $P = 0.032$, Cohen's $d_s = -0.87$ [-1.56, -0.16]), with no significant changes in the delta (one-sample Wilcoxon signed-rank test, $V = 38$, $\text{DoF} = 10$, $P = 0.700$, Cohen's $d_s = 0.20$ [-0.41, 0.79]) or beta bands (one-sample Wilcoxon signed-rank test, $V = 28$, $\text{DoF} = 10$, $P = 0.700$, Cohen's $d_s = 0.08$ [-0.52, 0.67]).

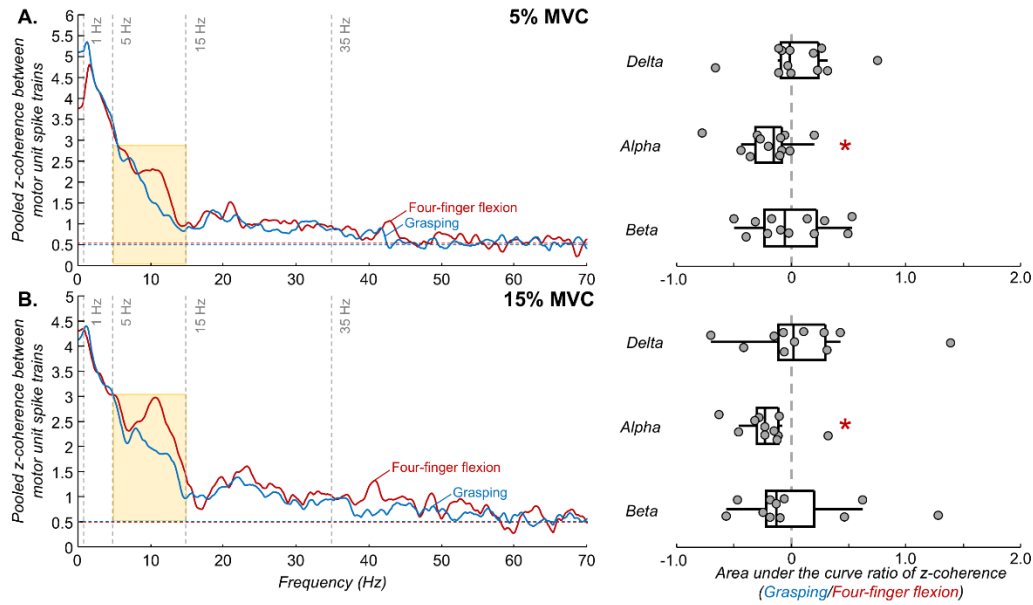


Figure 7.3: Motor unit coherence results. The left panel shows pooled z-coherence profiles including all participants for 5% MVC (A) and 15% MVC (B). Red and blue traces denote four-finger flexion and grasping tasks, respectively. The horizontal dashed line indicates the confidence level of coherence. Vertical dashed lines highlight the three frequency bandwidths analyzed: delta (1–5 Hz), alpha (5–15 Hz), and beta (15–35 Hz) bands. Yellow boxes denote statistical differences in the area under the curve between four-finger flexion and grasping tasks. The right panel displays group results of the area under the curve ratio of coherence for 5% MVC (top) and 15% MVC (bottom). Circles identify individual participants. Vertical black traces, boxes, and whiskers denote the median value, interquartile interval, and distribution range, respectively. * $P < 0.05$.

To determine whether the observed reductions in alpha-band coherence were driven by differences in motor unit discharge rate, we conducted a correlation analysis between the grasping/four-finger flexion change ratios in coherence and mean discharge rate. At both 5% and 15% MVC, no significant correlation was observed (5% MVC: $R = 0.22$, $t = 0.712$, $P = 0.493$; 15% MVC: $R = 0.35$, $t = 1.117$, $P = 0.293$).

7.3.3 Coupling between neural drive and force oscillations

To assess if the observed changes in the alpha band of common synaptic inputs between the four-finger flexion and grasping tasks were accompanied by alterations in the linear coupling between the neural drive and the force oscillations, we calculated the z-coherence between the CST and the force signals. For this analysis, only the bandwidths relevant to force production were examined (delta and alpha bands). For both 5% MVC (**Figure 7.4A**) and 15% MVC (**Figure 7.4B**) force levels, the CST-force z-coherence within the alpha band was significantly lower during the grasping task compared to four-finger flexion (one-sample Wilcoxon signed-rank test; 5% MVC: $V = 13$, $\text{DoF} = 11$, $P = 0.043$,

Cohen's $d_s = -0.55$ [-1.15, -0.07]; 15% MVC: $V = 9$, $DoF = 10$, $P = 0.032$, Cohen's $d_s = -0.97$ [-1.68, -0.23]), with no significant changes for the delta band (one-sample Wilcoxon signed-rank test; 5% MVC: $V = 30$, $DoF = 11$, $P = 0.519$, Cohen's $d_s = 0.20$ [-0.41, 0.79]; 15% MVC: $V = 14$, $DoF = 10$, $P = 0.102$, Cohen's $d_s = -0.49$ [-1.11, 0.15]).

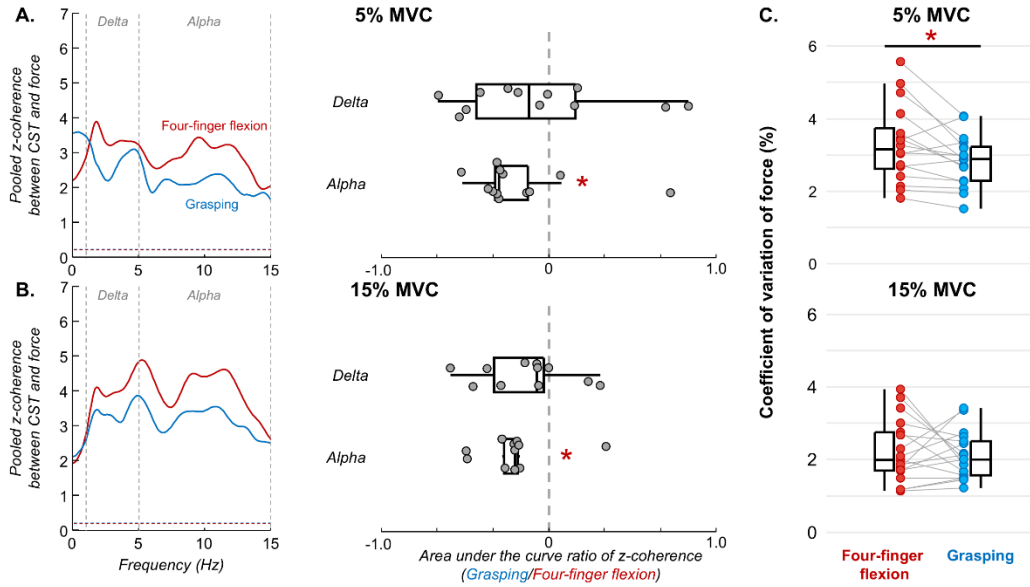


Figure 7.4: Results of force steadiness and coherence between neural drive and force oscillations. A-B, left panel shows pooled z-coherence profiles between the motor units cumulative spike train (CST) and force for 5% MVC (A) and 15% MVC (B). Red and blue traces denote the four-finger flexion and grasping tasks, respectively. The horizontal dashed line indicates the confidence level of coherence. Vertical dashed lines highlight the two frequency bandwidths analyzed: delta (1–5 Hz) and alpha (5–15 Hz) bands. Note that the frequency bandwidth analyzed was limited to the frequency bandwidth of force. The right panel shows group results of the area under the curve ratio of z-coherence for 5% MVC (top) and 15% MVC (bottom). Circles identify individual participants. Vertical black traces, boxes, and whiskers denote the median value, interquartile interval, and distribution range, respectively. C, group results of the coefficient of variation of force (force steadiness) for 5% MVC (top) and 15% MVC (bottom). Coloured circles and horizontal grey lines indicate individual participants. $*P < 0.05$.

