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Implanted high-density electrode arrays to connect with the human spinal cord

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Ai miei cari genitori

Volare per vivere

Moony Witcher, alias Roberta Rizzo

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Abstract

Neural interface technologies aim to bridge the gap between the nervous system and external environment, offering both clinical applications for individuals with impairments and enhancement capabilities for healthy users. While various interface approaches exist across different neural pathway levels, muscle-level interfaces through electromyography (EMG) present a particularly promising avenue by capturing neural signals as they transform into physical actions. Although surface EMG provides insights into global muscle activity, its effectiveness is limited by filtering of the signal through the conductive tissue. Intramuscular EMG, especially when recorded via multichannel electrodes, offers more direct access to neural signals—yet the full potential of intramuscular multichannel electrodes remains unexplored, with current decoding algorithms not optimized for these advanced recording capabilities.

This thesis advances the field through the characterization of a novel intramuscular multichannel micro-electrode array. I collected a novel dataset of multichannel intramuscular EMG recordings from forearm and leg muscles in healthy participants, which supported several key contributions: (1) development of a novel algorithm to extract (decode) the neural information from high-density intramuscular EMG, later adapted for surface EMG applications; (2) systematic analysis of electrode parameters' impact on decoding performance; and (3) demonstration of a proof-of-concept offline system for gesture control using motor neuron decoding and mapping. These advances lay the groundwork for more sophisticated neural interfaces that could enhance both clinical applications and human-machine interaction.

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List of Abbreviations

ACh acetylcholine. 23

AP action potential. xvii, 17–20, 23, 24, 26, 27, 29, 37, 38, 58, 63–66, 68, 69, 75, 79, 83, 171

ATP adenosine triphosphate. 19, 23

BSS blind source separation. 10, 28, 35, 49, 58, 60, 63–66, 77, 83, 86, 88, 89, 112–114, 135, 171, 174, 176

Ca²⁺ calcium. 19, 23, 26, 27

cBSS convolutive blind source separation. xxiv–xxvi, 49, 52, 54, 55, 75–78, 89, 112, 114, 115, 122–137

CKC convolution kernel compensation. 49, 51, 53–55, 57, 89

CNS central nervous system. 16, 24, 27

CoV coefficient of variation. 54, 56, 57, 70–72, 75–78, 148

ECRB extensor carpi radialis brevis. xxi, xxvii, 74, 150, 151

EDC extensor digitorum communis. xviii, xxi, xxvii, 30, 73, 74, 76, 150, 151

EDM extensor digiti minimi. xxvii, 150, 151, 156

EMG electromyography. xv, xix, xx, xxv, xxviii, 6–10, 15, 16, 24, 27, 28, 30, 32–39, 42, 43, 48, 49, 54, 56, 59–61, 64–67, 72, 73, 76, 83, 86–88, 90, 110–114, 116, 119, 120, 127–129, 139, 141, 142, 144, 146–148, 156, 157, 163, 165–167, 170–172, 176, 180, 182

EPL extensor pollicis longus. xxi, xxvii, 73, 74, 76, 150, 151, 153

fastICA fast independent component analysis. 52–54

FDS flexor digitorum superficialis. xxi, xxvii, xxviii, 73, 74, 117, 150, 151, 155

FR firing rate. 56, 57, 95, 116, 148

HD high-density. 10, 29, 38, 61, 63, 64, 66, 74, 86, 88, 139, 144, 148

HD-iEMG high-density intramuscular EMG. xv, xviii, xix, xxi–xxiii, xxvii, xxix, xxx, 8–11, 28–31, 57, 58, 61, 63, 69, 70, 72, 73, 75–78, 81, 83, 84, 86–92, 95–100, 102, 106–109, 111, 120, 122, 130, 131, 136, 138, 141, 142, 144, 146–149, 151–154, 156, 158, 159, 161, 163–166, 168–174, 176–178, 180, 182

HD-sEMG high-density surface EMG. xv, xviii, xix, xxii–xxiv, xxix, 9, 11, 29–31, 39, 58, 61, 86, 87, 89–92, 95–101, 106–109, 111–114, 116, 120–122, 130, 131, 135, 136, 138, 141–144, 148, 149, 151, 152, 154, 156, 158, 159, 161, 163–165, 171–174, 180

ICA independent component analysis. xx, xxi, 41–43, 48, 49, 51, 54, 64, 65, 69–71, 80, 81, 83, 112, 114, 115, 124

IED interelectrode distance. xv, xxii, xxiii, xxix, 28, 84, 86, 88, 90, 92–94, 100, 103–105, 107, 144, 148, 149, 158

iEMG intramuscular EMG. xviii, xxvii, xxix, 6, 7, 29, 30, 37, 38, 56, 60, 61, 142, 143, 147, 148, 156, 158, 166, 171, 176

IPT innervation pulse train. xxviii, 54, 147, 157

IQR interquartile range. xv, 78, 82

K⁺ potassium. xvii, 17, 19, 20

MIMO multiple-input-multiple-output. 33

MN motor neuron. x, xviii, xix, 6–9, 15–19, 21, 24, 26–28, 35, 42, 43, 48, 54–61, 66, 111, 112, 117, 118, 137, 138, 142, 144, 146, 149, 151, 152, 156, 163, 165–167, 169, 170, 172

MU motor unit. xv, xviii, xix, xxii–xxix, 7, 11, 15, 19, 21, 24, 26–29, 31–35, 37–39, 43, 55, 56, 58, 61, 63, 66, 70, 76, 78, 84, 86–89, 91, 93, 95–101, 103, 104, 106–109, 111–119, 121–139, 145–149, 152–159, 161, 163–167, 169, 171–176

MUAP motor unit action potential. xviii, xix, xxiv–xxvi, 24, 25, 29, 31–35, 37–39, 43, 56, 58, 59, 66, 88, 111–114, 116, 117, 122, 123, 125, 128–138, 171

MUAPT motor unit action potential train. 32

MVC maximum voluntary contraction. xxii–xxv, 26, 72, 73, 76, 77, 82, 86, 87, 90, 91, 93, 97–109, 118–124, 126, 128, 131, 180

Na⁺ sodium. xvii, 17–20, 23

NMJ neuromuscular junction. 19, 24, 30, 31

PNR pulse-to-noise ratio. 56, 57

pps pulses per second. 27, 43, 118

PSO particle swarm optimization. xx, 65, 70, 71, 76, 77, 80, 81

RoA rate of agreement. xv, xxiii–xxvi, 55, 76, 78, 83, 95, 96, 98, 103, 106, 121–123, 125–134, 136

SCD Swarm-Contrastive Decomposition. xxi, xxiv–xxvi, 65, 70, 72, 74, 75, 83, 89, 95, 109, 111, 113–116, 122–138, 146, 171, 172, 174–177

sEMG surface EMG. xviii, xxvii, xxix, 6–8, 29, 30, 38, 56, 60, 61, 111–113, 142, 143, 149, 156, 158, 160, 162, 166, 171

SFAP single fiber action potential. 23, 24, 30–32

SIL silhouette measure. xxiii, 56, 57, 72, 76, 77, 91, 95, 98, 100, 102, 103, 105, 107, 115, 116, 148

SNR signal-to-noise ratio. 6, 38, 82, 118

SoA state-of-the-art. 8, 38, 65, 75, 77, 83, 111, 113, 114, 135, 137, 141, 145, 171, 172, 174, 176

TA Tibialis Anterior. xv, xxii, xxiv, xxx, 57, 72, 73, 76–78, 81, 82, 84, 86, 90, 92, 109, 119–121, 173–175, 182, 184

*But how could you live and have
no story to tell?*

Dostoevsky, Fyodor

1

Introduction

1.1 Motivation

Movement shapes how humans interact with the world around them. A simple smile can require the activation of up to 53 facial muscles, while a handshake coordinates muscles spanning from the back to the fingertips. These movements, whether simple or complex, emerge from a sequence of precisely timed neural events that begin with action potentials in the central nervous system and culminate in precisely coordinated muscle contractions.

Movement control follows a clear neural pathway. The brain processes sensory input, past experience, and current objectives to generate motor plans. The motor commands travel through the

spinal cord, where they undergo continuous modification based on sensory feedback. The refined signals then propagate through the peripheral nervous system to activate target muscle groups.

The hierarchical organization of movement control, from initial intent to final execution, provides multiple potential access points for neural interfaces (Farina et al., 2023). While initially developed for medical rehabilitation and assistive devices, neural interfaces now support broader applications including human-machine interaction, tele-operation systems, and virtual reality control.

The most direct approach to accessing movement intentions is through brain-computer interfaces (BCIs) that target the cortical origins of motor commands. Invasive BCIs, which require surgical implantation, can capture high-resolution neural signals directly from the brain (Leuthardt et al., 2004). This rapidly advancing field is benefiting from innovations in sensor design and insertion techniques. However, despite substantial progress, these systems remain highly invasive, costly, and associated with considerable risk, limiting their use to only the most critical cases. Non-invasive methods like electroencephalography (EEG) (Wolpaw et al., 1991) avoid surgery but face technical limitations. Significant hurdles include prolonged user training times, user fatigue, and critically, the low signal-to-noise ratio (SNR) that complicates reliable signal interpretation (Abiri et al., 2019). These limitations have historically constrained the widespread adoption of EEG-based neural interfaces, despite their potential for less invasive neural communication.

Peripheral interfaces offer alternative methods for movement detection with varying degrees of invasiveness. Current technologies include ultrasound imaging for muscle deformation detection (X. Yang et al., 2024), inertial measurement units (IMUs) for joint angle tracking (Bennett & Goldfarb, 2017), mechanomyograms and EMG for muscle contraction detection and monitoring (Castillo et al., 2020; Farina & Holobar, 2016). Among these, the EMG stands out as it indirectly measures the electrical activity of spinal MNs through the muscle fibers, providing a direct window into the neural control of movement.

EMG signals can be accessed through two distinct approaches, each offering unique insights into neural control. iEMG, recorded via electrodes placed within the muscle tissue, captures electrical signals that closely mirror the original neural commands from MNs. This proximity to the source provides high signal quality but requires invasive implementation. In contrast, sEMG uses electrodes

placed on the skin surface, offering a non-invasive alternative. The biological tissues between the muscle fibers and the electrodes act as a volume conductor, effectively filtering and attenuating the signal. Regardless of the recording method, the EMG signal comprises both a neural component (the MNs discharge series) and a muscle component (the action potential waveforms). Various decoding algorithms have been developed to isolate the neural component by removing or minimizing the impact of the muscle component.

The earliest attempts at EMG decomposition focused on iEMG signals due to their superior signal quality compared to sEMG. However, recent technological advancements have made multichannel sEMG systems a primary focus in neuromuscular signal processing research. These systems, offering tens of recording sites and relative accessibility, have gained significant attention thanks to their non-invasive nature and simple recording setup. As a result, numerous algorithms have been developed to extract neural information from interference signals.

Parallel advancements in iEMG technology have also emerged, producing micro-electrode array systems with tens of recording sites (Farina et al., 2008; Muceli et al., 2015, 2022). These innovations have addressed the limitations of high selectivity in iEMG recordings, expanding their capabilities to include a larger number of recording sites. Despite these advancements, the high production costs associated with manufacturing multi-channel micro-electrode arrays remain a significant barrier, limiting their commercialization and widespread use in large-scale research studies.

With these multi-channel micro-electrode arrays, researchers have begun adapting sEMG decoding algorithms for iEMG signals. However, current decomposition methods struggle to fully capture the complexity of muscle activation patterns, typically isolating only a fraction of the active MUs present in a signal (Negro et al., 2016). This technical limitation represents a significant obstacle in maximizing the potential of intramuscular recordings.

The practical implications of these limitations extend into the clinical and human-interfacing applications. While initial explorations have begun to use MN signals decoded from sEMG as potential control mechanisms (C. Chen, Yu, et al., 2020; C. Chen et al., 2023; Farina et al., 2017; Tanzarella et al., 2023), the application of MNs extracted from iEMG remains virtually unexplored. This gap presents a critical opportunity for innovative research in neural interfacing, particularly in developing more

precise and physiologically accurate control systems for prosthetics, rehabilitation technologies, and human-machine interfaces.

In this thesis, I aimed to develop a novel EMG interface based on high-density intramuscular micro-electrode arrays. I collected an unprecedented dataset from various human muscles, providing the first comprehensive recordings with over 100 electrode points directly inside muscle tissue. This dataset served as a critical validation foundation for the subsequent analyses.

I developed a novel decomposition algorithm specifically designed for intramuscular recordings, demonstrating state-of-the-art (SoA) performance. I then conducted a systematic analysis of the intramuscular micro-electrode array configurations, rigorously investigating the parameters most significantly affecting decoding performance. Building on these insights, I adapted the algorithm for sEMG, demonstrating SoA performance for this recording modality too. Lastly, I developed a proof-of-concept interface that leverages MNs extracted from HD-iEMG recordings to generate control signals for gesture recognition.

Taken together, these developments have advanced the field of HD-iEMG, offering new insights into optimal electrode configurations, providing highly optimized decoding algorithms, and presenting a vision for more intuitive human-computer interaction.

1.2 Aims and Objectives

The aim of this PhD is to:

- Develop an innovative EMG interface that comprises advanced decoding algorithms and multi-channel intramuscular micro-electrode EMG arrays to enable precise neural signal extraction, interpretation, and decoding/mapping.

Specific sub-objectives of this PhD are to:

1. Establish a comprehensive HD-iEMG signal database across diverse experimental conditions to enable robust algorithms development and validation. This objective encompasses:

- (a) Training in intramuscular electrode insertion techniques
 - (b) Developing an integrated hardware platform for synchronized recording of finger forces and EMG signals
2. Develop and validate a novel HD-iEMG signal decomposition algorithm for improved neural information extraction. This involves:
 - (a) Conducting a systematic review of state-of-the-art decomposition methodologies, identifying both achievements and limitations in current approaches
 - (b) Designing and implementing advanced decoding algorithms optimized for HD-iEMG signals to achieve superior neural information extraction compared to existing methods
 - (c) Validating algorithm performance across diverse invasive multi-channel datasets to demonstrate robustness and adaptability across different recording conditions
3. Quantify and characterize the channel capacity of HD-iEMG signals in comparison with HD-sEMG signals through:
 - (a) Synthesizing current methodologies for channel capacity analysis through comprehensive literature review
 - (b) Conducting systematic evaluation of how recording configuration parameters (channel count, density, and location) impact decomposition performance, with direct comparison to concurrent HD-sEMG recordings
4. Establish a proof-of-concept for neural-based human-machine interfaces through:
 - (a) Reviewing state-of-the-art methods for interfacing with MNs
 - (b) Developing and implementing motion classification algorithms based on MNs activity and benchmarking against traditional gesture recognition approaches
 - (c) Validating the interface system using HD-iEMG data to demonstrate practical viability for real-world applications

Figure 1.1 presents a flowchart of the aims and objectives of this PhD.

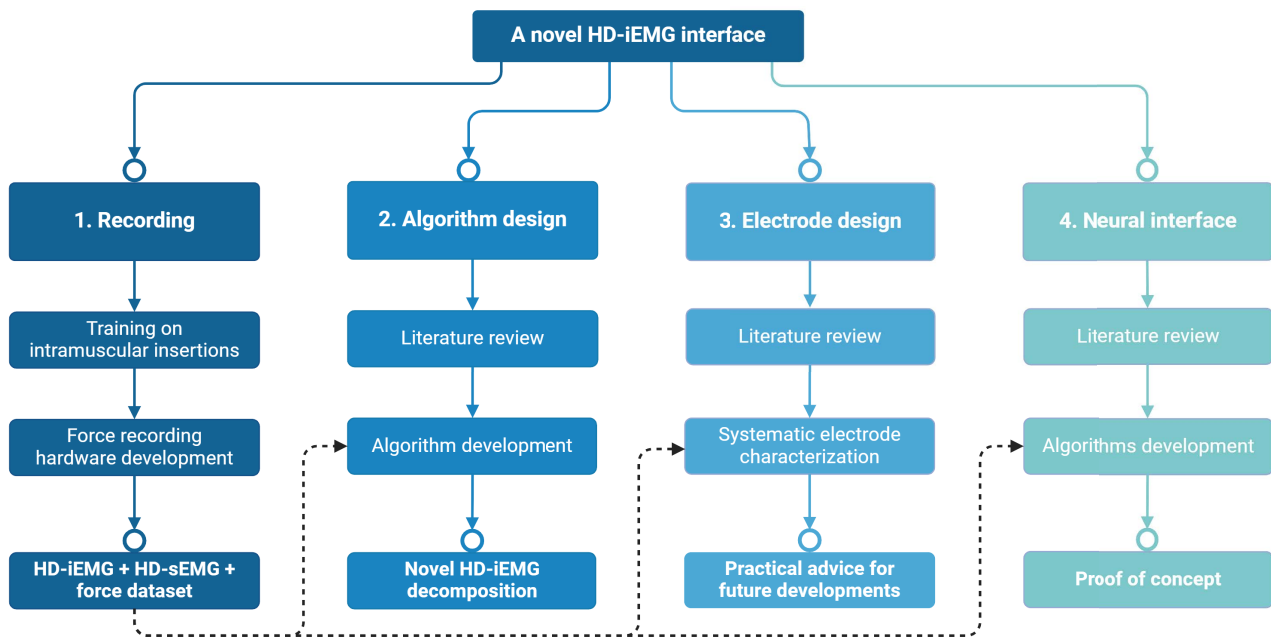


Figure 1.1: Flowchart of thesis aims and objectives.

1.3 Thesis Outline

Chapter 1 establishes the foundational role of EMG as a peripheral interface, demonstrating its distinct advantages over alternative interfaces. The chapter concludes by exploring diverse potential applications of EMG technology across multiple domains.

Chapter 2 presents a comprehensive analysis of neuromuscular anatomy and physiology, tracing the signal pathway from individual neurons to their manifestation in EMG recordings. The chapter systematically examines various recording modalities and introduces the EMG generative model. Through critical evaluation of current methodologies for extracting neural information from interference signals, it identifies both successful algorithmic features and persistent limitations that have hindered widespread adoption, thereby fulfilling Objective 2 (a) of this thesis.

Chapter 3 introduces a novel blind source separation (BSS) decomposition algorithm for HD-iEMG signals, featuring an adaptive function for enhanced source separation (Objective 2 (b)). The algorithm's effectiveness is validated using diverse HD-iEMG datasets, including data collected as part of a comprehensive data collection study (fulfilling Objective 2 (c)), and complemented by proof-of-concept demonstrations using high-density (HD) intracortical signals. The results demonstrate

significant improvements over existing methods in three key areas: elimination of expert manual intervention, increased quantity of detectable sources, and enhanced decomposition accuracy.