To assess changes in force steadiness between four-finger flexion and grasping tasks, we calculated the coefficient of variation of force. Significant reductions in the coefficient of variation of force were observed between the four-finger flexion and grasping tasks for 5% MVC (**Figure 7.4C**; Wilcoxon signed-rank test, $V = 130$, $DoF = 16$, $P = 0.009$, Cohen's $d_z = -0.41$ [-0.90, 0.09]), but not for 15% MVC; Wilcoxon signed-rank test, $V = 81$, $DoF = 16$, $P = 0.854$, Cohen's $d_z = -0.11$ [-0.58, 0.37]) force level.

7.4 Discussion

The main purpose of this study was to investigate changes in common synaptic inputs to spinal motor neurons between a functionally relevant and frequently used grip (grasping)

and a less functionally relevant hand task (four-finger flexion without thumb involvement). Our results demonstrated a reduction in alpha band (5-15 Hz) oscillations during grasping compared to the four-finger flexion task. These differences were reflected in force oscillations, revealing decreased force-neural drive coupling within the alpha band and increased force steadiness during grasping. These findings suggest that a more functionally relevant and natural grip involves specific neural control mechanisms to enhance force control.

Considering the complex biomechanical and neural structure of the hand, even frequently performed daily tasks, such as grasping an object, require precise coordination of multiple degrees of freedom that must be controlled simultaneously (Santello et al., 2013; Fuglevand, 2011). Over the past three decades, researchers have sought to understand how the fine coordination of multiple hand muscles is achieved during finger and hand movements (Santello & Soechting, 2000; Tanzarella et al., 2021; Hockensmith et al., 2005; Keen & Fuglevand, 2004; Schieber, 1990; Kilbreath & Gandevia, 1994). As reviewed by Schieber (1990), two (non-mutually exclusive) models describe how descending pathways may be organized to achieve this control. In the first model, each muscle or digit is controlled individually, with corticospinal pathways projecting separately to each motor neuron pool. In the second model (proposed as model 3 in Schieber (1990)), a small set of muscle activity patterns, or synergies, generate the full repertoire of hand movements, with corticospinal pathways diverging across individual motor neuron pools within and across muscles. Compelling evidence from force (Li et al., 1998; Reilly & Hammond, 2000; Santello & Soechting, 2000), kinematic (Soechting & Flanders, 1997; Santello et al., 1998; Santello et al., 2002), muscle activation (Maier & Hepp-Reymond, 1995; Weiss & Flanders, 2004; Tanzarella et al., 2021), and motor unit (Tanzarella et al., 2021; Hockensmith et al., 2005; Winges & Santello, 2004) recordings suggest that the latter model likely exemplifies hand control, with the synaptic inputs that are shared across spinal motor neurons playing a key functional role in grip force coordination (Santello & Fuglevand, 2004). However, the degree of commonality across motor units within and between hand muscles appears to be muscle- and task-dependent. For instance, while extrinsic hand muscles show a significant degree of common synaptic inputs between motor units, some studies suggest that intrinsic hand muscles exhibit lower commonality (Hockensmith et al., 2005; Winges & Santello, 2004; McIsaac & Fuglevand, 2008; Gandevia & Rothwell, 1987). Interestingly, in the current study it was demonstrated that grip type – highly functional (grasping) versus less frequently

performed (four-finger flexion) – also modulates common synaptic inputs across motor units of the extrinsic flexor muscles of the hand. Specifically, we observed a reduction in alpha band coherent oscillations between tracked motor units during grasping compared to four-finger flexion (**Figure 7.3**).

Although the physiological basis of the reduction in alpha band oscillations during grasping remains challenging to pinpoint, several potential mechanisms could explain this finding. First, the addition of thumb flexion alongside four-finger flexion (i.e., grasping) compared to four-finger flexion alone may introduce an additional source of common synaptic input to spinal motor neurons, leading to a reduction in alpha band coupling. This hypothesis aligns with previous evidence that reported when a new common input is introduced independently to a motor neuron pool, it can decorrelate motor unit spike train output within specific frequency bands (Negro & Farina, 2011). In addition, this hypothesis is supported by recent findings showing task-dependent motor unit recruitment patterns between multi-digit grasping and single-digit tasks, implying functional modulation in common synaptic inputs to the extrinsic hand muscles (Oßwald et al., 2025). Second, previous studies suggested that spinal interneurons may phase-cancel cortical oscillatory inputs in the alpha frequency range, thereby reducing force tremor, which subsequently enhances movement precision (Cabral et al., 2024a; Williams & Baker, 2010; Koželj & Baker, 2014). This mechanism could contribute to improved force control in grasping. Third, considering that learning of a motor task is mediated by reductions in alpha-band oscillations to spinal motor neurons (Cabral et al., 2024a), it is possible that the observed results are driven by a learning effect, as grasping is a more frequently performed task in activities of daily life. Finally, given that modulations in the gain of the Ia afferent feedback loop can directly influence alpha band inputs (Halliday & Redfearn, 1956; Lippold, 1971; Christakos et al., 2006; Laine et al., 2016), it is possible that grasping involves a reduction in Ia afferent gain due to muscle mechanic variations between the two tasks. Since grasping is more frequently performed in daily life, it may rely less on afferent feedback compared to a less applied and less functional grip. Nevertheless, it remains possible that more complex neural mechanisms are involved, integrating both cortical and spinal pathways.

Our findings have directly applied relevance, as the observed reductions in alpha band oscillations during grasping were reflected in force fluctuations. Since alpha band fluctuations in the effective neural drive to the muscle are not fully canceled by the muscle's twitch contractile properties, which act as a low-pass filter with a cutoff frequency of ~12

Hz (Bawa & Stein, 1976; Baldissera et al., 1998), they are consequently transmitted to force, contributing to its variability (Negro et al., 2009; Farina et al., 2014). Thus, the reduction in alpha band coherence during grasping may represent a neural strategy to enhance force steadiness in a frequently performed hand task, effectively filtering out frequency oscillations from the control signal responsible for force modulation (i.e., delta band coherence) (Farina et al., 2014; Hug et al., 2023). Supporting this interpretation, the observed decrease in the coefficient of variation of force (indicative of increased force steadiness) during grasping with respect to four-finger flexion at low force levels (**Figure 7.4C**). This was further supported by a decrease in alpha band coupling between the cumulative spike train and force during grasping compared to four-finger flexion (**Figure 7.4A and 7.4B**). These results align with previous studies and support the findings that various factors, such as sensorimotor integration (Laine et al., 2014), muscle contractile properties (Cabral et al., 2024a), pain (Yavuz et al., 2015), or learning a new motor skill (Cabral et al., 2024b), modulate the alpha band in the common synaptic input to motor neurons, directly influencing optimal force control. Our findings extend this evidence by demonstrating that grip type affects common synaptic oscillations to spinal motor neurons, further influencing force regulation in functionally relevant tasks. It is important to note, however, that differences in force variability may also be partially attributable to biomechanical factors. Specifically, during isometric grasping, activation of the flexor pollicis longus muscle during thumb flexion, which spans both the thumb and the wrist (Chao, 1989), likely contributes to passive wrist stabilization. The synergistic action between this muscle and other wrist flexors during grasping may help counteract wrist extension torques, thereby improving the mechanical stability of the wrist. This stabilization effect could, in turn, reduce the variability in force. Future studies could implement task variations that maintain identical wrist posture while selectively manipulating functional demands or stabilization requirements. In this way it is possible separate mechanical effects from neural adjustments in common synaptic input.