Chapter 4 conducts a systematic investigation of HD-iEMG electrode configurations and their impact on decomposition performance. The analysis focuses on how the number and location of detection points within the muscle affect decomposition yield and accuracy. The chapter presents a comparative analysis between information extracted from different HD-iEMG configurations within the same muscle, as well as between HD-iEMG and HD-sEMG signals. This comprehensive characterization fulfills Objective 3 of the thesis.

Chapter 5 extends and adapts the novel decomposition algorithm for HD-sEMG signals. The algorithm undergoes systematic evaluation using both simulated signals and experimental datasets. The results establish a new state-of-the-art in HD-sEMG decomposition by addressing key limitations of previous approaches. Specifically, the algorithm demonstrates superior performance against the current methods in three critical areas: increased detection of low-amplitude motor units, reduction of the sex-based performance disparities, and improved decomposition accuracy during rapid force development. These advances significantly expand the utility and reliability of HD-sEMG decomposition techniques. While this extension to HD-sEMG signals was not an original objective of this thesis, it represents a valuable contribution to the field by adapting the core algorithm to a more widely accessible recording modality, thereby broadening its potential impact and practical applications.

Chapter 6 presents the development and implementation of an innovative system that translates MU activity extracted from HD-iEMG signals into motion commands. The system achieved exceptional classification accuracy, reaching nearly 100% for up to 12 gesture classes and maintaining accuracy above 96% for up to 16 classes. The chapter includes a comparative analysis between the neural classifier and traditional approaches using global features from raw signals. Additionally, it demonstrates a proof-of-concept for human-machine interaction (the prediction of gestures for prosthetic control) using interpretable physiological features, thereby fulfilling Objective 4 of the thesis.

Chapter 7 synthesizes the key contributions and findings of this thesis, evaluates their implications for the field, and outlines promising directions for future research and development.

The dataset collection, while fulfilling Objective 1, is not presented in a dedicated chapter as the experimental protocols and acquisition details are integrated into the relevant technical chapters. The custom-developed hardware platform and detailed experimental protocols for all data collections are documented in Appendices A.1 and A.2. Although this thesis uses only a subset of the data collected, the comprehensive dataset represents a valuable resource for future research initiatives and collaborations.

1.4 Publications

1.4.1 Papers/Manuscripts

- **A. Grison**, I. Mendez Guerra, A. K. Clarke, S. Muceli, J. Ibanez Pereda, D. Farina, “Unlocking the Full Potential of High-Density Surface EMG: Novel Non-Invasive High-Yield Motor Unit Decomposition”, Accepted pending minor revisions in the Journal of Physiology. arXiv:2410.14800 (2024).

Contributions of Agnese Grison: Conceptualization, Investigation, Analysis, Validation, Visualisation, Writing

- **A. Grison**, J. Ibanez Pereda, S. Muceli, A. Kundu, F. Baracat, G. Indiveri, E. Donati, D. Farina, “Intramuscular High-Density Micro-Electrode Arrays Enable High-Precision Decoding and Mapping of Spinal Motor Neurons to Reveal Hand Control”, arXiv preprint arXiv:2410.11016 (2024).

Contributions of Agnese Grison: Conceptualization, Investigation, Analysis, Validation, Visualisation, Writing

- **A. Grison**, J. Ibanez Pereda, D. Farina, “Motor unit sampling from intramuscular micro-electrode array recordings”, IEEE Transactions on Neural Systems and Rehabilitation Engineering (2025)

Contributions of Agnese Grison: Conceptualization, Investigation, Analysis, Validation, Visualisation, Writing

- A. K. Clarke, **A. Grison**, I. Mendez Guerra, P. Mamidanna, S. Ma, S. Muceli, D. Farina, "HarmonICA: Neural non-stationarity correction and source separation for motor neuron interfaces", arXiv preprint arXiv:2406.19581 (2024).

Contributions of Agnese Grison: Conceptualization, Investigation, Validation, Writing

- **A. Grison**, A. K. Clarke, S. Muceli, J. Ibanez Pereda, A. Kundu, D. Farina, "A Particle Swarm Optimised Independence Estimator for Blind Source Separation of Neurophysiological Time Series", IEEE Transactions on Biomedical Engineering (2024).

Contributions of Agnese Grison: Conceptualization, Investigation, Analysis, Validation, Visualisation, Writing

- R. J. Varghese, M. Pizzi, A. Kundu, **A. Grison**, E. Burdet, D. Farina, "Design, Fabrication and Evaluation of a Stretchable High-Density Electromyography Array". Sensors, 24.6 (2024): 1810.

Contributions of Agnese Grison: Conceptualization, Investigation, Writing

1.4.2 Workshops

- "Applications of decomposition in the field of rehabilitation and prosthetics", ISEK 2024.

1.4.3 Conference Abstracts

- **A. Grison**, A. K. Clarke, I. Mendez Guerra, J. Ibanez Pereda, D. Farina, "A Particle Swarm Optimised Nonlinearity for High Yield Surface EMG Decomposition", Proceedings, The International Society of Electrophysiology & Kinesiology (ISEK2024).
- A. K. Clarke, **A. Grison**, I. Mendez Guerra, D. Farina, "Fast and Stable Decomposition of Unwhitened Surface EMG Signal by Random Projection", Proceedings, The International Society of Electrophysiology & Kinesiology (ISEK2024).

- J. Chartier, C. Kao, R. Rajalingham, C. Warriner, D. Morrison, E. Formento, M. Bräcklein, C. McConkey, D. Barsakcioglu, **A. Grison**, B. G. Sgambato, M. Glueck, D. Farina, D. Sussillo, “Towards human motor augmentation by learning to control novel wrist muscle co-contractions”, Neural Control of Movement (NCM2024).
- **A. Grison**, A. K. Clarke, S. Muceli, J. Ibanez Pereda, A. Kundu, D. Farina, “New insights and methods for automatic and reliable decomposition of the spiking activity of populations of neurons”, Society for Neuroscience 2023 (SfN23).
- **A. Grison**, A. Kundu, B. G. Sgambato, S. Muceli, D. Farina, “A Platform for Real-Time Recording of Finger Forces and Surface EMG in Forearm Muscles”, 9th International IEEE EMBS Conference on Biomedical Robotics and Biomechatronics (BioRob2022).
- A. Kundu, J. Gmaz, S. Avrillon, R. Varghese, **A. Grison**, D. Farina, “A platform to elicit and record intramuscular EMG signals from anesthetized mice”, 11th International IEEE/EMBS Conference on Neural Engineering (NER23).

*Un mondo el xe che noi
se lo capissi 'pena*

*(It's a world that we
barely understand)*

Giotti, Virgilio

2

Background Theory

This chapter provides a broad overview of the essential background concepts. It begins with an explanation of force generation, the foundation of all movement. Next, the principles of electromyography (EMG) are introduced, followed by a discussion of current methods used to extract motor units (MUs) from the interference signal. Finally, human-machine interaction techniques based on motor neurons (MNs) are examined. The chapter concludes by identifying gaps in the existing literature and outlining the aims of this thesis.

While the content introduced in this chapter provides a comprehensive foundation for understanding the original work presented in Chapters 3, 4, 5, 6, each of these chapters (excluding the final discussion) will offer its own background, expanding on the context provided here and explaining the relevant literature in more detail.

2.1 Neuromuscular System

Human movement is the result of complex interactions within the nervous system, where coordinated signals transform motor intentions into physical actions. These neural commands, refined by continuous sensory feedback, are transmitted to the musculoskeletal system for execution.

This section explores the anatomy and physiology of the neuromuscular system, which underpins the extraction and interpretation of neural motor commands from EMG signals. It provides an in-depth look at how neural activity is converted into force and movement, a process known as neuromechanical transduction, while also examining the various factors that influence the precision and efficiency of this translation. By understanding the mechanisms of motor control, we gain insights into how EMG signals can be used to decode MN behavior and how this knowledge can be applied in areas such as movement analysis and human-machine interfaces.

2.1.1 The Motor Neuron and Muscle Action

Human movement is driven by the complex interaction between the central nervous system (CNS) and the musculoskeletal system, where motor commands originating from the CNS are translated into precise muscle contractions. At the heart of this process are alpha MNs, specialized neurons located in the spinal cord or brain stem that serve as the final point of summation for all descending and reflex input (Moritani et al., 2004).

Like other neurons, alpha MNs have distinct structural components: branching dendrites that receive inputs from other neurons, a cell body (soma) that integrates these signals, and a long axon that conducts electrical signals to the target muscle. The axon is wrapped in myelin, a fatty insulating sheath formed by Schwann cells. This myelin sheath is interrupted at regular intervals by nodes of Ranvier, small exposed segments of the axon that enable rapid signal conduction. The axon extends from the spinal cord to the muscle, where it branches into terminal boutons that form synapses with muscle fibers (Koester & Siegelbaum, 2012a) (Figure 2.1).

Alpha MNs integrate inputs from supraspinal centers, spinal interneurons, and peripheral afferents

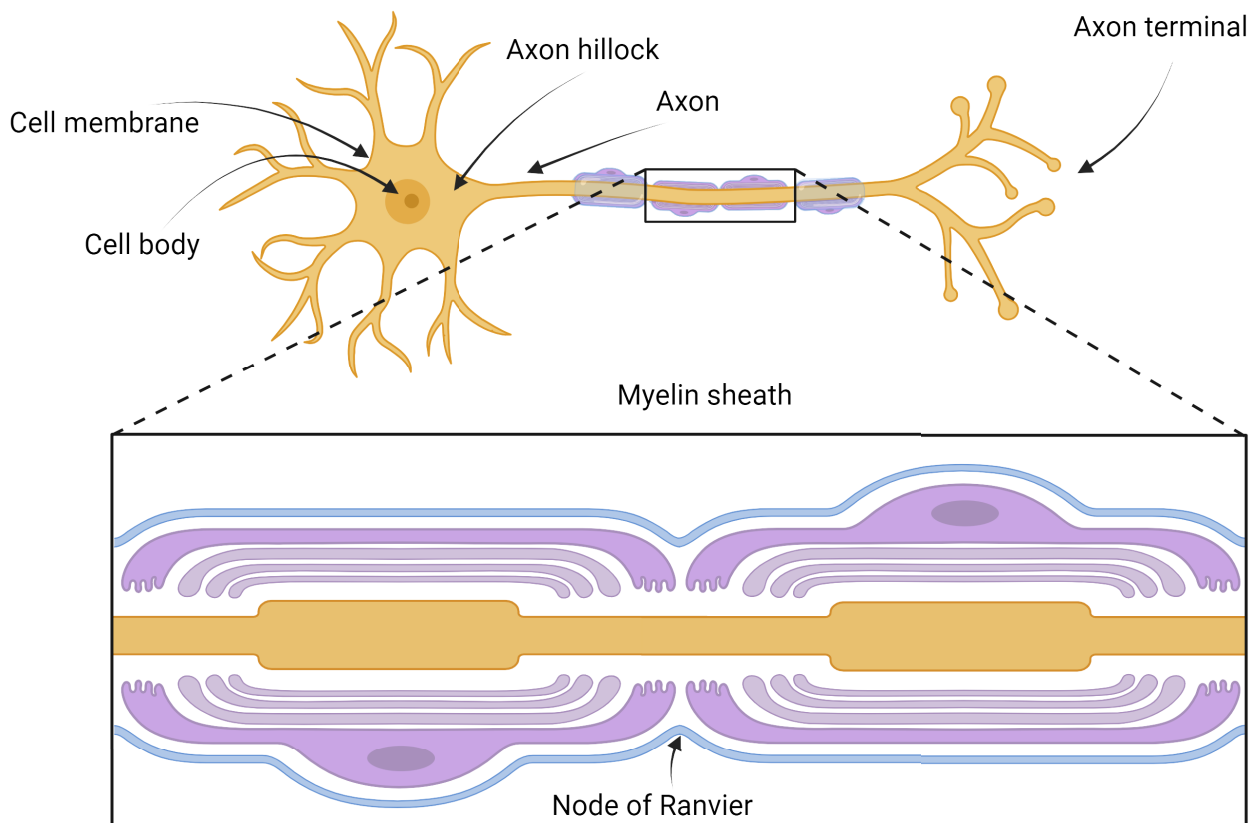


Figure 2.1: Anatomy of a neuron. Created with BioRender.com.

to drive muscle contraction. At rest, neurons maintain a negative membrane potential of approximately -70 mV, established by the active transport of sodium (Na^+) and potassium (K^+) ions across their membrane by Na^+/K^+ pumps and the selective permeability of the membrane to K^+ ions via leak channels (Figure 2.2 **a**, **b**). Alpha MNs primarily receive synaptic signals through ionotropic receptors, also known as ligand-gated ion channels. These specialized membrane proteins open to allow ion flow when neurotransmitters bind to them. The main excitatory neurotransmitter, glutamate, depolarizes the neuron by making the internal charge less negative, moving it closer to the firing threshold of approximately -55 mV. When this threshold is reached, voltage-gated Na^+ channels open, triggering an action potential (AP). In contrast, inhibitory neurotransmitters such as glycine and GABA reduce the likelihood of firing through hyperpolarization, which moves the membrane potential further from the threshold, closer to -75 mV (R. Enoka & Duchateau, 2016; Heckman & Enoka, 2004) (Figure 2.2 **a**).

For an AP, or nerve impulse, to occur, the combination of excitatory and inhibitory inputs must depolarize the neuron's membrane potential to approximately -55 mV, at the axon hillock, a key lo-

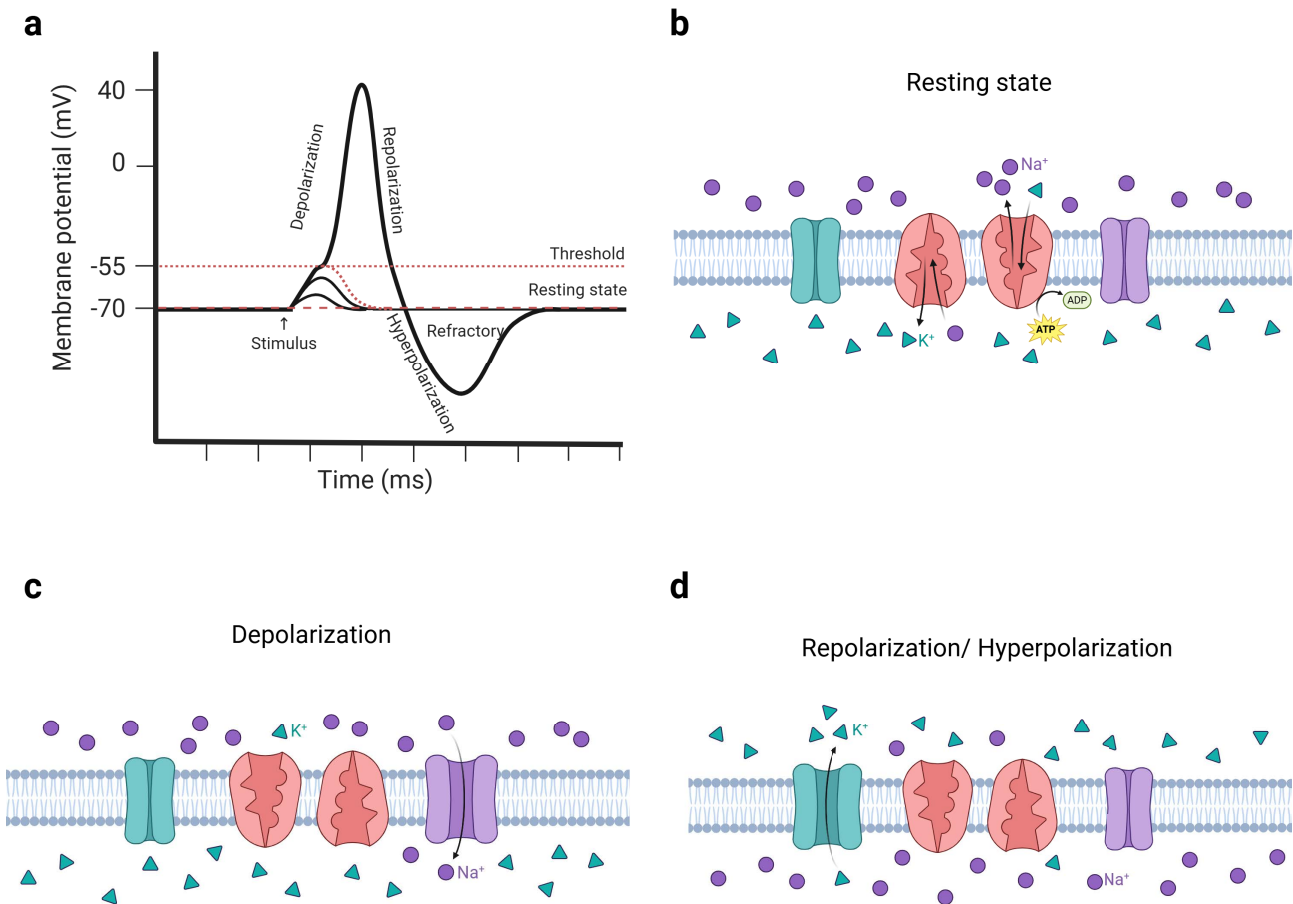


Figure 2.2: Action potential. **a** Schematic of an AP. Membrane potentials during resting state, depolarization and repolarization/hyperpolarization in **b**, **c**, **d**. Created with BioRender.com.

cation densely packed with voltage-gated Na⁺ channels (Heckman & Enoke, 2004). The axon hillock acts as a decision point, determining whether the neuron will fire based on the balance of inputs it receives. In addition to these immediate excitatory and inhibitory effects, MN activity is modulated by neuromodulatory inputs. Chemicals like serotonin and noradrenaline bind to different receptors on the neuron, initiating complex internal processes that alter how sensitive the neuron is to further inputs (Koester & Siegelbaum, 2012b). These processes fine-tune the neuron's overall responsiveness, ensuring precise control over muscle activation. In certain motor circuits, electrical synapses also play a role by directly coupling neurons through gap junctions, enabling rapid and synchronized communication. While less common than chemical synapses, these specialized connections are crucial for coordinating rhythmic motor patterns and reflexive responses, providing an additional layer of efficiency in motor control.