While these experiments do not allow us to directly address this question, a key issue arising from the findings in this study is whether the observed modulation in common synaptic inputs reflects a hard-wired neural control strategy or emerges due to task constraints (i.e., soft-assembled control), which is a topic of ongoing debate in the literature (Tresch & Jarc, 2009; Kutch & Valero-Cuevas, 2012; DERNONCOURT et al., 2025). Recently, using a combination of kinematic, electromyography, and functional magnetic resonance imaging data, Leo, Handjaras (2016) demonstrated that human motor cortical areas encode

hand movement synergies, supporting the idea that modular hand control is represented at higher motor centers. Similarly, Takei, Confais (2017) reported that modular control of hand movements is implemented at the spinal level in primates, suggesting that the nervous system organizes motor outputs into functional modules to simplify the control of voluntary hand movements. In addition, unlike other primates, humans have a fully opposable thumb, while at the same time exhibiting a limited ability to fully individuate finger movements (Napier, 1956; Napier, 1960; Napier, 1965; Marzke, 1997; Susman, 1979; Marzke & Shackley, 1986). This evolutionary predisposition is further reflected in the fact that grasping is an innate reflex in infants (Dennis, 1943; Twitchell, 1965). Consequently, grasping can be considered an evolutionarily relevant and deeply consolidated movement, and it is reasonable to assume that it may rely on facilitatory mechanisms along the corticospinal pathway, which enhance the optimized synergistic expression of force during functional grips. However, further research is needed to validate this hypothesis. Specifically, future studies could combine motor unit recordings with neuroimaging techniques (e.g., functional magnetic resonance imaging or electroencephalography) or neurostimulation techniques (e.g., transcranial magnetic stimulation) to examine whether the observed modulation in common synaptic inputs during grasping is mirrored by changes in cortical activity. Additionally, longitudinal experimental designs involving targeted motor training could help dissociate the effects of task familiarity from hard-wired control strategies. Finally, it may be relevant to assess, during functional tasks, the difference between neural control modulated through potential facilitatory mechanisms and the control directed toward individuated finger movements, which is not an innate ability and is characterized by more limited capabilities.

A final consideration regarding differences in motor unit mean discharge rates. This study reported a reduction in mean discharge rate during grasping compared to four-finger flexion alone, though with relatively small effect sizes (see Results). Given the complexity of intrinsic and extrinsic muscle coordination required for hand control, some motor units within the extrinsic flexor muscles primarily contribute to force production, while others play a role in force distribution across muscles and mechanical stabilization (Li, 2002; Kinoshita et al., 1995; Butler et al., 2005). Therefore, the observed small reduction in mean discharge rate may reflect mechanical adaptations rather than direct changes in neural control. Notably, this decrease was contrasted by an increase in mean discharge rate when the thumb was flexed alone compared to grasping. This suggests that the nervous system

dynamically adjusts motor unit activity, whether for force generation or mechanical stabilization, to maintain the required force target across different grip conditions.

Due to the complex anatomy and close proximity of the extrinsic hand flexor muscles, a limitation of this study was the inability to precisely identify the specific forearm muscles from which non-invasive HDsEMG signals were recorded. However, this limitation does not substantially affect our interpretation, as our primary interest was in within-subject, between-task differences rather than muscle-specific differences. Importantly, the HDsEMG grids were positioned in the exact same location across tasks, as grids were not removed in between tasks, ensuring that relative comparisons were not confounded by spatial differences in electrode placement. While intramuscular EMG could provide muscle-specific recordings as has been done in previous studies (Hockensmith et al., 2005; Keen & Fuglevand, 2004; McIsaac & Fuglevand, 2008), it typically yields a limited number of motor units per participant. In contrast, HDsEMG decomposition allowed us to analyze a substantially larger motor unit population (average ~18 motor units per participant and task), which justifies why we chose this method to address our research question. Another limitation is that the estimation of common synaptic input using coherence analysis is inherently influenced by motor unit discharge rate and cannot be entirely disentangled from it (Negro & Farina, 2012; de la Rocha et al., 2007). Therefore, the observed reductions in alpha-band coherence between the four-finger flexion and grasping tasks could, in principle, be attributed to the corresponding reductions in mean discharge rate. However, our correlation analysis indicated that these reductions in alpha-band coherence were not significantly associated with changes in motor unit discharge rate at either 5% or 15% MVC, suggesting that the differences in coherence reflect alterations in common synaptic input.

In conclusion, the findings of this study demonstrate that grasping, which is a functionally relevant and habitually performed hand movement, exhibits distinct neural control mechanisms compared to less commonly used grips. Therefore, the reduction in alpha band oscillations during grasping suggests a task-dependent modulation of common synaptic input, which has functional implications for enhanced force steadiness and optimized neural drive-force coupling.

7.5 References

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8 Study 4: Rate coding of the deltoid

Title: Rate coding of the deltoid in two angles of shoulder abduction

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Manuscript in preparation

Abstract: The glenohumeral joint performs a wide range of movements through the coordinated action of numerous muscles, with the deltoid being primarily responsible for shoulder abduction. Although traditionally divided into three heads, recent evidence suggests the presence of functionally distinct subregions, making the identification of individual motor unit activity using HD-sEMG particularly challenging. Moreover, only few studies investigated how deltoid motor units modulate their discharge behaviour with increasing force demands. This study involved two experimental sessions on consecutive days, one for each joint angle. Eighteen participants performed two maximal voluntary contractions (MVCs) and trapezoidal isometric contractions at 5%, 10%, 20%, and 30% MVC. HD-sEMG was recorded using two 64-electrode grids placed over the anterior and lateral deltoid, and signals were decomposed into motor unit spike trains using a convolutive blind source separation algorithm (Negro et al., 2016). Motor units were matched across force levels by reapplying separation vectors. Results showed a significant increase in mean discharge rate with higher force demands at both joint angles ($p < 0.001$). Laterally located motor units exhibited greater rate modulation between the lowest and highest force levels compared to anterior units, particularly at 30° of abduction ($p = 0.02$). In conclusion, increased force production in the deltoid is primarily achieved through rate-coding modulation at low to moderate range, especially in task-relevant motor units.

Keywords: Deltoid, functional differentiation, HDs-EMG, motor unit matching, rate coding

8.1 Introduction

The motor unit represents the final common pathway of the neuromuscular system, acting as a neuromechanical transducer that converts neural input from the central nervous system into force, thereby enabling movement (Liddell & Sherrington, 1925). Force production is mediated through two mechanisms: the recruitment of motor units and the modulation of their discharge rate (i.e., rate coding) (Avrillon et al., 2024). While both mechanisms contribute to force generation, rate coding plays a key role in adjusting force output once motor units are recruited, particularly across a wide range of contraction intensities (Enoka & Duchateau, 2017). Importantly, the modulation of motor unit recruitment and rate coding is not fixed, but varies according to the muscle involved, the force demand and the characteristics of the task (Heckman & Enoka, 2012).

In addition to neural factors, force output is influenced by biomechanical constraints, including muscle architecture, fibre orientation, and the direction of force production (Brown et al., 2007; Hill, 1938; Lieber & Fridén, 2001; Luff, 1981; Sharples & Miles, 2021; Taylor et al., 2009). These factors impose specific functional demands on the neuromuscular system, requiring flexible and task-dependent control strategies. As a result, neural control is not necessarily distributed uniformly within a muscle but can be spatially organized to meet the task-specific mechanical requirements (Gorelick & Brown, 2007; McMillan & Hannam, 1992; Urquhart & Hodges, 2005). In a review, Higham and Biewener (2011), in their review, summarized extensive research on regional muscle activation, highlighting how activation patterns and recruitment strategies differ across regions within a muscle depending on their functional role and the task being performed. Such regional control may confer functional advantages, including enhanced control of joint mechanics and increased diversity of movement patterns (Higham & Biewener, 2011).

The need to meet diverse biomechanical demands is especially evident in large muscles with complex architecture, such as those with multiple origins and insertions, where different regions can produce forces in distinct directions (Scholle et al., 2001). In this context, skeletal muscles are thought to be subdivided into neuromuscular compartments, defined as sub-volumes of muscle fibres that receive exclusive innervation from distinct groups of homogeneous motoneurons (Wickham & Brown, 2012). This compartmental organization allows the central nervous system to selectively modulate motor unit activity within specific muscle segments, enabling fine control of force direction and magnitude

(English et al., 1993). The contribution of each compartment depends on its anatomical location, movement direction, fibre orientation, and mechanical advantage, resulting in region-specific activation patterns during different motor tasks (Brown et al., 2007; Lieber & Fridén, 2001). Experimental evidence supporting this framework has been previously demonstrated in shoulder muscles across different isometric tasks (abduction, adduction, flexion, and extension) (Brown et al. 2007). Specifically, the onset, duration, and intensity of activation of different segments within shoulder muscles were largely determined by their functional role, supporting the idea that individual muscle segments can be controlled independently by the nervous system to achieve the desired force output (Brown et al., 2007).

The deltoid muscle is particularly well suited for such functional organization. Although commonly described as consisting of three heads (anterior, lateral and posterior) (Morgan et al., 2006b), several studies have demonstrated that it is composed of multiple functional segments that can be independently controlled (Huijing, 2003; Klepps et al., 2004; Lieber & Fridén, 2001; Moser et al., 2013). In particular, Wickham and Brown (1998) identified seven segments within the deltoid with specific functional roles during abduction and adduction shoulder movements. Of these seven segments, five were responsible for abduction and the remaining two for adduction, supporting the concept of region-specific activation within the deltoid depending on the movement direction. Despite this, the neural mechanisms underlying the control of these regions remain poorly understood. Specifically, the literature investigating motor unit behaviour in the deltoid are very limited, rely on invasive techniques such as needle electromyography, and are typically limited to moderate-to-high contraction intensities (Alpaydin Baslo et al., 2024a; de Luca et al., 1982c; Doherty & Stashuk, 2003a).

Therefore, the aim of the present study was to investigate motor unit discharge behaviour in the deltoid muscle across different force levels and to determine whether rate coding modulation depends on the spatial location of motor units. To this end, high-density surface electromyography (HD-sEMG) and signal decomposition techniques were used to identify motor unit activity and assess its spatial distribution across the muscle during isometric shoulder abduction at two functionally relevant shoulder abduction angles. It was hypothesized that the central nervous system modulates motor unit discharge rate in response to increasing force demands, and that this modulation varies according to the spatial location of motor units within the muscle.

8.2 Methods

8.2.1 Participants

This study included 19 healthy subjects (12 males and 7 females; age: 28 ± 7 years). None of the participants reported neuromuscular disorders or specific conditions affecting the upper limb. Before starting the experimental protocol, all subjects signed an informed consent form that included a detailed description of the experimental procedures. The study was approved by the ethical committee of the Università degli Studi di Brescia (code NP2490) and conducted in accordance with the latest Declaration of Helsinki.

8.2.2 Experimental protocol

The experimental protocol consisted of two sessions, each lasting approximately two hours. The sessions were identical in structure but performed at two different shoulder abduction angles: 30° and 65° . The order of the two angle conditions was randomized. Sessions were on consecutive days to minimize excessive muscular and mental fatigue in the participants. To ensure consistent electrode positioning across sessions, the placement of the HD-sEMG grids was marked on the skin with a pen.

Participants were seated comfortably in a rigid chair with the knee and hip joints positioned at 90° (0° corresponding to full extension) and secured with a Velcro strap to minimize compensatory torso movements. The right arm was positioned in a custom-made apparatus connected to a six-degrees-of-freedom load cell (© 2025 Advanced Mechanical Technology Inc., Watertown MA, USA, model #MC3A) to measure isometric force produced by the deltoid muscle. The load cell was mounted on a robotic arm (Kawasaki Robotics Inc., Wixom MI, USA), allowing precise adjustment of the apparatus to ensure proper shoulder joint alignment according to the experimental session. Details on this methodological framework to assess neural control of shoulder muscles can be found in Inglis et al. (2026).

The experimental protocol began with participants performing three maximal isometric voluntary contractions (MVCs) of shoulder abduction, each lasting 3-5 seconds, with two minutes of rest between trials. Among the three MVCs, the highest value was used as the reference MVC for the submaximal protocol. Subsequently, participants performed isometric submaximal voluntary contractions at 5%, 10%, 20%, and 30% of MVC (Figure 8.1A). Each contraction was repeated twice, with one minute of rest between trials. The order

of the force levels was randomized. Submaximal contractions followed a trapezoidal profile consisting of: (i) a ramp-up phase reaching the target force at a rate of 2% MVC/s for 5% MVC and 10% MVC, and at 3% MVC/s for 20% MVC and 30% MVC; (ii) a 30-second plateau phase during which the target force was maintained as steadily as possible; and (iii) a ramp-down phase at the same rate as the ramp-up. The selected joint angles and force levels were chosen to reflect conditions commonly encountered during activities of daily living (Gates et al., 2016; Nam et al., 2019).

Visual feedback was provided through a customized graphical user interface (GUI). The interface consisted of a horizontal axis representing the abduction movement and a target square indicating the target force level. A circle represented the participant's force output, and participants were instructed to maintain the circle within the target area. When the error exceeded 2.5% MVC, the target square changed colour to red. Trials were repeated if participants were unable to maintain the circle within the required target.

8.2.3 Data collection

During isometrical tasks, surface myoelectric signals were recorded from the deltoid muscle using HD-sEMG. Prior to electrode placement, the skin area was shaved, abraded (EVERI, Spes Medica, Genoa, Italy), and cleaned with distilled water. Two two-dimensional adhesive grids, each consisting of 64 electrodes arranged in a 13×5 layout (HD08MM1606; 8 mm inter-electrode distance; OT Bioelettronica, Turin, Italy) were positioned over the deltoid muscle (Figure 8.1B). An adhesive foam layer (FOA08MM1305, OT Bioelettronica, Turin, Italy) was applied to the grids, and conductive cream (AC cream, Spes Medica, Genoa, Italy) was used to fill the cavities of the foam and ensure optimal electrode-skin contact. Electrode placement followed the guidelines described by Inglis et al. (2026). The anterior margin of the deltoid and the acromion were first identified manually. The first grid was placed horizontally, 2 cm below the acromion, with its medial edge aligned with the anterior margin of the muscle. The second grid was placed directly below to the first and shifted laterally by two electrode columns (Fig. 1) (Inglis et al., 2026). Reference electrodes were positioned over the acromion (T3545, OT Bioelettronica, Turin, Italy) and the right elbow (WS1.1, OT Bioelettronica, Turin, Italy).

HD-sEMG signals were recorded in monopolar configuration and digitized at a sampling frequency of 2000 Hz with a 16-bit A/D converter (10-500 Hz bandwidth; Novecento+, OT Bioelettronica, Turin, Italy). Force signals were acquired synchronously.

Data were subsequently processed in MATLAB (version 2024a) using custom-written scripts.