When the excitatory threshold is reached, voltage-gated Na⁺ channels open, allowing an influx of

Na^+ ions into the neuron (Figure 2.2 c). This influx causes a rapid depolarization of the membrane, which triggers a positive feedback loop that amplifies the increase in membrane potential. At the same time, depolarization activates voltage-gated calcium (Ca^{2+}) channels, allowing Ca^{2+} ions to enter the neuron (Heckman & Enoke, 2012). Following this, the neuron enters the repolarization phase. This occurs when the voltage-gated Na^+ channels close and voltage-gated K^+ channels open, allowing K^+ ions to flow out of the cell (Figure 2.2 d). This return of K^+ to the extracellular space helps restore the negative membrane potential. During this phase, known as the absolute refractory period, the neuron is unable to fire another AP because the voltage-gated Na^+ channels remain inactive. Once these channels reset, the neuron enters the relative refractory period, during which a stronger-than-usual stimulus is required to trigger a new AP. To restore the balance of Na^+ , K^+ , and Ca^{2+} ions inside and outside the cell, the neuron relies on Na^+/K^+ -adenosine triphosphate (ATP) and Ca^{2+} pumps, which use energy from ATP to re-establish the ion gradients (Koester & Siegelbaum, 2012b). This careful regulation ensures that the neuron maintains the ion gradients necessary for repeated and continuous firing of APs over time. While a single AP can propagate along the axon without immediate restoration of equilibrium, these homeostatic mechanisms are essential for sustaining neural activity during prolonged signaling. The process is further enhanced by the myelin sheaths insulating the axon, which allow the signal to "jump" between nodes of Ranvier, speeding up transmission through a mechanism known as saltatory conduction (Koester & Siegelbaum, 2012a) (Figure 2.3).

The axon terminals of MNs form specialized synaptic contacts with muscle fibers at the neuromuscular junction (NMJ). At these junctions, the motor axon terminal branches into multiple endings that align precisely with folded regions of the muscle fiber membrane, creating a highly reliable synaptic connection. A single alpha MN can innervate varying numbers of muscle fibers, from as few as 3-5 in muscles requiring precise control (like eye muscles) to several thousand in large limb muscles (R. M. Enoke & Pearson, 2012). Together, an alpha MN and all the muscle fibers it innervates form a MU, which represents the basic functional unit of motor control. This one-to-many arrangement allows a single MN to coordinate the simultaneous contraction of multiple muscle fibers (R. M. Enoke & Pearson, 2012) (Figure 2.4).

Muscle contraction occurs at the level of the sarcomere, the functional unit of muscle fibers. Each

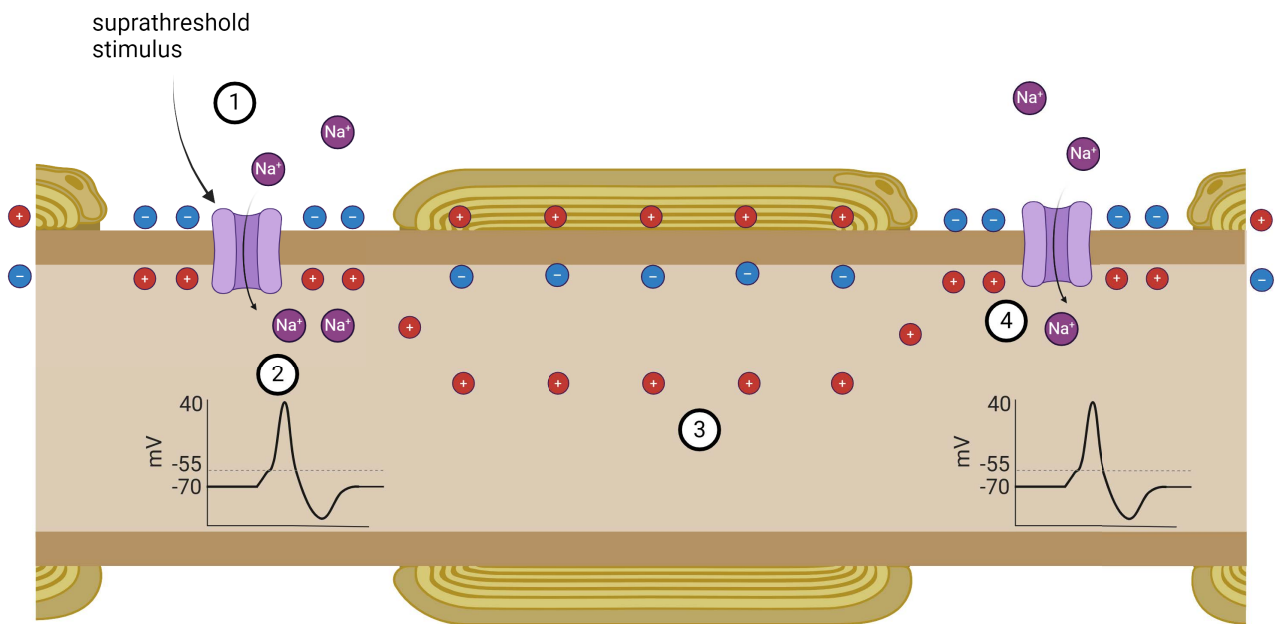


Figure 2.3: Saltatory conduction in myelinated neurons. A suprathreshold stimulus triggers Na^+ channels to open at one node of Ranvier (1), allowing Na^+ ions to flow into the axon, causing depolarization (2). During this depolarization phase, the positive change in membrane potential creates a local electrical gradient between nodes. This electrical gradient drives local circuit currents through the axoplasm to the next node (3), while the myelin sheath provides high resistance insulation, preventing current leakage and ensuring efficient signal propagation. When the local circuit current reaches the next Node of Ranvier, it causes initial depolarization. Once this depolarization reaches threshold, Na^+ channels open, triggering a new AP (4). Meanwhile, the previous node undergoes repolarization as K^+ channels open and Na^+ channels inactivate. This saltatory ("jumping") conduction repeats along successive nodes, enabling rapid and energy-efficient signal transmission along the axon. Created with BioRender.

sarcomere, bounded by Z disks, ranges in length from $1.5\ \mu\text{m}$ to $3.5\ \mu\text{m}$ and is composed of thick and thin filaments that interact to generate force. Sarcomeres are arranged in series to form myofibrils, which in turn align in parallel to form a muscle fiber. The thick filament is made up of myosin molecules, with each molecule consisting of paired coiled-coil domains ending in two globular heads. These heads protrude from the filament and interact with the surrounding thin filaments. Each thick filament is surrounded by six thin filaments, which consist primarily of F-actin, a helical strand of fibrous protein. Superimposed on the F-actin are regulatory proteins, tropomyosin and troponin, which control the interaction between actin and myosin.

The thin filaments are anchored at the Z disk, while the thick filaments occupy the center of the sarcomere (Figure 2.5 a). This arrangement creates the characteristic light and dark bands of striated muscle: the light bands contain only thin filaments, while the dark bands contain both thick and thin

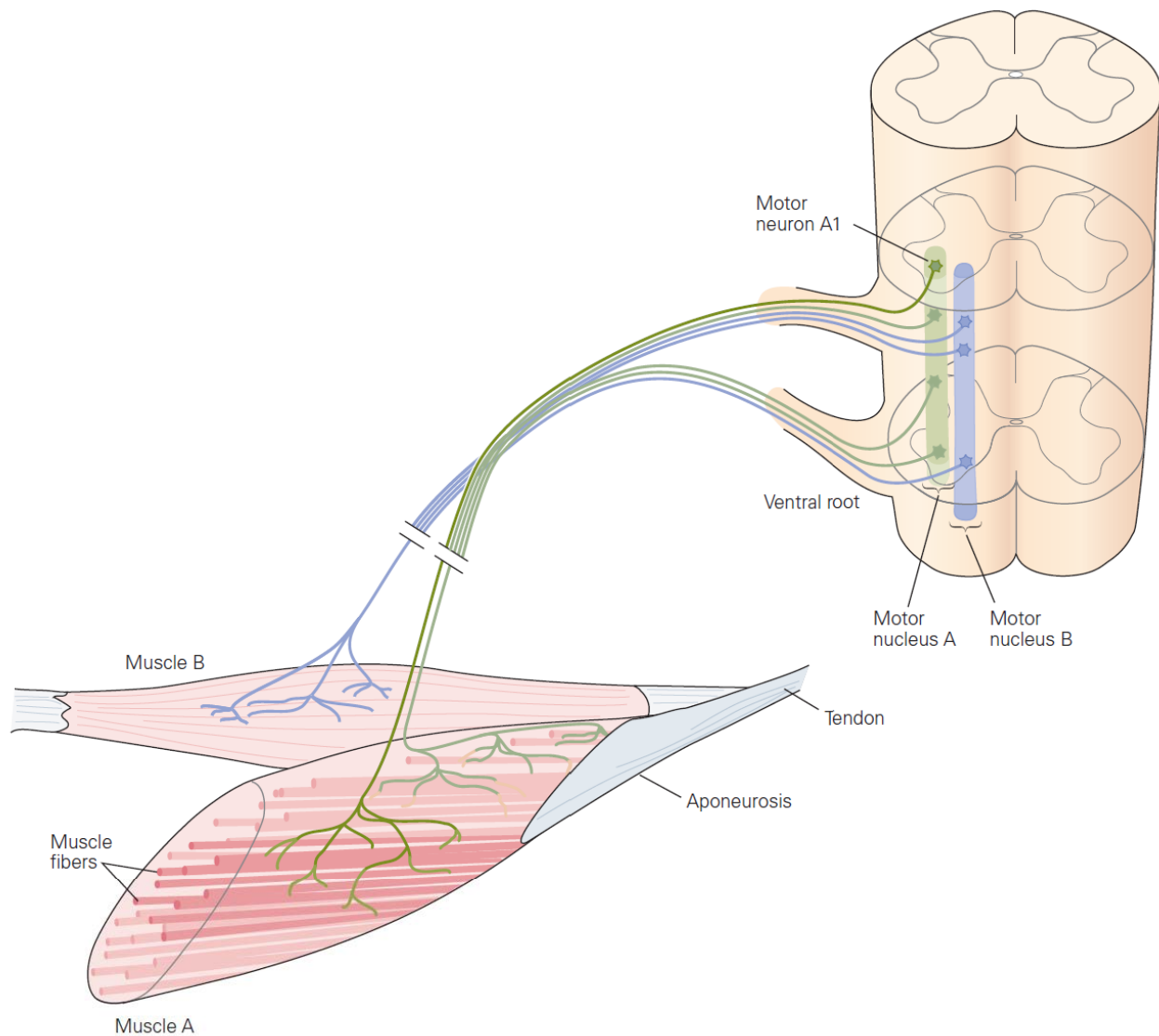


Figure 2.4: Neuromuscular system. A MU consists of a MN (e.g., MN A1) and the muscle fibers it innervates. The MNs innervating one muscle cluster in the spinal cord into a motor nucleus. The axons from a motor nucleus exit the spinal cord and are collected into one nerve bundle near the innervated muscle. Reproduced with permission from Enoka et al. (2012).

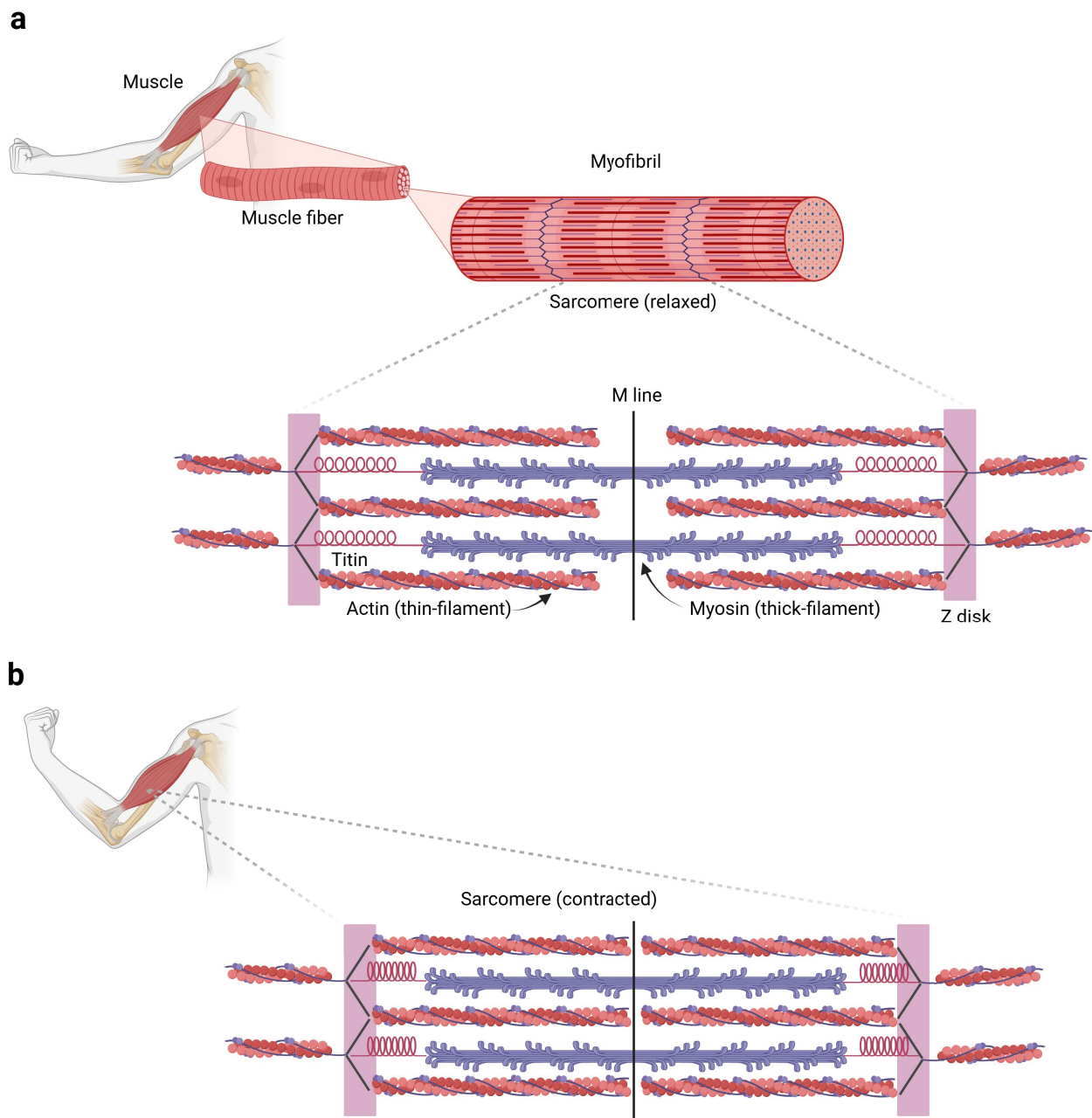


Figure 2.5: Hierarchical organization of skeletal muscle and contractile mechanism. **a** Relaxed state. Skeletal muscle structure showing its nested arrangement, from the whole muscle down to its fundamental contractile unit. The muscle consists of bundled muscle fibers, each containing multiple myofibrils. Myofibrils are composed of repeating sarcomeric units, which contain interdigitating thick (myosin) and thin (actin) filaments. **b** Contracted state. Sarcomere configuration during muscle contraction. Created with BioRender.com.

filaments. During muscle contraction, the thick and thin filaments slide past each other (Huxley & Niedergerke, 1954), shortening the sarcomere without changing the width of the dark band (Figure 2.5 b).

The sliding of the filaments is triggered by the release of Ca^{2+} into the sarcoplasm, which occurs in response to an AP traveling along the sarcolemma, the membrane of the muscle fiber. This AP prompts the release of the neurotransmitter acetylcholine (ACh) into the synaptic cleft via exocytosis of synaptic vesicles at the neuromuscular junction. The binding of ACh to receptors on the muscle fiber's membrane generates an excitatory postsynaptic potential, which is typically large enough to activate Na^+ channels, thereby producing an AP along the sarcolemma. This intracellular AP follows a monophasic profile when measured relative to the extracellular space and is referred to as the intracellular AP, while the corresponding extracellular potential constitutes the single fiber action potential (SFAP).

As the intracellular AP propagates through the sarcolemma, it triggers the release of Ca^{2+} from the sarcoplasmic reticulum into the sarcoplasm, initiating the excitation-contraction coupling process. The transverse tubules, sarcoplasmic reticulum, and terminal cisternae work together to rapidly release Ca^{2+} from storage sites in the terminal cisternae into the sarcoplasm. Once in the sarcoplasm, Ca^{2+} binds to troponin, displacing the troponin-tropomyosin complex, initiating the sliding of the thick and thin filaments. The strength of muscle contraction depends on the frequency of APs, as a single AP does not release enough Ca^{2+} to saturate all troponin binding sites.

The mechanical work of muscle contraction is performed by the globular heads of myosin through a process known as the *cross-bridge* cycle. This cycle, fueled by ATP, involves the myosin heads attaching to, pulling on, and releasing the actin filaments, with each cycle resulting in a 5 to 10 nm displacement. The contraction continues as long as sufficient levels of Ca^{2+} and ATP are present in the sarcoplasm.

The transmission of signals from nerve to muscle is highly reliable, even during extreme voluntary exertion (S. J. Wood & Slater, 2001). This reliability is due to the fact that the motor nerve terminal releases more ACh than is required to initiate an AP in the muscle fiber. This excess of neurotransmitter ensures that neuromuscular transmission remains effective, even under conditions of

prolonged, high-frequency stimulation. This mechanism is known as the safety factor (or safety margin), a term used to describe the additional capacity for neurotransmitter release that guarantees successful signal transmission at the NMJ (S. J. Wood & Slater, 2001).

The robust nature of AP transmission at the NMJ means that MNs and their innervated muscle fibers function essentially as a single unit, known as the MU (Liddell & Sherrington, 1925). The motor unit action potential (MUAP) represents the combined electrical activity of all SFAPs controlled by a single MN (Figure 2.6). These MUAPs are amplified versions of the original neural signals, occurring with a brief delay (≈ 1 ms) (Katz & Miledi, 1965), and maintain a strict one-to-one relationship with MN firings (R. Enoka & Duchateau, 2016). This natural amplification by muscle fibers allows electrodes, whether intramuscular or surface-mounted, to detect the spatio-temporal summation of nearby MUAPs. The detected signals are attenuated by intervening tissues—muscle fibers and connective tissue for intramuscular recordings, or multiple tissue layers including muscle, adipose tissue, and skin for surface recordings—resulting in the EMG signal (R. Enoka & Duchateau, 2016; Merletti et al., 2001). Modern signal processing techniques can now extract individual MN firing patterns from the EMG signals, effectively creating a window into the CNS's output. The temporal relationship between muscle activation and force generation is crucial, with approximately 200 ms of neuromechanical delay between the muscle fiber AP and force production (Del Vecchio et al., 2018). This delay establishes the maximum acceptable processing time for decoding algorithms, as longer delays result in perceptible lag in action execution (Farrell & Weir, 2007).