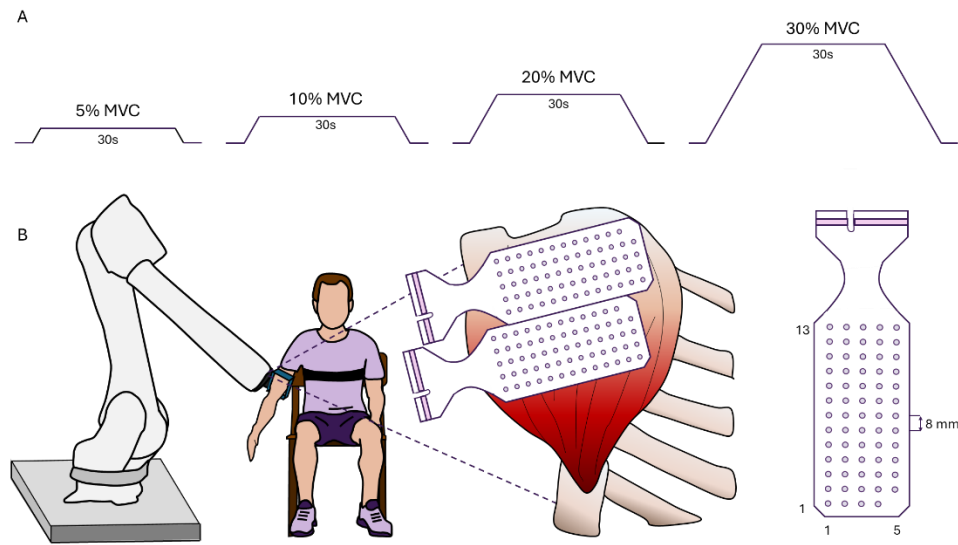


Figure 8.1: Experimental setup and protocol. A, Experimental protocol. Participants performed submaximal isometric contractions at 5%, 10%, 20%, and 30% of MVC. B, Participant positioning during the experimental task. The participant's arm was fixed to adjustable supports connected to load cells to measure the produced isometric force. The load cell was attached to a robotic arm, which was used to determine the appropriate arm position to allow participants to perform the task at two different degrees of lateral arm abduction (30° and 65°). Muscle activity from the anterior and lateral heads of the deltoid was recorded using two high-density electrode grids, each consisting of 64 electrodes.

8.2.4 Data Analysis

8.2.4.1 Force. The force recorded during the contractions was low-pass filtered using a third-order Butterworth filter with a cutoff frequency of 15 Hz. The force values recorded during the MVCs were used to assess potential differences between the peak force in two joint angles.

8.2.4.2 HD-sEMG decomposition. HD-sEMG monopolar signals were initially band-pass filtered using a third-order Butterworth filter with a cut-off frequency of 20-500 Hz. Signals were then visually inspected and channels exhibiting excessive noise (e.g., poor skin-electrode contact or artifacts) were removed. Subsequently, motor unit spike trains were decomposed from HD-sEMG signals using a convolutive blind source separation algorithm (Negro, Muceli, et al., 2016). Only motor units with a silhouette (SIL) value of at least 0.87 were retained for further analysis. The SIL threshold provides a quantitative index of the separation between motor unit spike trains and noise, thereby serving as an objective measure of decomposition accuracy (Negro, Muceli, et al., 2016). The extracted spike trains were further visually examined by an experienced operator, who added misidentified

discharges and removed non-physiological discharges (<4 pulses per second or >50 pulses per second, or inter-spike intervals <20 ms or >250 ms; (Negro et al., 2009)). This method has been shown to be highly reliable across operators (Hug et al., 2021).

8.2.4.3 Motor units tracking. Decomposed motor units were matched across different force levels by pairs (i.e., 5-10%, 10-20%, and 20-30% MVC). This tracking procedure was performed by reapplying the motor unit separation vectors from one trial (e.g., 5% MVC) to the other (e.g., 10% MVC), and vice versa (Cabral et al., 2025). To maximize the number of matched motor units, all possible combinations of trials were considered. Considering each force level was recorded twice, the matching procedure involved four possible trial combinations (e.g., trial01_5%MVC-trial01_10%MVC, trial01_5%MVC-trial02_10%MVC, trial02_5%MVC-trial01_10%MVC, and trial02_5%MVC-trial02_10%MVC). Since motor units were matched across three different force-level pairs, a total of twelve matching procedures were performed.

All matched motor units were visually inspected (Figure 8.2) and, to be considered eligible, the motor unit had to discharge for at least 20 seconds within the plateau. During this procedure, the spatial representation of the motor unit action potential (MUAP) location was calculated using the spike-triggered averaging technique (Hu et al., 2013) and shown to the operator. The motor unit was then classified as located at the anterior or lateral region of the deltoid based on its MUAP amplitude distribution. Finally, following the matching procedure, duplicate motor units identified across the two matrices were detected (i.e., units that shared at least 30% of their discharge timings), and for subsequent analyses the motor unit exhibiting the lower coefficient of variation of the interspike interval was maintained.

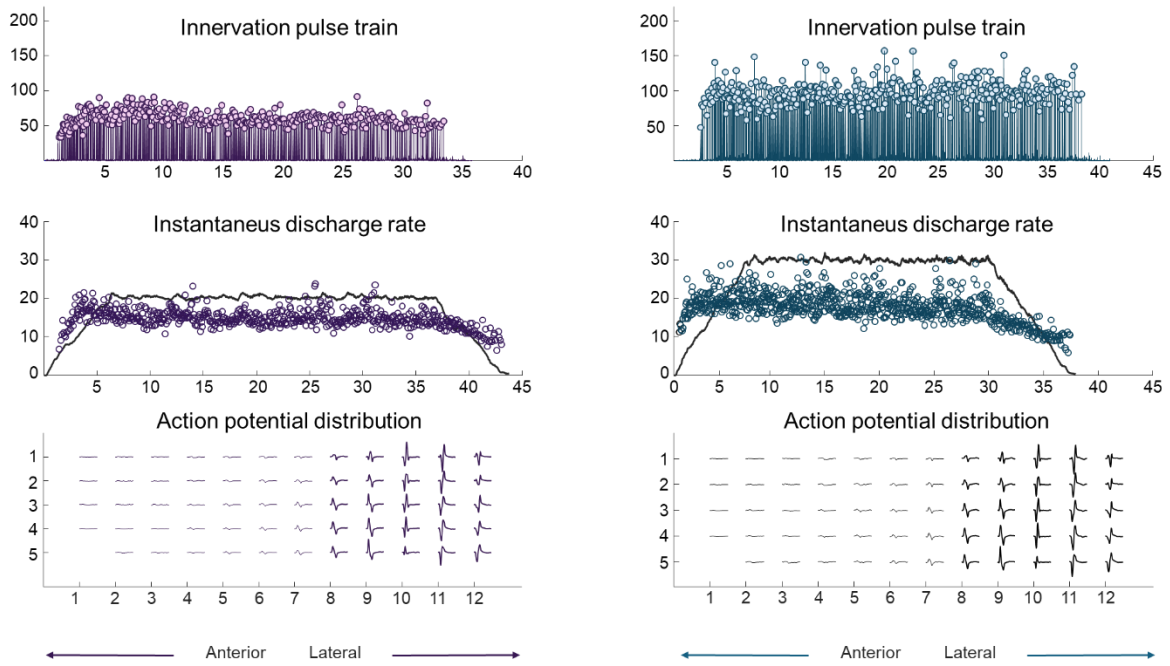


Figure 8.2: Representative example of the decision-making process for motor unit territorial classification. The figure displays the innervation pulse train (temporal spike sequence), the instantaneous discharge rate superimposed on the force profile, and the action potential distribution for the same motor unit matched across two distinct recruitment levels (purple: lower force level; blue: higher force level). Following a quality assessment of the innervation pulse train and the stability of the instantaneous discharge rate, the operator performed a manual selection to retain or discard the unit. Once validated, the spatial distribution of the action potential across the electrode grid was analyzed to determine the motor unit's anatomical territory (anterior or lateral)