2.1.2 Force Modulation

The force generated by a muscle is fundamentally determined by two factors: the number of cross-bridges formed and the force each cross-bridge produces (R. M. Enoka & Pearson, 2012). This mechanical output is precisely modulated through multiple physiological mechanisms that operate at both the cellular and neural levels (Lieber & Ward, 2011).

At the cellular level, force production is governed by the length-force relationship, which depends on the geometric arrangement of myosin and actin filaments within the sarcomere. Maximum force

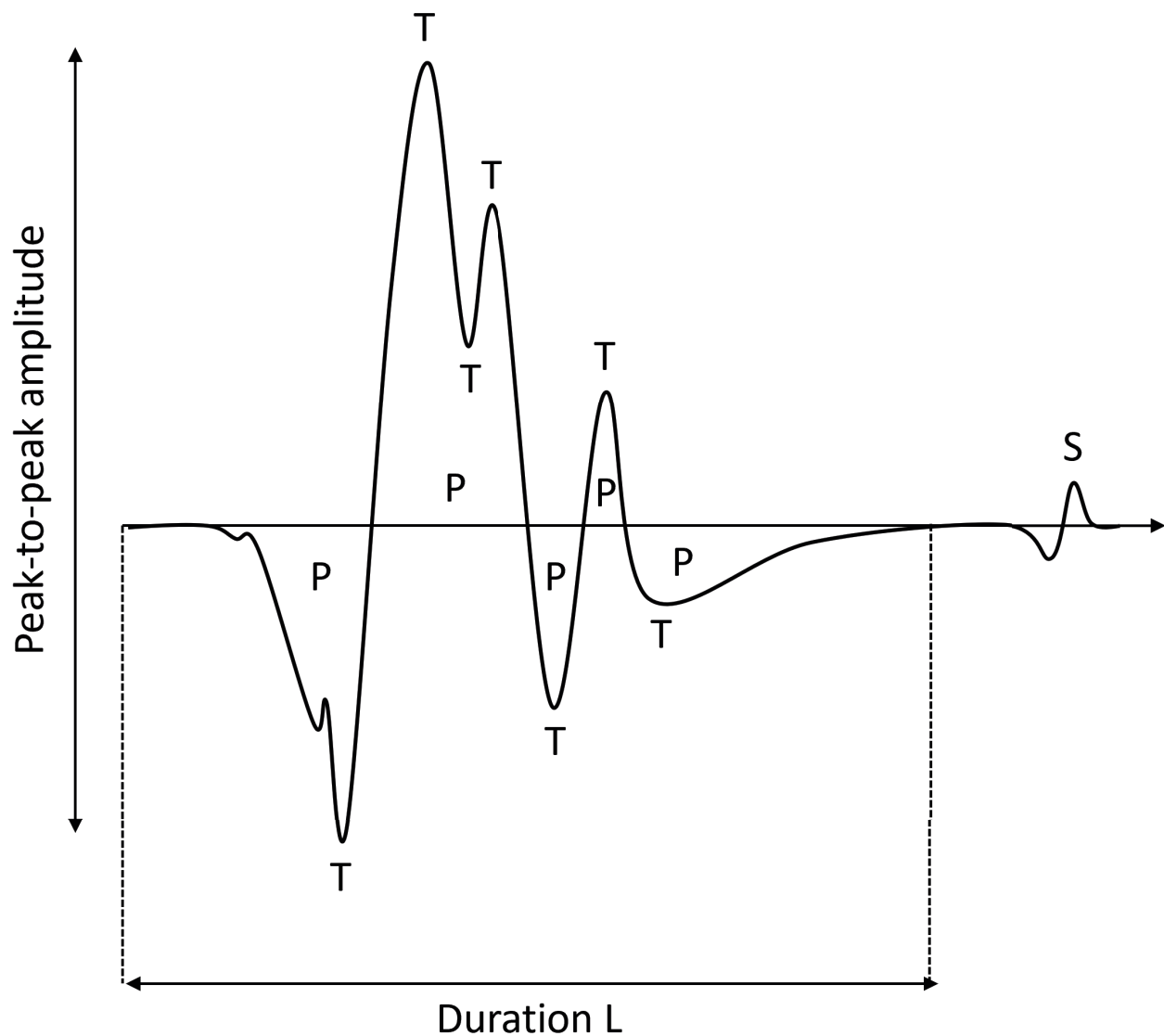


Figure 2.6: Intramuscular MUAP. The MUAP parameters describe the temporal coordination of muscle fiber activation and their conduction velocities. The phases (P) and turns (T) reflect the degree of asynchrony in the firing of muscle fibers. The peak-to-peak amplitude of the main spike is mainly influenced by the closest muscle fibers, while the duration correlates with the number of fibers detected by the electrode. A "satellite" (S) component, a delayed waveform, can be produced by a muscle fiber that is either innervated by a slowly conducting terminal axon or conducting more slowly due to muscle atrophy. Figure inspired by (Trontelj et al., 2004).

occurs at intermediate sarcomere lengths where cross-bridge formation is optimal, while force diminishes when muscles are either too shortened or overstretched (R. Enoka & Duchateau, 2016). Notably, this optimal length for peak force production is not static but shifts based on the MN's activation rate, with lower firing rates resulting in peak force at longer sarcomere lengths. This relationship remains consistent across different MUs, reflecting a fundamental property of sarcomere dynamics (R. Enoka & Duchateau, 2016; Filippi & Troian, 1994). While MUs typically operate on the ascending and plateau regions of the length-force curve, the relationship can be modified by the history of contractile activity (Herzog et al., 2010).

The force-generation process becomes more complex during dynamic movements, as force output is significantly influenced by contraction velocity. During muscle shortening, force decreases due to reduced cross-bridge formation and cycling efficiency, as cross-bridges have less time to complete their cycle before further shortening occurs. Conversely, during lengthening contractions, force increases due to enhanced Ca^{2+} release from the sarcoplasmic reticulum, improved cross-bridge cycling efficiency, and increased work per cross-bridge (Colombini et al., 2007). This velocity-dependent response varies among MUs, with slower-contracting units showing a more rapid force decline during shortening compared to faster units, leading to different optimal velocities for power generation (Petit et al., 1993).

At the neural level, force is controlled through two primary mechanisms: recruitment and rate coding of MUs (Duchateau & Enoka, 2011). Following the size principle (Henneman, 1985), smaller MNs innervating weaker muscle units with longer contraction times are recruited first, followed by progressively larger MNs controlling stronger, faster-contracting units (Henneman, 1957). This orderly recruitment pattern stems from differences in MN size, as smaller neurons require less current to reach their voltage threshold. Rather than classifying MUs as simply fast or slow, which is considered unreliable (Burke et al., 1973), they are better differentiated by their recruitment threshold—the force level at which they begin firing APs repetitively during gradual force increases. The upper limit of MU recruitment varies across muscles in the range of 50% to 90% maximum voluntary contraction (MVC) (R. Enoka & Duchateau, 2016). However, whilst the MNs tend to be recruited in order, their de-recruitment threshold is variable (Heckman & Enoka, 2012). While most MUs are recruited at lower forces, higher force production increasingly relies on rate coding—the modulation

of MN discharge rates (Heckman & Enoka, 2012).

In addition to this orderly recruitment pattern, the discharge rate of MN APs serves as a crucial secondary neural mechanism for force modulation through its effect on intracellular Ca^{2+} concentration, which directly influences cross-bridge formation probability (Heckman & Enoka, 2012). This rate coding mechanism is highly adaptable, with discharge patterns varying based on both contraction type and task demands. During slow isometric force production, peak discharge rates typically range from 30-50 pulses per second (pps) (R. Enoka & Duchateau, 2016), but can exceed 120 pps during rapid, ballistic contractions (Desmedt & Godaux, 1979; Van Cutsem & Duchateau, 2005). The relationship between recruitment order and discharge rate follows a systematic pattern: during moderate force production, earlier-recruited units achieve higher discharge rates, complementing their lower recruitment thresholds, but this pattern reverses near maximal voluntary contraction (Moritz et al., 2005; Oya et al., 2009). Movement type also significantly influences rate coding; shortening contractions require higher discharge rates and additional unit recruitment due to reduced force capacity, while lengthening contractions exhibit lower discharge rates due to enhanced force production capabilities (Duchateau & Enoka, 2008). During sustained, fatiguing contractions, the CNS employs complex adjustments, including concurrent decreases in active unit discharge rates while recruiting additional units (Carpentier et al., 2001). These adaptations reflect the intricate interplay between ionotropic and neuromodulatory inputs, rather than voluntary control strategies (Bawa & Murnaghan, 2009; Westad et al., 2003), highlighting how recruitment and rate coding work in concert to achieve precise force control.

2.2 Electromyography

The EMG signal captures the combined electrical potential generated by all active MUs as they evolve across the muscle in space and time. EMG can be recorded invasively using intramuscular electrodes (Adrian & Bronk, 1929; Muceli et al., 2022) or non-invasively from the skin surface (Merletti & Hermens, 2004). The difference between the two acquisition modalities is given by the distance between the sources (the muscle fibers) and the recording points (the electrodes) (Merletti & Farina, 2009).

The following subsections will outline the key differences between these two recording techniques, including the specific information each method provides and the inherent limitations of both approaches. Afterward, the generative model of the EMG will be explained, followed by a brief history of decomposing EMG signals into their constituent MNs, leading into a more detailed exploration of blind source separation (BSS) methods.

2.2.1 Recording Modalities

Nearly a century ago, Adrian and Bronk (1929) introduced the concentric needle electrode, marking the beginning of *in vivo* MU investigations in humans (Farina & Enoka, 2023). Since then, both intramuscular and surface recording techniques have evolved significantly, each offering distinct advantages and limitations in studying muscle activity.

The evolution of intramuscular recordings has seen several key developments. The original concentric needle electrode, which recorded signals between an insulated wire tip and metal cannula, was expanded by Buchthal et al. (1957) to include 12 insulated wires, increasing the detectable area inside the muscle. De Luca & Forrest (1972) later introduced the quadripolar needle electrode, providing four distinct perspectives of the same MU. To address limitations of the concentric needle such as pain and movement-related recording variability, wire electrodes were developed (Basmajian, 1962), though these presented their own challenges in repositioning and creating consistent multi-channel detection systems (Merletti & Farina, 2009).

A fundamental limitation of these early systems was their low spatial sampling due to the low number of channels (Figure 2.7 **b**). To address this issue, recent advances in thin-film technology have revolutionized intramuscular recordings (Farina et al., 2008; Yoshida et al., 2000), culminating in flexible electrodes with 16 or 40 detection sites that establish the first instances of really high-density intramuscular EMG (HD-iEMG) systems (Muceli et al., 2015, 2022), now being adapted for animal studies (B. Chung et al., 2023) (Figure 2.7 **d**). However, a systematic investigation into channel configuration, optimal interelectrode distance (IED) spacing, and the necessary number of channels to optimize the amount of information recorded has yet to be conducted. In this thesis, all original work utilized the 40-channel micro-electrode arrays developed by Muceli et al. (2022).

Parallel to these developments, surface EMG (sEMG) techniques emerged as non-invasive alternatives, particularly gaining prominence from the 1980s (Farina & Enoka, 2023; Masuda et al., 1985). The progression from simple monopolar electrodes (Merletti & Hermens, 2004) to bipolar configurations (Campanini et al., 2022) and eventually to sophisticated high-density (HD) arrays has recently led to modern innovations like wearable sleeves (Tacca et al., 2024; Varghese et al., 2024) and tattoo-like electrodes (Chandra et al., 2020; Liu et al., 2020; K. Yang et al., 2019). sEMG systems using conductive gel have proven superior to dry electrode systems in maintaining stable skin-electrode contact and reducing movement artifacts (Lapatki et al., 2004). Modern HD arrays enable sampling over large surface areas, providing two spatial dimensions of muscle activity measurement alongside the temporal dimension. Figure 2.7 shows a schematic of the detection area by the bipolar sEMG (**a**) and the high-density surface EMG (HD-sEMG) systems (**b**) on the cross section of the forearm.

The fundamental differences between these recording modalities manifest in their respective advantages and limitations. Intramuscular EMG (iEMG) provides superior bandwidth (exceeding 1 kHz) (Merletti & Farina, 2009) and more reliable detection of individual MU discharge patterns (LeFever & De Luca, 1982) due to minimal low-pass filtering effects from direct muscle fiber contact. It enables access to deep muscles and offers diagnostic capabilities through AP shape analysis. However, its high selectivity means recordings typically capture only nearby active MUs, potentially providing an incomplete picture of total MU activity.

In contrast, sEMG offers indirect measures of the neural drive to muscles but faces several inherent challenges: low specificity, known as crosstalk (De Luca & Merletti, 1988; Farina, Merletti, Indino, et al., 2002; Solomonow et al., 1994), amplitude cancellation (Keenan et al., 2005), high levels of MUAP overlaps (Farina, Merletti, & Enoka, 2014), and volume conductor effects on signal amplitude and power spectrum (Farina, Cescon, & Merletti, 2002; Farina, Fosci, & Merletti, 2002), limiting the signal bandwidth to approximately 400 Hz (Merletti & Farina, 2009).

Recent advances in both technologies have begun to address their respective limitations. HD-iEMG systems have expanded the detection volume of intramuscular recordings, while improvements in surface electrode design and signal processing continue to enhance the capability of non-invasive approaches. These parallel developments offer complementary tools for understanding neural con-

trol of movement in both clinical and research applications (Figure 2.8).

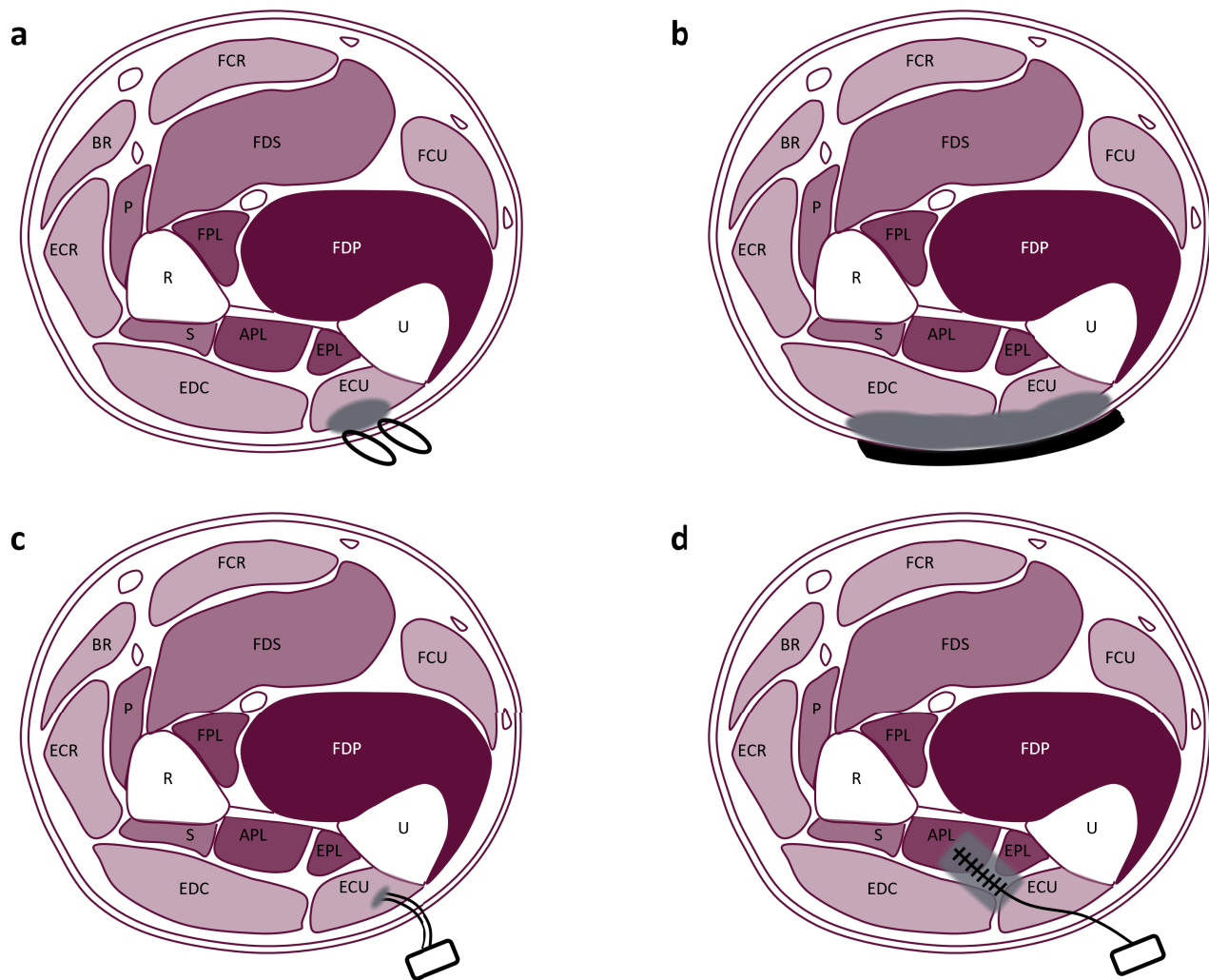


Figure 2.7: Detection area (shaded in grey) for different recording modalities. **a** Bipolar sEMG: Captures electrical activity from a limited area of superficial muscles, demonstrated here using the Extensor Carpi Ulnaris (ECU) as an example. **b** HD-sEMG: Expands detection to cover multiple superficial muscles, such as the extensor digitorum communis (EDC) and ECU, while providing higher spatial resolution. **c** Bipolar iEMG: Records electrical activity directly from muscle fibers with high spatial selectivity, targeting only a few muscle fibers. **d** HD-iEMG: Also records directly from muscle fibers but with lower spatial selectivity, as it covers a broader area using multiple channels.