8.2.4.4 Motor unit discharge behaviour. Motor unit discharge behaviour was characterized by calculating the mean discharge rate and the coefficient of variation of the inter-spike interval. Mean discharge rate was computed as the average of the inverse inter-spike intervals. The coefficient of variation of the inter-spike interval was calculated as the ratio of the standard deviation to the mean inter-spike interval (%). In addition, the slope of the mean discharge rate was calculated for each matched motor unit, defined as a normalized measure indicating the rate of increase in discharge rate per %MVC. These parameters were computed over the 20s plateau interval where the maximal number of motor units was active, which was defined visually. Furthermore, the mean force exerted by the subject during the plateau was calculated to assess potential deviations from the target force

8.2.5 Statistical analysis

Statistical analyses were performed in R (v. 4.3, R Core Team, 2023) using the RStudio environment (v. 2023.03.1).

The Wilcoxon signed-rank test was performed to compare the MVCs in the two different angles.

Linear Mixed-Effect Models (LMMs) were employed to analyse potential changes in motor unit discharge parameters (rate coding, slopes of the mean discharge rate and coefficient of variation of interspike interval). The models were fitted using the *lmerTest* package (Kuznetsova et al., 2017), applying the Kenward-Roger method to approximate degrees of freedom and to estimate *P*-values. Motor unit discharge behaviour was examined separately for each joint angle, with force range (5-10%, 10-20%, and 20-30% MVC), force level (lower and upper limit within each range), matched trial (e.g., trial01_5%MVC-trial01_10%MVC, trial01_5%MVC-trial02_10%MVC, trial02_5%MVC-trial01_10%MVC, trial02_5%MVC-trial02_10%MVC) and their interaction as fixed effects. Participants were included as a random-intercept effect. Similar models were applied to investigate variations in the slope of discharge rate (pps/%MVC). For the analysis considering different MUAP locations, the motor unit region (anterior or lateral) was further included as a fixed effect in the model. Statistical significance was set at $P < 0.05$. All force and motor unit yield results are reported as mean $\pm$ standard deviation. Motor unit discharge behavior results are reported as estimated marginal means along with 95% confidence intervals.

8.3 Results

8.3.1 Peak MVC

To evaluate potential differences in MVC between the two shoulder angles, peak MVC values were calculated and compared between joint positions. At 30° and 65°, average MVC values ($\pm$ standard deviation) were 29.80 ± 12.00 N and 32.91 ± 13.22 N, respectively. A Wilcoxon signed-rank test revealed a significant increase in peak MVC at 65° compared to the 30° of lateral abduction ($p = 0.025$).

8.3.2 Force

To verify that the force produced by participants was consistent with the required target force level, the mean force during the plateau was calculated for each trial. At 30°, the force values for 5%, 10%, 20%, and 30% MVC were 5.08 ± 0.35 , 10.16 ± 0.32 , 20.07 ± 0.69 , and 30.14 ± 0.62 %MVC, respectively. At 60°, the values were 5.06 ± 0.47 , $9.84 \pm$

0.50, 19.84 ± 0.84 , and 29.51 ± 1.55 %MVC. At both angles, mean force produced by participants closely matched the target force values.

8.3.3 Motor unit yield

In total, 1553 motor units were decomposed from the deltoid muscle at the two different shoulder abduction angles (908 and 645 in the proximal and distal grids, respectively). At 30° of abduction, a total of 791 motor units were decomposed (461 and 330 in the proximal and distal grids, respectively), corresponding to an average ($\pm$ standard deviation) of 5 ± 7 motor units per subject (6 ± 7 and 4 ± 6 in the proximal and distal grids, respectively). The average number of motor units extracted per force level (average across the two trials) was 5 ± 7 at 5% MVC (6 ± 8 and 4 ± 7 in the proximal and distal grids, respectively), 5 ± 7 at 10% MVC (6 ± 8 and 4 ± 7 in the proximal and distal grids, respectively), 5 ± 7 at 20% MVC (6 ± 7 and 4 ± 6 in the proximal and distal grids, respectively), and 5 ± 6 at 30% MVC (5 ± 6 and 4 ± 6 in proximal and distal grids, respectively). Average SIL values across force levels were 0.91 ± 0.02 .

At 65° of abduction, a total of 762 motor units were decomposed (447 and 315 in the proximal and distal grids, respectively), with an average of 5 ± 7 motor units per subject (6 ± 7 and 4 ± 6 in the proximal and distal grids, respectively). The average number of motor units per force level was 5 ± 7 at 5% MVC (6 ± 8 and 4 ± 7 in the proximal and distal grids, respectively), 5 ± 8 at 10% MVC (6 ± 8 and 4 ± 7 in the proximal and distal grids, respectively), 5 ± 7 at 20% MVC (6 ± 7 and 4 ± 6 in the proximal and distal grids, respectively), and 5 ± 6 at 30% MVC (5 ± 6 and 4 ± 6 in the proximal and distal grids, respectively). Average SIL values were 0.91 ± 0.02 across all force levels.

8.3.4 Motor unit matching yield

Motor units were matched across three different force-level pairs (5-10%, 10-20%, and 20-30% MVC) and considering the two trials of each force level. As no significant effects of matching trial were observed in the linear mixed model analyses ($p > 0.20$), motor units from the four trials (e.g., trial01_5%MVC-trial01_10%MVC, trial01_5%MVC-trial02_10%MVC, trial02_5%MVC-trial01_10%MVC, and trial02_5%MVC-trial02_10%MVC) were pooled for the subsequent analyses. The average number of matched motor units per subject was therefore calculated by averaging the motor units identified across the four trials. For the 5-10% MVC force range, 11 ± 13 motor units were

matched at 30° and 11 ± 16 at 65° (average SIL value of 0.89 ± 0.02 for both angles). For the 10-20% MVC force range, the average number of matched motor units was 10 ± 14 at 30° and 11 ± 11 at 65° (SIL value of 0.88 ± 0.02 for both angles). For the 20-30% MVC force range, the average number of matched motor units was 9 ± 12 at 30° and 8 ± 10 at 65° (SIL value of 0.88 ± 0.01 for both angles).

8.3.5 Motor unit discharge behaviour

To assess motor unit behavior at different force levels and across the two joint angles, mean discharge rate, slope of mean discharge rate and coefficient of variation of the inter-spike interval were calculated. These parameters were compared across different force ranges (5-10%, 10-20%, and 20-30% MVC) separately for 30° and 65° shoulder angles. The same analyses were repeated considering motor unit territory as a factor (anterior vs. lateral).

8.3.5.1 Mean discharge rate. For both 30° and 65° of shoulder abduction, there were significant main effects of force range, force level, and their interaction ($p < 0.001$ for all the analysis). Within the 5-10% MVC force range, mean discharge rate at 30° of lateral abduction increased from 14.2 pps [13.4, 15.1] at 5% MVC to 15.1 pps [14.2, 16.0] at 10% MVC. At 65° of lateral abduction, mean discharge rate increased from 14.8 pps [13.6, 16.0] at 5% MVC to 15.7 pps [14.5, 16.9] at 10% MVC. In the 10-20% MVC force range, mean discharge rate at 30° increased from 13.9 pps [13.0, 14.8] to 15.6 pps [14.8, 16.5]. At 65° of abduction, mean discharge rate increased from 14.8 pps [13.7, 16.0] to 16.2 pps [15.0, 17.4]. Finally, within the 20–30% MVC force range, mean discharge rate at 30° increased from 14.6 pps [13.6, 15.5] at 20% MVC to 17.0 pps [16.1, 17.9] at 30% MVC. Similarly, at 65° of lateral abduction, mean discharge rate increased between 20% and 30% MVC, rising from 15.3 pps [14.1, 16.5] to 17.9 pps [16.7, 19.1].

8.3.5.2 Slope of mean discharge rate. To compare the rate of increase of the mean discharge rate across different force levels, the slope of the mean discharge rate was calculated (pps/%MVC). For both joint angles, a progressive increase was observed in relation to force level ($p < 0.001$). At 30°, the slopes were 0.158 pps/%MVC between 5–10 and 10–20 MVC, and 0.232 pps/%MVC between 10–20 and 20–30 MVC. At 65°, the slope was 0.169 pps/%MVC between 5–10 and 10–20 MVC, while it reached 0.242 pps/%MVC between 10–20 and 20–30 MVC.

8.3.5.3 *MUAP location*. For both 30° and 65° shoulder angles, motor units located in the lateral region exhibited significantly higher mean discharge rates than anterior units across all force ranges ($p < 0.0001$) (Figure 8.3).

At 30° of lateral abduction, a significant interaction between force level and region ($p = 0.02$), indicating a different modulation of mean discharge rate between the lowest and highest force levels compared. Motor units in the anterior region exhibited mean discharge rates of 13.5 pps [12.7, 14.3] in the lower force level and 14.8 pps [14.0, 15.6] in the highest force level, with an average increase of 1.3 pps between the two trials. In contrast, units in the lateral region showed mean discharge rates of 14.6 pps [13.9, 15.4] in the lower force level and 16.4 pps [15.6, 17.2] in the highest force level, with an average increase of 1.8 pps. Consequently, the increase between the two force levels was significantly greater in lateral units ($p < 0.001$). These results were supported by the analysis of the slope of mean discharge rate across different force levels. Overall, slopes were significantly higher for motor units in the lateral region compared to the anterior region ($p < 0.0001$), with lateral units showing an increase of 0.221 pps/%MVC [0.183, 0.259] versus 0.146 pps/%MVC [0.108, 0.184] for anterior units.

At 65°, mean discharge rates for anterior motor units were 14.7 pps [13.4, 15.9] at the lower force level and 15.8 pps [14.6, 17.0] at the higher force level, with an average increase of 1.2 pps. Motor units in the lateral region, in contrast, exhibited mean discharge rates of 15.3 pps [14.1, 16.5] at the lower force level and 16.9 pps [15.7, 18.1] at the higher force level, with an average increase of 1.6 pps. Although there was a significant effect of both force level (lowest vs highest) and region ($p < 0.001$ for both), no significant interaction between these factors was observed. However, contrasts indicated a tendency for a greater increase in mean discharge rate between the two force levels in units located in the lateral region. This tendency was further supported by the slope of mean discharge rate, where there was a significant effect of both force range and motor unit region, as well as an interaction ($p < 0.004$). Specifically, motor units in the lateral region of the deltoid exhibited steeper slopes compared to units located in the anterior region (0.191 [0.133, 0.249] pps/%MVC for lateral; 0.134 [0.076, 0.192] pps/%MVC for anterior; $p = 0.003$).

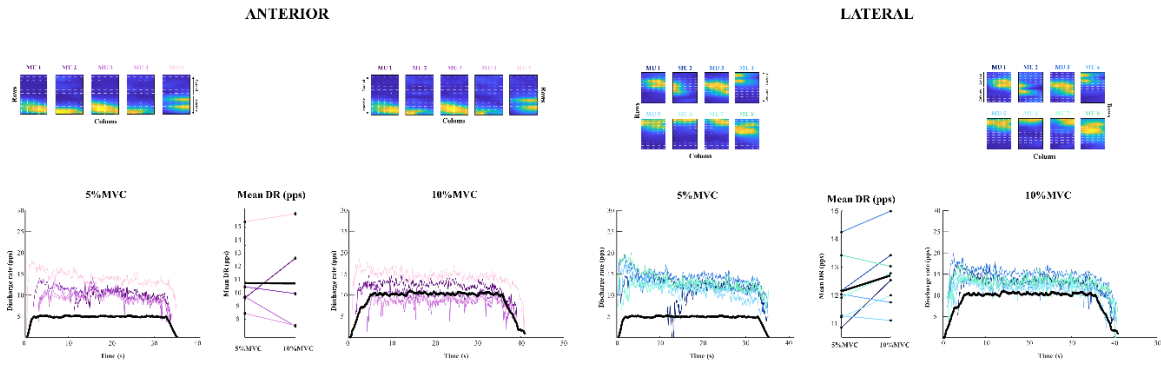


Figure 8.3: Representative example of the discharge behaviour of territory-based motor units. The figure illustrates the motor unit population of a representative subject, categorized according to their anatomical region of belonging. The left panel displays the motor units located within the anterior head of the deltoid, while the right panel shows those identified within the lateral head. The top section of each panel provides the spatial activation maps for each individual motor unit, defining its electrophysiological territory across the electrode grid. In the lower section, the smoothed discharge rates are displayed superimposed on the force profile, alongside the mean discharge rate values, highlighting the firing behaviour of the units as they transition from the low force level (5% MVC) to the high force level (10% MVC).

8.3.5.4 Coefficient of variation of the interspike interval. Motor unit discharge variability was quantified by calculating the coefficient of variation of the interspike interval. For both 30° and 65° shoulder angles, a significant interaction between the force range analyzed and force level was found ($p = 0.009$ at 30° and $p = 0.015$ at 65°).

At 30° in the 5–10% MVC range, the mean coefficient of variation was 18.7 [17.0, 20.5] at 5% and 18.9 [17.1, 20.6] at 10%. When comparing 10% and 20% MVC, the values were 20.4 [18.7, 22.2] at 10% MVC and 20.6 [18.9, 22.4] at 20% MVC. Finally, between 20% and 30% MVC, the mean value increased from 21.4 [19.6, 23.2] at 20% MVC to 23.3 [21.5, 25.1] at 30% MVC. Only the comparison between 20–30% MVC showed a significant increase in the coefficient of variation of the interspike interval between the trial performed at the lower force intensity and higher force intensity ($p = 0.71$ in the 5–10% MVC range, $p = 0.65$ in the 10–20% MVC range, and $p = 0.0001$ in the 20–30% MVC range).

At 65°, in the 5–10% MVC range, the mean coefficient of variation was 19.2 [17.2, 21.1] at 5% and 20.2 [18.3, 22.2] at 10%. In the 10–20% MVC range, the mean coefficient of variation was 20.9 [18.9, 22.9] at 10% to 20.2 [18.2, 22.2] at 20%. Lastly, in the 20–30% MVC range, the mean coefficient of variation was 22.4 [20.4, 24.5] at 20% and 22.1 [20.1, 24.1] at 30%. Contrary to 30°, at this shoulder abduction angle, only the comparison within the 5–10% MVC range revealed a significant difference between the lower and higher force levels ($p = 0.01$), whereas no significant differences were observed in the 10–20% MVC ($p = 0.15$) or 20–30% MVC ranges ($p = 0.54$).

8.4 Discussion

The present study aimed to investigate a muscle that is underrepresented in the literature, the deltoid, and to characterize the discharge behaviour of its motor units under conditions reflecting those encountered during activities of daily living. Accordingly, to achieve this objective, the same motor units were tracked while comparing isometric contractions performed at low-to-moderate force levels (5–10%, 10–20%, and 20–30% MVC) and executed at two different joint angles (30° and 65°).