2.2.2 Electromyography Generative Model

EMG electrodes capture the electrical activity of multiple muscle fibers that are active during a muscle contraction (Farina & Holobar, 2016). The electrical signal generated by each muscle fiber is characterized by a SFAP, which propagates from the NMJ towards the tendons (Farina & Merletti, 2004; Harris et al., 2005; Ruff, 2003). As the SFAP travels, its amplitude attenuates with increasing

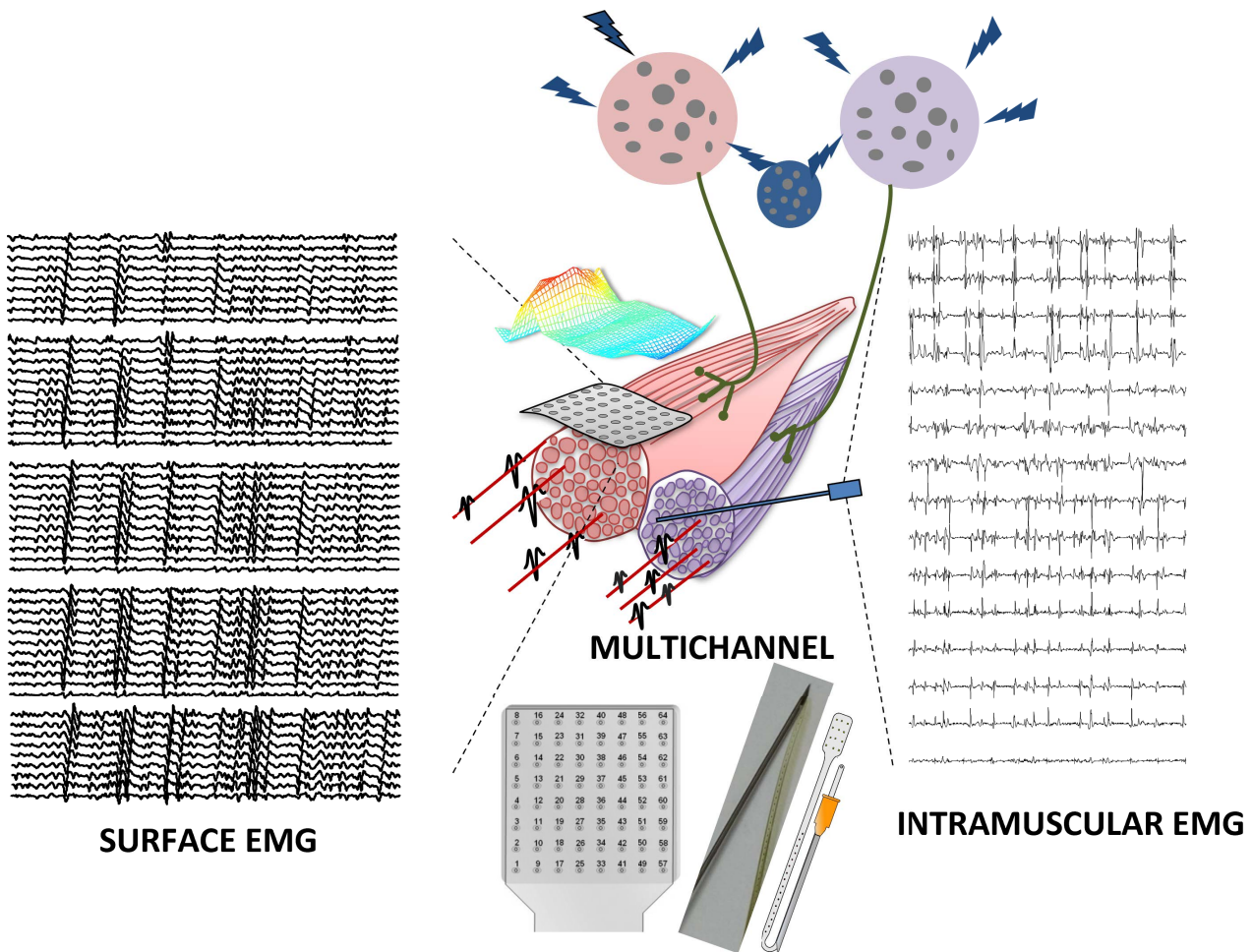


Figure 2.8: Schematic of recording modalities. Simplified pathway from motor cortex to muscle, illustrating HD-sEMG on the muscle surface and HD-iEMG within the muscle, visually highlighting differences in the characteristics of the signals. Adapted with permission from Negro et al. (2016).

distance from the fiber, primarily due to the diffusion of the signal through the surrounding tissues, which act as a passive volume conductor (Albers et al., 1989; Van Veen et al., 1994). Consequently, the SFAP recorded by the electrode exhibits spatial dependence, influenced by the distance between the electrode and the muscle fiber, as well as the conductivity of the interposed tissue layers.

The SFAPs belonging to the same MU propagate approximately synchronously along the muscle fibers with the same conduction velocity, influenced by the scatter of the NMJs within the innervation zone of the MU. As such, they can be considered phase-locked and mathematically modeled as a common response to a single MU discharge. Therefore, at any point on the muscle or skin surface, the SFAPs of individual MUs can be assumed to sum together, forming an L -sample-long MUAP:

$$\mathbf{h}_{ij} = [h_{ij}(0) \ h_{ij}(1) \dots h_{ij}(L-1)] \quad (2.1)$$

where $h_{ij}(l)$ is the l th MUAP sample of the j th MU, as detected by the i th uptake electrode. The shape of MUAPs change across different recording points due to the combined influence of multiple spatially filtered SFAPs. This variability is mainly caused by the in-homogeneous properties of the volume conductor and the varying distances between the muscle fibers and the electrodes. As a result, the MUAPs recorded by different electrodes are not simple scaled or temporally shifted versions of each other, but have distinct waveforms. Additionally, factors like muscle fatigue, MU firing rates, and changes in muscle geometry during non-isometric contractions further affect the MUAP shape.

The recorded EMG signals arise from the spatial and temporal summation of the contributions from all excited muscle fibers, irrespective of their originating MU. Such an aggregate signal can be conceptually understood as a cumulative summation of motor unit action potential trains (MUAPTs) from all activated MUs, providing an overview of the MU activation status and the resulting muscle response.

As a result, the EMG, recorded either invasively or non-invasively, is typically modelled by the time-varying convolution of MUAP shapes:

$$x_i(t) = \sum_{j=1}^N \sum_{k=1}^K \mathbf{h}_{ij,k} * \delta(t - \tau_j(k)) + \xi_i(t), \quad i = 1, \dots, M, \quad t = 1, \dots, T \quad (2.2)$$

where $*$ stands for convolution, $x_i(t)$ is the EMG signal recorded at location i , M is the number of uptake electrodes, t is continuous time, T is the total duration of the recording, δ is the unit-sample pulse, $\xi_i(t)$ is the additive noise and the k th MUAP of the j th MU appears at time $\tau_j(k)$, and N is the number of MUs generating the EMG signal.

As shown in Equation 2.2, the discharge patterns of the MUs can be modeled as delta trains, where a '1' indicates a discharge and a '0' indicates no discharge at that time point (Holobar & Zazula, 2007a;

Holobar & Farina, 2014). Due to the inherent properties of the neuromuscular system (Merletti & Parker, 2004), such as the refractory periods, these spike trains are sparse, with each spike typically followed by several zeros. All anatomical characteristics of each detected MU, such as its position within the muscle, the orientation of its fibers, and its conduction velocity, are modeled by $\mathbf{h}_{ij,t}$. Additionally, this term incorporates the properties of the volume conductor, such as the thickness and in-homogeneities of the subcutaneous tissue and its conductivity, as well as the parameters of the detection system, such as the shape and size of the electrodes, the distance between them, and their configuration.

Frequently, and throughout this thesis, the EMG is measured in isometric conditions. This is a convenient approach to interpret EMG activity, as it reduces the variations of detected MUAP shapes due to changes in muscle geometry. Moreover, by assuming that the observed recorded time is short enough to limit the effect of muscle fatigue, and there are no other parameters that modify the shapes of MUAPs, the detected MUAP shapes can be assumed stationary.

The EMG can then be approximated by a linear time-invariant convolution multiple-input-multiple-output (MIMO) model, with the i th output defined as:

$$x_i(t) = \sum_{j=1}^N \mathbf{h}_{ij} * \delta(t - \tau_j) + \xi_i(t) \quad (2.3)$$

$$= \sum_{j=1}^N \sum_{l=0}^{L-1} h_{ij}(l) s_j(t - l) + \xi_i(t), \quad i = 1, \dots, M, \quad t = 1, \dots, T \quad (2.4)$$

where $h_{ij}(l)$ is the MUAP of the j th MU as recorded at the i th location, L is the duration (in samples) of the MUAPs, N is the number of MUs, and

$$s_j(t) = \sum_r \delta(t - \tau_{jr}), \quad j = 1, \dots, N \quad (2.5)$$

is the spike train of the j th MU where the r th spike occurs at time τ_{jr} ,

The EMG signal can be compactly expressed in matrix form as:

$$\mathbf{x}(t) = \sum_{l=0}^{L-1} \mathbf{H}(l)\mathbf{s}(t-l) + \boldsymbol{\xi}(t) \quad (2.6)$$

where $\mathbf{x}(t) = [x_1(t), x_2(t), \dots, x_M(t)]^T$ is the vector of M EMG channels, $\mathbf{s}(t) = [s_1(t), s_2(t), \dots, s_N(t)]^T$ represents the N MU spike trains, and $\boldsymbol{\xi}(t)$ is a vector with different noise realizations per recording channel. The matrix $\mathbf{H}(l)$ has size $M \times N$ and contains the l th sample of the L -sample-long MUAP waveforms that appear for N MUs across M channels. A schematic representation of the convolution between the discharge patterns of two MUs and their MUAP shapes is presented in Figure 2.9. The general EMG convolutive model is represented in Figure 2.10 and Figure 2.11.

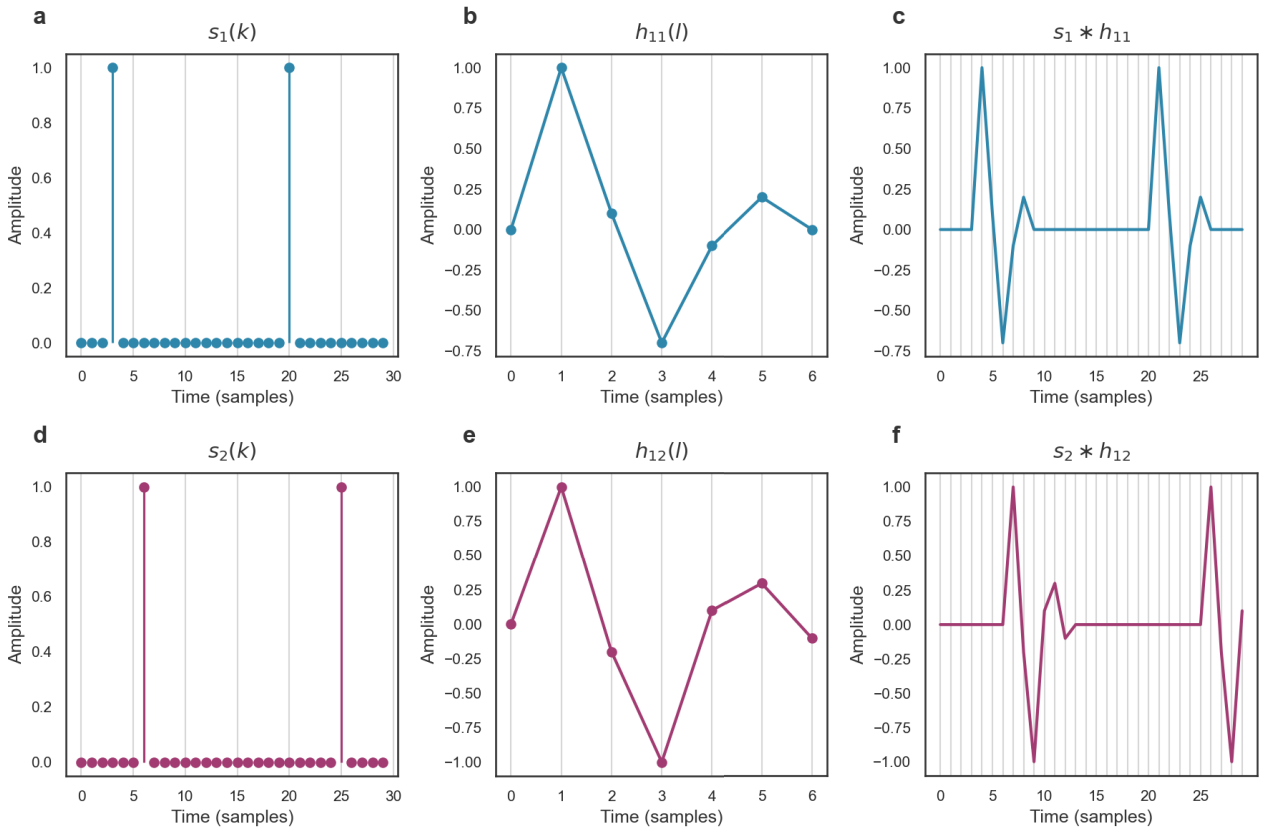


Figure 2.9: Schematic representation of the discharge times and the produced MUAPs. Source signals s_1 and s_2 each discharge with an initial 'one' followed by a sequence of zeros (**a**, **d**). The corresponding MUAPs shapes for each source are illustrated in **b** and **e**. The convolutions between the sources and the MUAPs are shown in **c** and **f**. For clarity of visualization, time is expressed in samples $k = 0, \dots, Duration$.

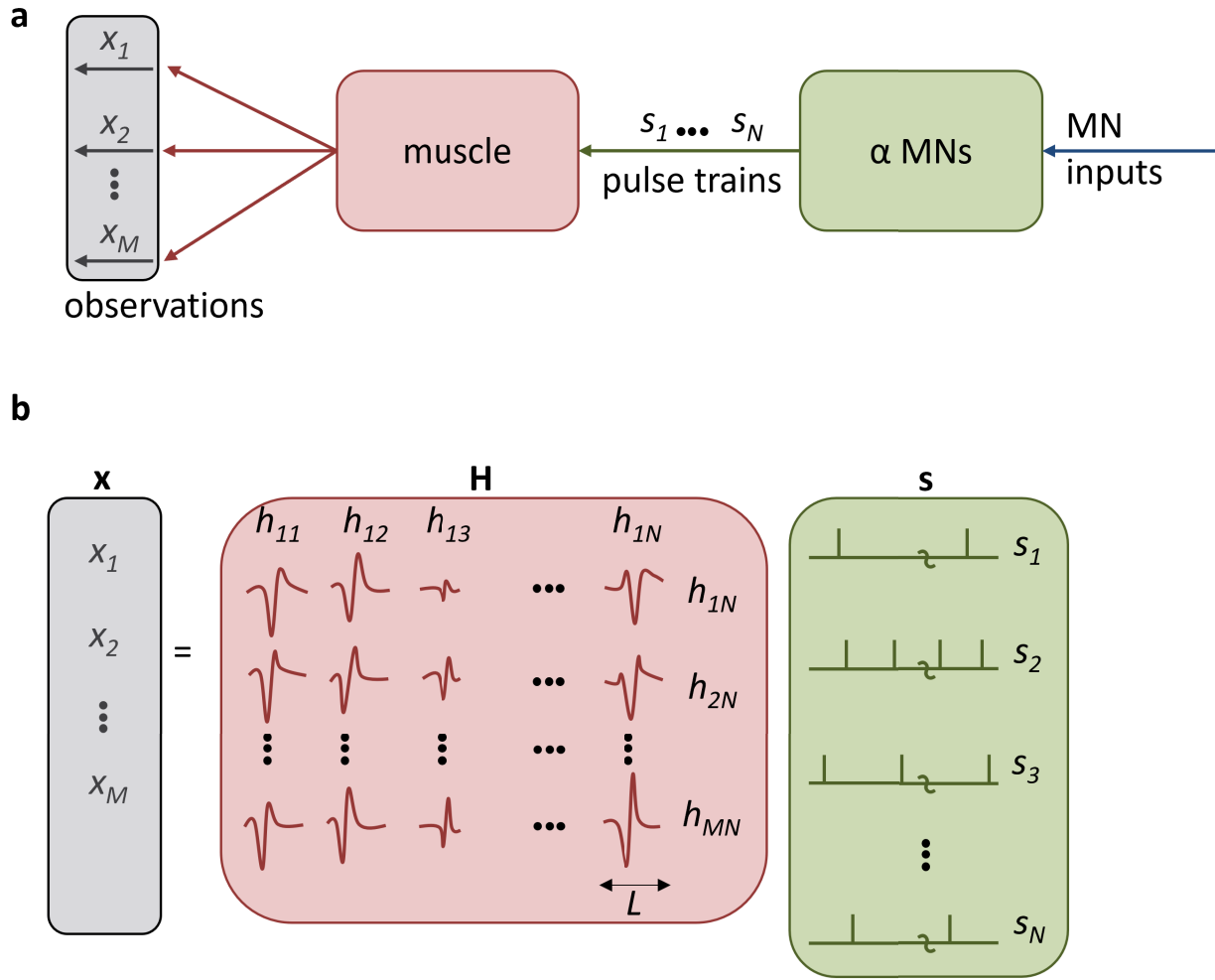


Figure 2.10: EMG convolutive model. **a** N MNs initiate electrical activity in muscle fibers, with MUs distributing and amplifying neural drive signals. **b** The activity of alpha MNs is represented as a sequence of discrete delta functions, which MUs convolve with their specific shape, i.e., the L -sample long MUAPs. The volume conductor serves as a low-pass filter, leading to variations in MUAPs shapes recorded across the M electrodes, and combined in the matrix $\mathbf{H}$.

2.2.3 Electromyography Decomposition

EMG decomposition aims to reverse the mixing process described in Equation 2.3 to retrieve the underlying sources from the observed signals. Various methods have been developed to address this challenge, and they can generally be divided into two main categories: template matching techniques and latent component analysis approaches, the latter also referred to as BSS. This section provides an overview of the main methods used for both template matching and BSS approaches. The emphasis will be on BSS, as it is the method utilized in the remaining chapters of this thesis.