The main results showed that peak MVC increased from 30° to 65°. Although the deltoid operates at a more favourable length for force production at lower abduction angles, effective force generation also depends on the stabilizing action of the supraspinatus. At approximately 65°, the supraspinatus allowing the deltoid to operate with a more advantageous moment arm despite its shortened length (Yanagawa et al., 2008). This biomechanical configuration ultimately enables greater force production at 65°. In addition, at both lateral arm abduction angles, the nervous system relied on rate coding as a strategy to respond to increased force demands and to modulate force within the investigated ranges. These findings are consistent with the literature, where it is well established that, although both recruitment and rate coding contribute to force production, rate coding plays a dominant role in fine force control, particularly in tasks requiring precise modulation (de Luca et al., 1982; Enoka & Duchateau, 2017). Furthermore, despite the deltoid muscle being a large, proximal muscle typically associated with gradual recruitment over a wide force range (Fuglevand et al., 1993; Garnett et al., 1979; Kukulka & Clamann, 1981), rate coding emerged as a crucial mechanism during low-to-moderate force tasks. This likely reflects the functional demands of the task, with low-intensity contractions prioritizing precise and controlled force output over gross maximal force production.

In addition to the analyses performed across the entire pool of decomposed and matched motor units, this study also examined the influence of motor unit territory on discharge behaviour. Consistent with previous studies suggesting functional compartmentalization within the deltoid to enable fine control of its mechanical action during shoulder joint movement (Brown et al., 2007; Gorelick & Brown, 2007; Wickham & Brown, 1998, 2012), motor units located in the lateral region exhibited higher mean discharge rates and steeper slopes of discharge rate compared to anterior units, particularly at 30° of abduction. These lateral units correspond to segments primarily involved in

abduction and, given their multipennate architecture, are structurally optimized for generating greater force (Peterson & Rayan, 2011). In contrast, anterior units displayed smaller increases in discharge rate with increasing force. These findings support the idea that the central nervous system coordinates motor unit activity in a region-specific manner, selectively modulating compartments that are functionally relevant for the task.

Another interesting finding from the analysis of the slope of the mean discharge rate, it is that the magnitude of increases in rate coding across force depends on the force range, becoming progressively higher as contraction intensity increases. This suggests that the central nervous system might modulate synaptic input more strongly in response to greater mechanical demands. In addition, this effect was consistent across the lateral abduction angles of the shoulder, indicating some degree of replicability and generalizability. However, as the difference between slopes at 20-30% and 5-10% is small in magnitude despite being statistically significant, further research is required to determine its practical implications.

In conclusion, the present study highlights that rate coding is a fundamental strategy for force modulation during low-to-moderate intensity contractions involving the deltoid. Importantly, this study further demonstrates the feasibility of distinguishing the spatial location of deltoid motor units, revealing that their discharge rate is modulated across force gradation depending on their anatomical location.

Of practical relevance, understanding how the nervous system orchestrates shoulder movement through the coordinated activity of distinct muscle segments, and elucidating the neural mechanisms underlying motor control, is of considerable importance. Among shoulder muscles, the deltoid is frequently affected by painful conditions and musculoskeletal pathologies (Mitchell et al., 2005). Therefore, insights into region-specific neural control may provide a valuable foundation for designing targeted preventive and rehabilitative interventions focused on the specific muscle segments engaged in a given task.

8.5 Limitations

Although this study represents a first step toward understanding a largely unexplored muscle, it has some limitations. First, while shoulder abduction angles were measured for each participant and the literature shows that changes in joint angle are associated with alterations in muscle length and moment arms (Hik & Ackland, 2019; Levin et al., 2023),

no ultrasound measurements were performed to verify actual fibre shortening. This limitation is largely due to the complex architecture of the deltoid, which, as described in this study, is composed of multiple compartments with distinct characteristics. Consequently, it would have been extremely challenging to quantify the shortening of individual compartments and to perform analyses based on the relationship between fiber length and force output. Second, motor units were matched exclusively across force levels and not across the two joint angles. This was because motor unit shapes differ when contractions are performed at different positions, making it difficult to track the same units across varying joint angles (Cudicio et al., 2022). Third, the experimental protocol included only low-to-moderate force levels. While this range reflects functionally relevant intensities for daily activities, future studies should explore higher force levels to determine the motor unit behaviour. Finally, although the present work focused on rate coding modulation, additional motor unit parameters could further clarify the neural mechanisms underlying regional differences. In particular, adding recruitment and de-recruitment thresholds, as well as the evaluation of common synaptic input of the motor units divided by territory, could represent fundamental tools to comprehensively understand how regional organization influences the motor unit parameters extracted from HD-sEMG decomposition.

Together, these considerations outline directions for future research aimed at deepening our understanding of how force production, regional muscle activation, and neural input interact in the deltoid.

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Part 4: Conclusion

The primary objective of this doctoral thesis was to investigate how neural control and connectivity between spinal motoneurons are organized and modulated during voluntary contractions across various functional contexts and body regions. This was achieved through the design and application of protocols based HD-sEMG, which allowed for the assessment of the neural drive to motor units during different types of motor tasks. A "top-down" approach was employed throughout the research, starting from the broad objective of gaining a comprehensive understanding of the existing literature on motor units, and progressing toward specific experimental studies increasingly focused on the final investigation of the shoulder.

The findings, particularly those from the systematic literature review (chapter 5), highlighted a significant gap in current research regarding motor unit analysis in most muscles of the upper limb. Specifically, it emerged that the shoulder region, despite being fundamental to a wide range of daily activities and sport gestures, and frequently being a site of pain and dysfunction, remains almost entirely unexplored in literature.

Subsequent experimental studies analyzed a broad range of muscles during various motor tasks. The first study (chapter 6) investigated potential changes in neural drive following a motor learning task in muscles of the lower limb that are functionally similar to those of the shoulder. It demonstrated that although the investigated muscles share most of the neural input sent by the nervous system, motor learning leads to a modulation of this input that depends on muscle architecture and, consequently, its functional role.

The following study further explored the concept of function-dependent synaptic modulation in the muscles of the hand, an area characterized by a complex interplay between different muscles and fine-tuned neural control. The aim was to verify whether the upper limb exhibits different neural input modulation between functionally relevant tasks (grasping) and less complex ones (individual finger movements). This second study also highlighted how the nervous system adopts targeted and specific force-control strategies during the execution of innate, everyday motor gestures such as grasping.

Finally, the third experimental study, which represents the core of this doctoral project, shifted the focus to the shoulder region, characterizing the behavior of the deltoid muscle at two different joint angles and during low-to-moderate force production. In addition to providing reference values for the muscle's discharge parameters, this study confirmed

the hypothesis of function-dependent modulation in this district as well, showing a specific regulation of motor units in relation to their functional role.

Collectively, the results of this research confirm that neural control is not a rigid mechanism but rather an adaptive process, primarily dependent on the functional demands of the task. Indeed, the central nervous system appears to modulate the stability of the neural drive based on the goal of the movement: a greater demand for precision leads to a reduction in oscillations within specific frequency bands associated with the stability of the motor output. Furthermore, these studies revealed that learning protocols can modulate the neural response, increasing stability and reducing variability through practice. Finally, this project supports the hypothesis that the specific architecture and structure of the target muscle influence the reception and translation of the neural drive into action potentials, thereby determining the resulting force output.

In conclusion, this project contributes to the methodology of muscle investigation using HD-sEMG, with a particular focus on the upper limb. Furthermore, it establishes a foundation for future research into pathological conditions that may affect neural signaling to the shoulder muscles and compromise movement, ultimately informing the design and implementation of targeted rehabilitation protocols.