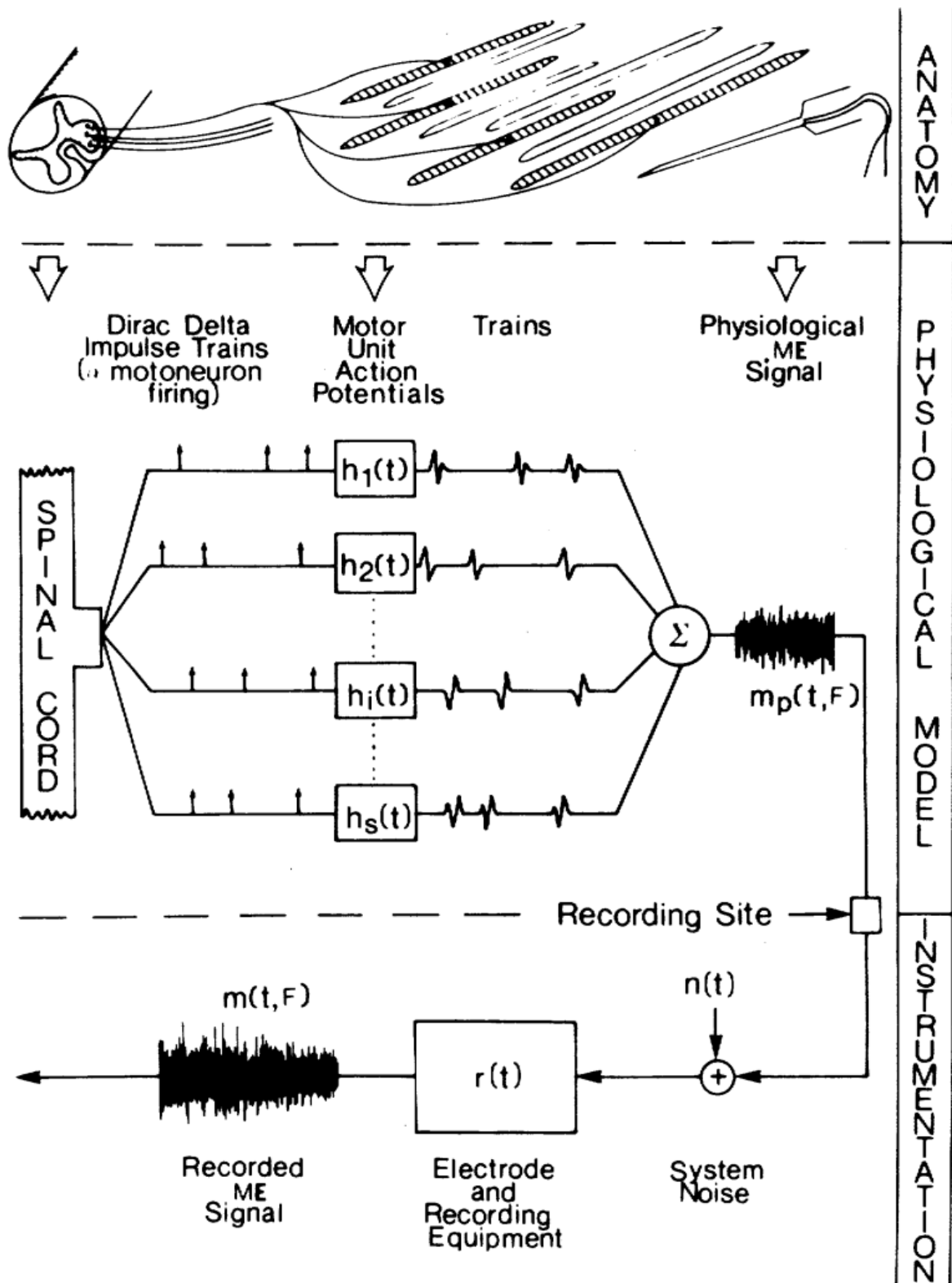


Figure 2.11: Schematic of EMG model. Figure reproduced with permission from De Luca (1979).

2.2.3.1 Template Matching

Template-matching approaches were initially developed for iEMG signals and typically consist of two key steps: detecting APs (segmentation) and clustering of the APs into distinct MU classes (classification) (Merletti & Farina, 2009). Ideally, complete EMG decomposition would detect all MUAPs generated by active MUs. However, in practice, smaller, low-frequency MUAPs from distant muscle fibers are often difficult to detect and may be missed, especially when they occur near larger MUAPs. As a result, decomposition algorithms tend to focus on reliably detectable MUAPs, ensuring more accurate classification. Detection methods usually involve calculating a statistic for each signal sample and comparing it to a threshold (D. W. Stashuk et al., 2004). Common approaches include amplitude (Christodoulou & Pattichis, 1999; Fang et al., 1999; LeFever & De Luca, 1982) or variance-based (Nikolic et al., 1997; Wellig & Moschytz, 1999; Wellig et al., 1998) detection of the raw or bandpass-filtered signal, or a combination of both raw signal slope and amplitude (Stålberg et al., 1995; D. Stashuk & De Bruin, 1988).

In the classification step, APs from the same MU must exhibit greater similarity in shape than those from different MUs to allow accurate clustering and differentiation between MUs. For automatic recognition, the detected MUAPs are represented as feature vectors in a multidimensional feature space, enabling the use of pattern recognition algorithms (D. W. Stashuk et al., 2004). To quantify these shape differences, various morphological features such as peak-to-peak voltage, number of phases, duration, and number of turns have been used (Loudon et al., 1992; Pattichis et al., 1995; Stålberg et al., 1995). Additional approaches have employed feature spaces based on Fourier transform coefficients (McGill et al., 1985; D. Stashuk & De Bruin, 1988), wavelet-based representations (Fang et al., 1999; Wellig et al., 1998), time samples of bandpass-filtered signals (Gerber et al., 1984; Nikolic et al., 1997), and lowpass-differentiated signal time samples (D. W. Stashuk, 1999).

When multiple MUs discharge simultaneously or in close succession, a superimposed MUAP is detected, which represents the algebraic summation of the individual MUAPs. To simplify the decomposition process, most algorithms first classify MUAPs that do not overlap in time. To resolve the superimposed MUAPs, two main strategies are commonly employed: the peel-off approach and the modeling approach. The peel-off method identifies the best match between a superimposed wave-

form and an AP from the classification phase, subtracting it to create a residual waveform, and repeating the process until the residual energy falls below a predefined threshold (LeFever & De Luca, 1982). Alternatively, the modeling approach constructs a superimposed waveform by combining AP templates shifted in time and searches for the best match between the synthesized and actual waveforms. While the modeling approach is theoretically capable of resolving all types of superimposed waveforms, it is computationally more intensive. Resolving superimposition is a major challenge in template-matching approaches, meaning that most of the signals that can be resolved need to be recorded at low-to-medium force contractions, as to minimize the amounts of overlaps in the signal (D. W. Stashuk et al., 2004).

The most common software for manual decomposition of intramuscular signals is EMGLAB (McGill et al., 2005), which integrates standard template-matching steps into an interactive program (Florestal et al., 2009). Validated for single and multi-channel recordings (6-8 channels), EMGLAB was shown to accurately decompose 75% of manually processed MUs with over 95% accuracy (Florestal et al., 2009). In parallel, template matching has been successfully applied in spike-sorting, with KiloSort (Pachitariu et al., 2016) being the most prominent algorithm. KiloSort has evolved over the years to include batch-based optimizations on GPUs and post-clustering steps to merge templates inspired by graph-based approaches (Pachitariu et al., 2016, 2024) and is showing state-of-the-art (SoA) performance for HD intracortical recordings. However, despite the importance of these methods, they come with significant limitations. They require expert intervention to re-cluster incorrectly grouped MUs, involve lengthy decomposition times, and often fail to fully resolve overlapping APs, restricting their efficacy in high-interference signals.

Although template-matching approaches were originally applied to iEMG signals, adaptations have been proposed for sEMG signals (De Luca et al., 2006; Nawab et al., 2010). However, these methods often fail when applied to high-interference EMG signals due to the low signal-to-noise ratio (SNR) caused by the low-pass filtering effect of the volume conductor, making it challenging to detect smaller MUs and resolve overlapping MUAPs (Lefebvre et al., 2016; Zhou & Rymer, 2004). This limitation is especially prominent in signals recording dynamic or high-intensity contractions (Garcia et al., 2005; Gazzoni et al., 2004; Kleine et al., 2007; Maathuis et al., 2008), preventing traditional methods from capturing the full range of MU activity. The challenges of sEMG signals, combined

with the inherent limitations of template-matching, make these approaches highly impractical for the decomposition of HD-sEMG signals.

2.2.3.2 Latent Component Analysis

A more promising direction was given by latent component analysis approaches, which invert the EMG model and retrieve the sources without any *a priori* information of the MUAP shapes. To simplify the inversion problem, the convolutional model can be transformed to an instantaneous mixture by augmenting the vector of sources to include the N original sources and their respective L delayed versions. The extension L is implicit, determined entirely by the shape of the MUAPs and inherently accounted for during the recording of the signal. To maintain a favorable ratio between the number of observations and sources, the observations are further extended by R delayed versions, which also implicitly extends the sources.

At each recording location i , the extended observation vector and the extended noise vector are:

$$\tilde{x}_i(t) = [x_i(t), x_i(t-1), \dots, x_i(t-R)], \quad i = 1, \dots, M \quad (2.7)$$

$$\tilde{\xi}_i(t) = [\xi_i(t), \xi_i(t-1), \dots, \xi_i(t-R)], \quad i = 1, \dots, M \quad (2.8)$$

After the extension of the observations, the extended instantaneous source vector for each j th MU is defined as:

$$\tilde{s}_j(t) = [s_j(t), s_j(t-1), \dots, s_j(t-(L+R-1))] \quad j = 1, \dots, N \quad (2.9)$$

Additionally, the extended MUAP $\tilde{h}_{ij}$ for the j th MU recorded at the i th channel, is defined as:

$$\tilde{h}_{ij} = \begin{bmatrix} h_{ij}[0] & \cdots & h_{ij}[L-1] & 0 & \cdots & 0 \\ 0 & h_{ij}[0] & \cdots & h_{ij}[L-1] & \cdots & 0 \\ \vdots & \ddots & \ddots & \ddots & \ddots & \vdots \\ 0 & \cdots & 0 & h_{ij}[0] & \cdots & h_{ij}[L-1] \end{bmatrix} \quad (2.10)$$

This reformulated extended instantaneous model can then be expressed as:

$$\tilde{\mathbf{x}}(t) = \tilde{\mathbf{H}}\tilde{\mathbf{s}}(t) + \tilde{\boldsymbol{\xi}}(t), \quad t = 1, \dots, T \quad (2.11)$$

where

$$\begin{aligned} \tilde{\mathbf{s}}(t) &= [\tilde{s}_1(t), \tilde{s}_2(t), \dots, \tilde{s}_f(t), \dots, \tilde{s}_N(t)]^\top \\ \tilde{\mathbf{x}}(t) &= [\tilde{x}_1(t), \tilde{x}_2(t), \dots, \tilde{x}_i(t), \dots, \tilde{x}_M(t)]^\top \\ \tilde{\mathbf{H}} &= \begin{bmatrix} \tilde{h}_{11} & \cdots & \tilde{h}_{1N} \\ \vdots & \ddots & \vdots \\ \tilde{h}_{M1} & \cdots & \tilde{h}_{MN} \end{bmatrix} \end{aligned} \quad (2.12)$$

with $\tilde{\mathbf{s}}(t)$ of size $N(L+R) \times 1$, $\tilde{\mathbf{x}}(t)$ of size $M(R+1) \times 1$ and $\tilde{\mathbf{H}}$ of size $M(R+1) \times N(L+R)$. In the absence of noise, the retrieval of discharge timings $\tilde{\mathbf{s}}(t)$ can be framed as the following inverse problem:

$$\tilde{\mathbf{s}}(t) = \mathbf{B}\tilde{\mathbf{x}}(t) \quad (2.13)$$

where $\mathbf{B}$ represents the approximate pseudo-inverse of matrix $\tilde{\mathbf{H}}$. The goal of the decomposition process is to determine the N separation vectors forming the mixing matrix.

Figure 2.12 presents a schematic of the extension of the sources. The extension of each source (refer back to Figure 2.9) by a factor L to transform the model from a convolutive to an instantaneous mixture is shown in Figure 2.12 **a, c**. The extension of the observations by the factor R is illustrated in Figure 2.12 **b, d**.

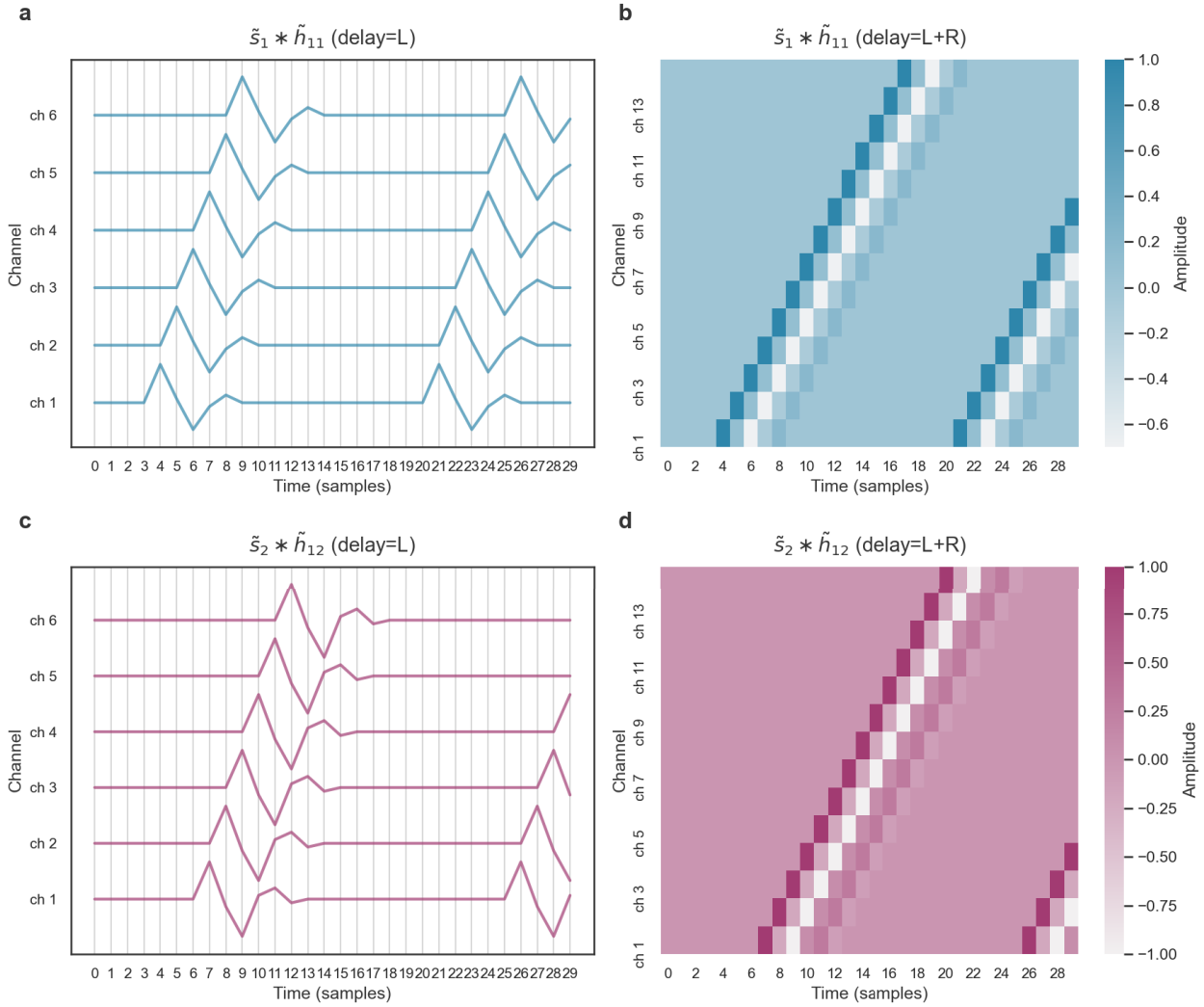


Figure 2.12: Schematic of transformation from convolutive to an instantaneous model. **a, c** The extension of the sources by factor L . In this example, $L = 6$. **b, d** The extension of the sources by factor $L + R$. For clarity of visualization, time is expressed in samples $k = 0, \dots, \text{Duration}$.

2.2.3.2.1 Independent Component Analysis

Since the model is now an instantaneous mixture of sources, independent component analysis (ICA)

approaches can be employed to extract the underlying sources. The primary assumptions of ICA are: 1) the sources are statistically independent, 2) the sources follow a non-gaussian distribution, and 3) the number of observations M is greater than or equal to the number of sources N (Hyvärinen et al., 2001f). Based on these assumptions, ICA can successfully separate the mixed signals into their independent sources. However, there are two inherent ambiguities in this separation. First, ICA cannot determine the original scaling of the sources, as multiplying a source by a constant while dividing its corresponding mixing vector by the same constant results in identical observations. Second, ICA cannot determine the original order of the sources, as permuting the order of sources while correspondingly permuting the columns of the mixing matrix produces the same observations. Therefore, while ICA recovers the independent sources, they may appear in any order and each may be scaled by an arbitrary non-zero constant (Hyvärinen et al., 2001f).

Two sources s_1 and s_2 are independent if knowing the value of s_2 does not give any information on the value of s_1 . Mathematically, this is expressed in terms of probability densities as follows:

$$p(s_1, s_2, \dots, s_N) = p_1(s_1)p_2(s_2) \dots p_N(s_N) \quad (2.14)$$

where $p_j(s_j)$ is the marginal density of the j th source and $p(s_1, s_2, \dots, s_N)$ is the joint density.

The core principle of ICA is grounded in the central limit theorem, which states that the sum of independent random variables tends to approach a gaussian distribution. By maximizing the non-gaussianity of the extracted sources, ICA is able to separate the independent components from the observed mixture (Hyvärinen et al., 2001a). The maximization of non-gaussianity as a criterion for independence is the most common formulation of ICA for solving the inverse problem.

From an EMG perspective, MN spike trains are highly sparse, producing super-gaussian distributions with positive kurtosis, where the majority of values cluster near zero, with occasional larger spikes. Although MNs are not entirely statistically independent due to the presence of common inputs within the MN pool and the concatenation of delayed versions of the sources in the model, the sparsity of spike trains, combined with the refractory period of MNs, results in weakly corre-

lated or nearly uncorrelated discharge times. Interestingly, in practice, the correlation between the original and delayed spike trains is always near-zero at time lag 0, regardless of the extension factor R . Consequently, the whitening process produces a nearly diagonal matrix for any R , as the minimal correlations between sources ensure negligible off-diagonal terms. The sparsity of the MNs and the weak correlation between delayed spike trains results in minimal overlap between spikes across different MUs (Farina & Holobar, 2016).

In this case, the concept of sparsity can be compared to independence: just as the sum of non-Gaussian sources tends toward a Gaussian distribution, the sum of sparse sources produces a less sparse signal than its individual components. For spike train sources, strict independence is not theoretically necessary; it suffices to assume that maximum sparsity occurs with a linear combination of sources (Farina & Holobar, 2016).

The impact of the MUAP length L and the extension factor R on the cross-correlation of the spike trains between the original and delayed version is minimal, provided that $L + R - 1 < \phi_j$, where ϕ_j is the average interspike interval of the j th MN (Farina & Holobar, 2016). Additionally, the extension factor must satisfy the condition $MR \geq N(L + R - 1)$, which ensures that the ratio of unextended observations M to the active sources N is sufficient (Negro et al., 2016). In practice, these conditions are typically met when using high-density systems with tens to hundreds of recording sites, where R is often set to $1000/M$. This results in extension factor values ≤ 25 , which are lower than the interspike interval of the fastest MNs (approximately 35 pps) (Negro et al., 2016). Theoretically, if one had N sources with MUAP length L (for example, $N = 100$, $L = 20$) and a large number of channels (e.g., $M = 1000$), the extension of the observations would not be necessary, as $MR \geq N(L + R - 1)$ would hold even for $R = 1$. The extension factor R is only needed to increase the number of observations relative to the number of sources, so if there were enough observations, it would not be required.

Therefore, in EMG decomposition using ICA, the algorithm maximizes the sparsity of the sources as a proxy for non-gaussianity, under the assumption that the identified sources are weakly correlated rather than fully independent. Moreover, each MN is expected to produce a unique MUAP signature (Farina & Holobar, 2016; Negro et al., 2016).

One way to quantify non-gaussianity is through the negentropy, J . Since gaussian distributions have the highest differential entropy, the negentropy is zero for gaussian variables and always non-negative for non-gaussian variables. The negentropy is defined as:

$$J(s_j) = H(s_{gauss}) - H(s_j) \quad (2.15)$$

where s_{gauss} is a gaussian source with the same covariance as s_j , and H is the differential entropy, defined as:

$$H(s_j) \approx - \int p_{s_j}(\zeta) \log p_{s_j}(\zeta) d\zeta \quad (2.16)$$

where p_{s_j} is the probability density function of s_j .

However, since direct computation of negentropy is challenging due to the need for an estimate of the probability density function, several approximations have been proposed to facilitate its use (Hyvärinen & Oja, 2000). A common approximation of the negentropy is given by the higher-order cumulants of the distribution (Hyvärinen et al., 2001a). Cumulants are statistical quantities that provide a systematic way to describe the shape characteristics of a probability distribution. Each cumulant captures a different aspect of the shape of a distribution - for instance, the first cumulant represents the mean, and the second represents the variance.

Mathematically, the i th cumulant (κ_i) is calculated using the cumulant generating function $K(\gamma)$, which is the natural logarithm of the moment-generating function:

$$K(\gamma) = \log E[e^{\gamma s_j}] \quad (2.17)$$

where $E[\cdot]$ denotes the expected value operator, and s_j represents the random variable of interest. The cumulants are the coefficients in the Taylor series expansion of $K(\gamma)$ around $\gamma = 0$:

$$K(\gamma) = \sum_{i=1}^{\infty} \kappa_i \frac{\gamma^i}{i!} = \kappa_1 \frac{\gamma}{1!} + \kappa_2 \frac{\gamma^2}{2!} + \kappa_3 \frac{\gamma^3}{3!} + \dots = \mu\gamma + \sigma^2 \frac{\gamma^2}{2!} + \dots \quad (2.18)$$

The i th cumulant is then obtained by differentiating the expansion above i times and evaluating the result at $\gamma = 0$:

$$\kappa_i = \left. \frac{d^i K(\gamma)}{d\gamma^i} \right|_{\gamma=0} \quad (2.19)$$

The derivations for the first three cumulants are:

$$\begin{aligned} \kappa_1(s_j) &= \left. \frac{dK(\gamma)}{d\gamma} \right|_{\gamma=0} \\ &= \left. \frac{d}{d\gamma} \ln(E[e^{\gamma s_j}]) \right|_{\gamma=0} \\ &= \left. \frac{\frac{d}{d\gamma} E[e^{\gamma s_j}]}{E[e^{\gamma s_j}]} \right|_{\gamma=0} \\ &= \left. \frac{E[s_j e^{\gamma s_j}]}{E[e^{\gamma s_j}]} \right|_{\gamma=0} \\ &= \frac{E[s_j \cdot 1]}{E[1]} = E[s_j] = \mu \end{aligned} \quad (2.20)$$

$$\begin{aligned}
\kappa_2(s_j) &= \left. \frac{d^2 K(\gamma)}{d\gamma^2} \right|_{\gamma=0} \\
&= \left. \frac{d}{d\gamma} \left(\frac{E[s_j e^{\gamma s_j}]}{E[e^{\gamma s_j}]} \right) \right|_{\gamma=0} \\
&= \left. \frac{\frac{d}{d\gamma} E[s_j e^{\gamma s_j}] \cdot E[e^{\gamma s_j}] - E[s_j e^{\gamma s_j}] \cdot \frac{d}{d\gamma} E[e^{\gamma s_j}]}{(E[e^{\gamma s_j}])^2} \right|_{\gamma=0} \\
&= \left. \frac{E[s_j^2 e^{\gamma s_j}] \cdot E[e^{\gamma s_j}] - E[s_j e^{\gamma s_j}] \cdot E[s_j e^{\gamma s_j}]}{(E[e^{\gamma s_j}])^2} \right|_{\gamma=0} \\
&= \frac{E[s_j^2 \cdot 1] \cdot E[1] - E[s_j \cdot 1] \cdot E[s_j \cdot 1]}{(E[1])^2} \\
&= E[s_j^2] - (E[s_j])^2 = \sigma^2
\end{aligned} \tag{2.21}$$

$$\begin{aligned}
\kappa_3(s_j) &= \left. \frac{d^3 K(\gamma)}{d\gamma^3} \right|_{\gamma=0} \\
&= \left. \frac{d}{d\gamma} \left(\frac{E[s_j^2 e^{\gamma s_j}] \cdot E[e^{\gamma s_j}] - E[s_j e^{\gamma s_j}]^2}{(E[e^{\gamma s_j}])^2} \right) \right|_{\gamma=0} \\
&= \left. \frac{d}{d\gamma} \left(E[s_j^2 e^{\gamma s_j}] - \left(\frac{E[s_j e^{\gamma s_j}]}{E[e^{\gamma s_j}]} \right)^2 \right) \right|_{\gamma=0} \\
&= E[s_j^3 e^{\gamma s_j}] - 2 \frac{E[s_j e^{\gamma s_j}]}{E[e^{\gamma s_j}]} \cdot \frac{d}{d\gamma} \left(\frac{E[s_j e^{\gamma s_j}]}{E[e^{\gamma s_j}]} \right) \Big|_{\gamma=0} \\
&= E[s_j^3] - 2E[s_j] \cdot (E[s_j^2] - E[s_j]^2) \\
&= E[s_j^3] - 2E[s_j]E[s_j^2] + 2(E[s_j])^3 \\
&= E[s_j^3] - 3E[s_j]E[s_j^2] + 2(E[s_j])^3
\end{aligned} \tag{2.22}$$

For a zero-mean standard distribution, the first six cumulants are as follows:

$$\begin{aligned}
\kappa_1(s_j) &= 0 \\
\kappa_2(s_j) &= 1 \\
\kappa_3(s_j) &= E\{s_j^3\} \\
\kappa_4(s_j) &= E\{s_j^4\} - 3 \\
\kappa_5(s_j) &= E[s_j^5] \\
\kappa_6(s_j) &= E[s_j^6] - 15E[s_j^4] + 30
\end{aligned} \tag{2.23}$$

Gaussian distributions have no higher order cumulants as they are entirely specified by the first and second cumulant, respectively the mean and variance. So by optimising the separation vector to maximise a higher order cumulant, most commonly the skewness κ_3 or the kurtosis κ_4 , non-gaussianity is also maximised and therefore the likelihood that the source is independent. Figure 2.13 presents the probability density functions of a representative example of gaussian, supergaussian, subgaussian and asymmetric distributions.

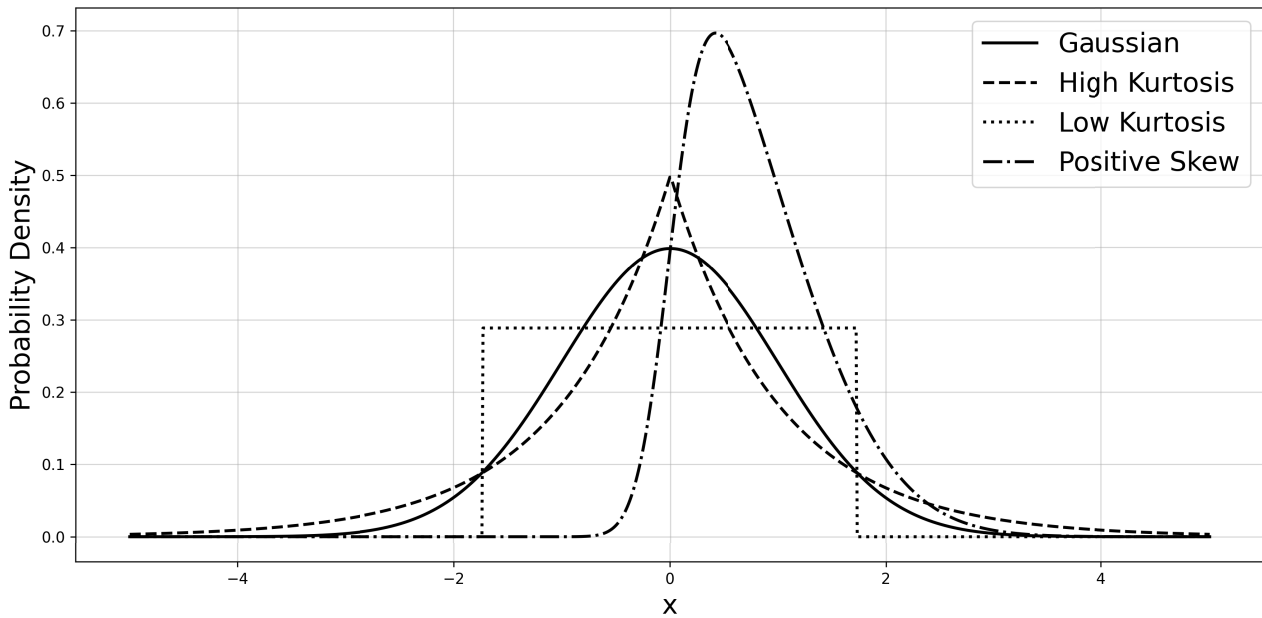


Figure 2.13: Probability density functions of 1) a zero-mean Gaussian distribution with a standard deviation of one, 2) a Laplacian distribution exhibiting a higher peak at 0 and fatter tails compared to the Gaussian, indicating higher kurtosis (supergaussian), 3) a uniform distribution as an example of low kurtosis (subgaussian), and 4) a positively skewed distribution with longer tails extending in the positive direction (asymmetric).

A computationally very simple approximation of the non-gaussianity as measured by the negentropy can then be given by:

$$J(s_j) \approx \frac{1}{12}\kappa_3(s_j)^2 + \frac{1}{48}\kappa_4(s_j)^2 \quad (2.24)$$

However, cumulant-based approximations are not widely used due to their high sensitivity to outliers, as a small number of large observations can disproportionately affect their values. Higher-order cumulants, such as κ_4 , predominantly measure the tails of a distribution. This is because data points far from the mean have a significantly larger influence on cumulant values than those close to zero, making them especially sensitive to extreme values.

Another approximation of entropy that is both more exact and more robust to outliers (Hyvärinen et al., 2001b) than the polynomial density approximations is given by:

$$J(s_j) \approx k_1[E\{G_1(s_j)\}]^2 + k_2[E\{G_2(s_j)\} - E\{G_2(s_{gauss})\}]^2 \quad (2.25)$$

where G_1 and G_2 are *odd* and *even* functions respectively. Therefore, in Equation 2.25 the first term is an estimate of the squared of the skewness, κ_3 (related to the symmetry of the underlying distribution), while the second one is an estimate of the squared of the kurtosis κ_4 . In practice, only one function is used depending on the properties of the sources. When ICA is applied on sparse sources like the MNs are in the EMG signals, common choices of contrast functions include (Caillet, Avrillon, et al., 2023; Negro et al., 2016):

$$G_1 = \frac{1}{6} s_j^3 \quad (2.26)$$

$$G_2 = \log \cosh(s_j) \quad (2.27)$$

$$(2.28)$$

Even though $G_1 = \frac{1}{6} s_j^3$ is a third-order polynomial proportional to the skewness, and not the square of the skewness, in practice it has shown fast convergence due to the sparsity of the sources (Negro et al., 2016).

The contrast function is then used as the objective function that measures non-gaussianity of the sources. ICA aims to find the separating matrix whose projections maximize this contrast function, thereby recovering the most non-gaussian and, consequently, the most statistically independent sources.

2.2.3.2.2 Blind Source Separation Algorithm Pipeline

Numerous BSS-based EMG decomposition algorithms have been introduced in the literature. Among the most notable are the convolution kernel compensation (CKC) method (Holobar & Zazula, 2004, 2007a; Holobar & Zazula, 2007b) and the convolutive blind source separation (cBSS) method (Negro et al., 2016). These algorithms share several common processing steps. In this section, we will outline these shared steps and highlight the key differences between the algorithms. Special emphasis will be placed on cBSS, as it serves as the benchmark automatic decomposition algorithm for the original work presented in Chapter 3 and Chapter 5.

In BSS of EMG signals, the decomposition is composed of three main aspects: 1) whitening of the centered and extended observations, 2) iterative optimization of the separation filters to identify the sources, and 3) spike detection to separate the firing times from the background noise.

A useful preprocessing method in ICA is to first whiten the observations. Whitening is a linear trans-

formation that transforms the observations $\tilde{\mathbf{x}}$ so its components are uncorrelated and their variances are equal to one (Hyvärinen & Oja, 2000) (Figure 2.14). The whitened and extended observations $\tilde{\mathbf{z}}$ are given by:

$$\tilde{\mathbf{z}}(t) = \mathbf{A}\tilde{\mathbf{x}}(t) \quad (2.29)$$

such that the covariance matrix of $\tilde{\mathbf{z}}(t)$ equals the identity matrix:

$$\mathbf{R}_{\tilde{\mathbf{z}}} = E\{\tilde{\mathbf{z}}(t)\tilde{\mathbf{z}}(t)^\top\} = \mathbf{I} \quad (2.30)$$

where $\mathbf{A}$ is the whitening matrix and $\mathbf{I}$ is the identity matrix. A popular method for whitening is with eigenvalue decomposition (Dai & Hu, 2019; Negro et al., 2016), where:

$$\mathbf{A} = \mathbf{U}\mathbf{D}^{-1/2}\mathbf{U}^\top \quad (2.31)$$

where $\mathbf{U}$ and $\mathbf{D}$ are the eigenvector and eigenvalue matrices of $\mathbf{R}_{\mathbf{z}} = \mathbf{U}\mathbf{D}\mathbf{U}^\top$, with $\mathbf{U}$ being an orthogonal matrix satisfying $\mathbf{U}^\top\mathbf{U} = \mathbf{U}^\top\mathbf{U} = \mathbf{I}$. Alternatively, for improved numerical stability, one can use singular value decomposition (Barsakcioglu et al., 2020; Peri et al., 2021):

$$\mathbf{A} = \mathbf{U}_1\mathbf{D}^{-1/2}\mathbf{U}_2^\top \quad (2.32)$$

where $\mathbf{U}_1$ and $\mathbf{U}_2$ are the orthonormal bases of $\mathbf{R}_{\mathbf{z}} = \mathbf{U}_1\mathbf{D}\mathbf{U}_2^\top$, and $\mathbf{D}$ is the diagonal matrix with the singular values of $\mathbf{R}_{\mathbf{z}}$.

In CKC, the whitening matrix $\mathbf{A}$ is given as the inverse of the covariance matrix (Holobar & Zazula, 2004).

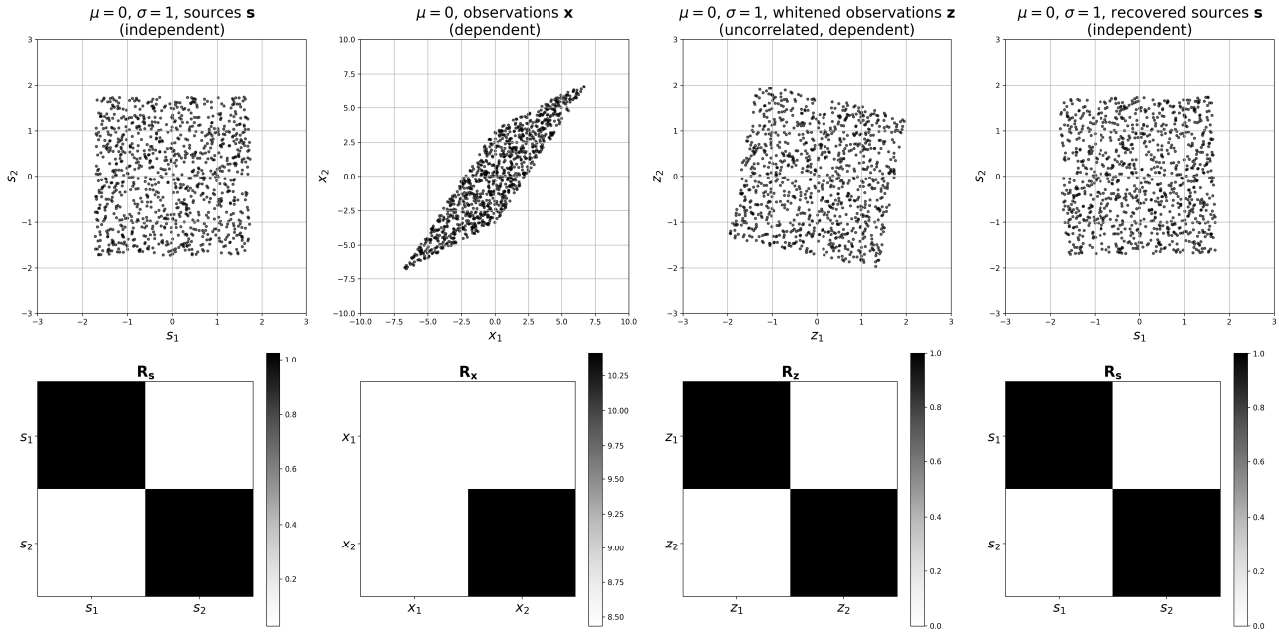


Figure 2.14: Effect of whitening and ICA on a mixture of independent sources. The first row shows the two sources plotted against each other, and the second row displays their covariance matrix $\mathbf{R}$. In the first column, two independent variables are generated from a uniform distribution with zero mean and standard deviation of 1. The second column shows a linear mixture of these variables, revealing a correlation between them. In the third column, the whitened mixture is presented, where the variables are no longer correlated but remain dependent. Finally, the last column shows the recovered sources after applying ICA, demonstrating that the variables are now both uncorrelated and independent.

An example of the whitening operation is given in Figure 2.14. Notably, Figure 2.14 shows why linear decorrelation, achieved through whitening, is not a strong enough property for recovering the sources. Indeed, two random variables that are uncorrelated (i.e., whitened) are not necessarily independent; however, two independent variables are necessarily uncorrelated. This limitation introduces the concept of ICA as nonlinear decorrelation: if two sources s_1 and s_2 are independent, then any nonlinear transformation $g(s_1)$ and $h(s_2)$ are uncorrelated (Hyvärinen et al., 2001c). Nonlinear decorrelation provides a natural extension of linear decorrelation by addressing higher-order statistical dependencies, ensuring that independence is captured beyond simple second-order relationships. Importantly, this approach is fundamentally linked to the maximization of non-gaussianity, as both methods achieve statistical independence by leveraging higher-order statistics. While nonlinear decorrelation ensures independence through transformations, maximizing non-gaussianity

achieves the same outcome by optimizing measures like negentropy, which are sensitive to deviations from gaussianity and capture the essence of statistical independence.

After whitening, the sources can be estimated as:

$$\tilde{\mathbf{s}}(t) = \tilde{\mathbf{B}}\tilde{\mathbf{x}}(t) = \mathbf{W}\mathbf{A}\tilde{\mathbf{x}}(t) = \mathbf{W}\tilde{\mathbf{z}} \quad (2.33)$$

where $\mathbf{W}$ represents the orthogonal separating matrix. $\mathbf{W}$ is computed by iteratively extracting separation vector $\mathbf{w}_j$ for each j th source. Whitening standardizes the data by fixing its second-order statistics (variances and covariances) and transforms the parameter estimation problem.

After whitening, the separating matrix $\mathbf{W}$ is orthogonal ($\mathbf{W}^\top \mathbf{W} = \mathbf{I}$), meaning that the vectors in $\mathbf{W}$ are orthonormal to each other. Therefore, orthogonality imposes two constraints. The first is on the norm of the vectors, which ensures that the matrix $\mathbf{W}$ is invertible, so it must be that each separation vector has unit length, and so imposes N constraints for a $N \times N$ matrix:

$$\|\mathbf{w}_j\|^2 = 1 \quad j = 1, 2, \dots, N \quad (2.34)$$

The second constraint is on the orthogonality. Each pair of vectors must be orthogonal to each other:

$$\mathbf{w}_j^\top \mathbf{w}_k = 0 \quad j \neq k \quad (2.35)$$

which imposes $\frac{N(N-1)}{2}$ constraints, roughly half because if vectors $\mathbf{w}_1, \mathbf{w}_2$ are orthogonal, so are $\mathbf{w}_2, \mathbf{w}_1$. Thus, the total number of constraints is $N + \frac{N(N-1)}{2} = \frac{N(N+1)}{2}$. Therefore, the N^2 parameters of $\mathbf{W}$ can be represented by $N^2 - \frac{N(N+1)}{2} = \frac{N(N-1)}{2}$, approximately halving the parameters to be estimated (Hyvärinen & Oja, 2000).

In cBSS, the separation vector is computed by maximizing an estimate of negentropy of the sources with fast independent component analysis (fastICA). The optimization of the separation vector is formulated in terms of a loss function $\mathcal{L}$:

$$\mathcal{L}(\mathbf{w}, \lambda) = -E\{G(\mathbf{w}^\top \tilde{\mathbf{z}})\} + \lambda[\|\mathbf{w}\|^2 - 1] \quad (2.36)$$

where $\mathcal{L}$ is the loss to maximize (hence, the negative sign), $\mathbf{w}^\top \tilde{\mathbf{z}}$ are the estimations of the sources, λ is the Lagrange multiplier that constrains the sources to have constant variance equal to one. Applying Newton's method, we get that the updated separation weight is given as:

$$\mathbf{w} \leftarrow \mathbf{w} - \left[\frac{\partial^2 \mathcal{L}(\mathbf{w}, \lambda)}{\partial \mathbf{w}^2} \right]^{-1} \frac{\partial \mathcal{L}(\mathbf{w}, \lambda)}{\partial \mathbf{w}} \quad (2.37)$$

By taking the first and second derivatives of $\mathcal{L}$, we get the final fastICA update rule (Hyvarinen, 1999):

$$\mathbf{w} \leftarrow E\{\tilde{\mathbf{z}}g(\mathbf{w}^\top \tilde{\mathbf{z}})\} - E\{g'(\mathbf{w}^\top \tilde{\mathbf{z}})\}\mathbf{w} \quad (2.38)$$

In the gradient version of CKC (Holobar & Zazula, 2007b), the separation vectors are updated by taking the natural gradient over the skewness contrast function:

$$\mathbf{w} \leftarrow \mathbf{w} - \eta E\{\tilde{\mathbf{z}}g(\mathbf{w}^\top \tilde{\mathbf{z}})\} \quad (2.39)$$

where η is the learning rate. In practice, fastICA is usually preferred because it ensures faster convergence to the source.

Since each separation weight is found iteratively, orthogonality of the vectors cannot be inherently guaranteed. Therefore, after each iteration, the newly found vector is decorrelated from the previously identified vectors using techniques such as Gram-Schmidt deflation or symmetric orthogonal-

ization. This process ensures uncorrelation after applying a nonlinear transformation, aligning with one of the formulations of ICA (Hyvarinen, 1999):

$$\mathbf{w}_j \leftarrow \mathbf{w}_j - \sum_{p=1}^P (\mathbf{w}_j^\top \mathbf{w}_p) \mathbf{w}_p \quad (2.40)$$

where $\mathbf{w}_j$ is the current separation vector and $\mathbf{w}_p$ represents the previously identified vectors. If the vector is then divided by its norm, the set will become orthonormal (Hyvärinen et al., 2001e). This process ensures that there is no repeated convergence to the same source. Furthermore, it makes fastICA more closely resemble *projection pursuit*, a method that seeks interesting, or non-gaussian, projections of the data rather than strictly independent components. When independence can be assumed in the model, optimizing non-gaussianity results in independent components. However, if this assumption does not hold, as in the case of EMG decomposition where common inputs to MNs exist, the algorithm finds projection pursuit directions (Hyvärinen et al., 2001a).

However, for spike sorting it is usually quite simple to estimate the contribution of a source to the signal by convolving the spike triggered average with the source (Farina & Holobar, 2016), which can then be subtracted from the signal, a process called peel-off. This process also avoids repeated convergence to the same source.

Once each separation vector is optimized—either by meeting a convergence threshold or reaching the maximum number of iterations—the vector, also referred to as an innervation pulse train (IPT), is divided into peak and noise components using clustering methods like *K*-means (Negro et al., 2016). The cluster with the largest centroid is selected as representing the firing of the MN.

In the probabilistic framework of CKC, the highest peaks are progressively included during the calculation of the separation vector (Holobar & Zazula, 2007a).

After detecting the spikes, in the cBSS method, the quality of detection is enhanced by iteratively updating the separation vectors. This process continues until the coefficient of variation (CoV) of the MN discharge times is minimized. The CoV is used to quantify the regularity of the firing patterns,

measured as the ratio between the standard deviation and the mean of the interspike intervals, which is a reliable assumption under isometric conditions.

The calculation of the separation vector in cBSS follows a similar approach to CKC (Holobar & Zazula, 2007a). However, a key distinction between these methods lies in the type of information they utilize. CKC leverages second-order statistical information about the distribution by calculating MN firings through cross-correlation between the sources and the observations, whilst cBSS utilizes higher-order information of the distribution (i.e., the skewness).

2.2.3.3 Metrics of Accuracy

To evaluate the quality of the decomposition, various methods are used depending on the nature of the recorded signal and the availability of ground truth data.

The most widely used metric to assess performance is the rate of agreement (RoA) (measured in percentage, %). This metric measures the agreement between the predicted firings and the true firings:

$$RoA = \frac{TP}{TP + FP + FN} * 100 \quad (2.41)$$

Here, TP represents the number of true positives, which are the predicted activations that match the true firings within a defined deviation margin, commonly set to 0.5 ms (Farina et al., 2001). FP refers to the false positives, indicating predicted discharge times that do not correspond to any true firing. FN represents the false negatives, which are true firing events that were not captured in the predicted firings.

For intramuscular signals, validation is relatively straightforward because manual decomposition is feasible due to the sparse nature of the data. The manually determined MU activity can then serve as ground truth to evaluate automatic decomposition methods. By using manual methods, such as

those described in (McGill et al., 2005), the agreement between predicted firings and true firings can be directly assessed using Equation 2.41.

In contrast, validating sEMG signals presents more challenges, as manual decomposition is impractical. This difficulty arises from the inability to easily isolate individual MUs from the interference signal. To address this, various approaches have been proposed. One of the most intuitive methods is the use of simulated signals. Models for generating surface EMG signals have become highly sophisticated, coupling accurate simulations of the underlying physiology of MN recruitment, firing patterns, and force generation (Fuglevand et al., 1993) with simulations of MUAPs propagating through tissue (Farina et al., 2004; Ma et al., 2024). Although these models sometimes oversimplify physiological processes, they offer a useful first step in validation before progressing to experimental signals (Farina & Holobar, 2016).

The gold standard approach for validating surface EMG decomposition involves recording iEMG signals simultaneously with sEMG and decomposing them independently, a process known as two-source validation. When both recordings capture the same MU activity, the results can be directly compared. For MUs detected by both methods, mismatches are considered errors, while agreements indicate correct decomposition. This approach is grounded in the assumption that if two independent methods applied to different signals produce the same result, that result is likely accurate. Unlike treating one method as a gold standard, this method cross-validates the surface and intramuscular decompositions against each other. Since errors from both methods are combined, the estimated error rate tends to be higher than that of either individual method (Farina & Holobar, 2016).

When concurrent intramuscular recordings are unavailable, metrics that estimate the likely accuracy of each decomposed MN can be used. These metrics assess both the amplitude of the background activity and the firing characteristics of the MUs. Common metrics include the silhouette measure (SIL) and pulse-to-noise ratio (PNR) for evaluating background noise, and the CoV and firing rate (FR) for analyzing firing regularity.

The SIL (measured in normalized units, n.u.) is typically calculated as:

$$SIL = \frac{\alpha_s - \alpha_n}{\max(\alpha_s, \alpha_n)} \quad (2.42)$$

where α_s represents the sum of within-cluster spike-to-cluster distances, and α_n denotes the sum of within-cluster noise-to-cluster distances (Negro et al., 2016). Typically, only sources with a SIL greater than 0.9 are retained for further analysis (Negro et al., 2016), although this threshold can be relaxed to 0.8 in certain cases (C. Chen, Ma, et al., 2020; Dai & Hu, 2019).

The PNR (measured in decibels, dB), used in CKC is computed as:

$$PNR = 10 \log \frac{E\{\mathbf{s}^2(t) | \mathbf{s}(t) \in \mu_s\}}{E\{\mathbf{s}^2(t) | \mathbf{s}(t) \in \mu_n\}} \quad (2.43)$$

where $\mathbf{s}$ are the sources, and μ_s and μ_n are the centroids of the spike and noise clusters, respectively. Typically, only units with a PNR greater than or equal to 30 dB are retained for analysis (Holobar & Farina, 2014).

Additionally, in isometric conditions, units with a CoV of 30% or less are generally kept for further analysis (Holobar et al., 2010), along with those that exhibit FRs lower than roughly 30 Hz (R. M. Enoka & Duchateau, 2017).

2.2.3.4 Limitations

In conclusion, EMG decomposition algorithms are essential for studying MN behavior.

For intramuscular signals, decomposition has traditionally been performed using template-matching methods. Although these methods are highly accurate, they are both time-consuming and labor-intensive (Farina & Enoka, 2023; McGill et al., 2005; Merletti & Farina, 2009), especially when dealing with HD-iEMG signals. For instance, manually decomposing a 10-second 32-channel recording during isometric contractions from the Tibialis Anterior (TA) muscle required approximately 130 hours

of expert effort. To overcome these limitations, BSS approaches have been increasingly adopted (Negro et al., 2016). However, these methods have so far only managed to decompose a fraction of the MUs compared to manual methods, revealing a significant limitation in the ability of current algorithms to effectively separate sources in HD-iEMG signals.

Similarly, when applied to HD-sEMG signals, current BSS algorithms are able to decompose only a small fraction of the MUs present in the signal. These algorithms tend to primarily identify MNs associated with high-amplitude MUAPs, either because these MNs innervate a larger number of muscle fibers and are thus recruited at higher force levels (Holobar et al., 2009), or because their MU territories are located closer to the recording electrodes (Holobar et al., 2010). To mitigate this limitation, when data from multiple force levels are available, one approach is to first decompose the signals at lower force levels and track the same MNs as the force increases (Frančič & Holobar, 2021). Another approach involves combining experimental recordings with realistic mathematical models to simulate a comprehensive pool of MNs (Caillet et al., 2022).

Another limitation of current decomposition methods is their inability to adapt to changes in muscle geometry. Such changes can occur during muscle fatigue, which leads to a reduction in AP amplitude and conduction velocity (R. M. Enoka et al., 1992), or when the muscle fibers shorten or lengthen during dynamic contractions (Merletti et al., 2001). Several approaches have been proposed to address this issue (C. Chen, Ma, et al., 2020; Glaser & Holobar, 2019; Glaser et al., 2013; Kramberger & Holobar, 2021; Mendez Guerra et al., 2024; Yeung et al., 2024). While these methods represent progress in improving decomposition during dynamic conditions, further validation is needed, particularly in faster movements and under a wider range of conditions.

Lastly, another limitation of BSS methods is their requirement for a greater number of sources than observations to maintain a well-conditioned system, which is also essential for accurately distinguishing between different MUAPs (Farina & Holobar, 2016). This requirement further increases the computational demands of the decomposition process. Moreover, as the field moves toward denser electrode configurations (with 2 mm spacing) and a higher number of channels (256), the computational complexity grows even more, despite the potential to significantly increase the number of decomposed MNs (Caillet, Avrillon, et al., 2023).

2.3 Human-machine Interaction based on MNs

MN activity offers critical insights into the neural processes that govern movement. This section first provides an overview of the most common alternatives to using MNs for control, and then delves into the primary methods employed for interfacing directly with MNs.

Human motor control can be studied and interfaced with at various physiological levels. At the highest level, brain activity can be recorded invasively, using methods like electrocorticography (ECoG), or non-invasively with electroencephalography (EEG). While ECoG provides detailed insights into motor planning and execution, it is highly invasive, whereas EEG is less invasive but suffers from a low signal-to-noise ratio (Tam et al., 2019). Peripheral nerve interfaces, such as peripheral nerve cuffs (Naples et al., 1988) or intraneural electrodes (Micera et al., 2010), offer more direct access to motor commands but require complex surgical procedures. Muscle state can be assessed using ultrasound imaging, which provides detailed architectural information, though its temporal resolution is limited (Botter et al., 2013). Additionally, movement kinematics can be captured through motion tracking systems, delivering high precision but without direct insight into the neural mechanisms driving movement.

MN activity presents a compelling alternative, representing a more direct window into the neural drive to muscles while being accessible through both surface and intramuscular electrodes. Recent advances in MN-based human-machine interfaces have demonstrated remarkable capabilities in gesture recognition or continuous control. Here, we will focus on gesture recognition as it is the topic discussed in the original work of Chapter 6 of this thesis. Interfacing with MNs typically involves decomposing EMG signals into their constituent MN spike trains, which are then used for movement classification.

Several studies have shown the effectiveness of MN-based gesture recognition. In Farina et al. (Farina et al., 2017), MNs were grouped based on the spatial location of their corresponding muscle units, and used to predict 11 classes in patients with targeted nerve reinnervation achieving over 97% accuracy. Chen et al. (2020) achieved over 95% accuracy in classifying 11 hand gestures by associating detected MNs with specific gestures based on their MUAP features and applying majority

voting. Similar performance was reported by Stachaczyk et al. (2020) when classifying individual finger movements and their contraction phases using MN firing features, including spike count, firing time variability, and inter-spike intervals. Recently, Mendez Guerra et al. (2022) used MNs for classification of individual and combined finger gestures achieving >95% classification accuracy for 10 classes and $\approx 93\%$ task completion rate for 10 classes in real-time settings. The field has recently embraced modern machine learning approaches, with both convolutional neural networks (Zhao et al., 2022) and spiking neural networks (Tanzarella et al., 2023) demonstrating high accuracy (>95%) in finger gesture decoding from MN activity.

The superiority of MN-based features over traditional EMG approaches has been particularly evident in complex tasks. Chen et al. (2023) showed that careful MN selection criteria led to significantly better classification accuracy compared to EMG-based features, especially when discriminating between more than 20 gestures. This finding was further supported by Yeung et al. (2023), who developed a comprehensive framework for optimal MN selection using mutual information criteria. Their results demonstrated remarkable efficiency, with only a 3% performance decrease when using just 25% of the available MN population for the classification of 18 motor tasks.

While these approaches show considerable potential for controlling motion intent through the neural drive, several challenges persist. One key issue is the reliability of the interface, especially when using sEMG electrodes, which come with limitations as previously discussed. These constraints often reduce the number of MNs that can be accurately decoded, limiting the precision and overall control of the interface. Furthermore, the translational potential of these systems remains uncertain, as factors such as long-term stability, ease of use in real-world environments, and adaptability to different users present significant challenges. These limitations highlight the need for further advancements to improve the practical application and robustness of these control methods.

This chapter provided an overview of the physiology underlying the neuromuscular system, establishing the basis for standard methods of recording EMG signals, including both sEMG and iEMG, as well as the mathematical foundations of EMG generative models. The chapter placed particular emphasis on decomposition techniques, with a focus on BSS approaches. Key limitations of each method were highlighted, such as the low-pass filtering effect imposed by the volume conductor

on sEMG signals, which reduces the information bandwidth of individual sources. Additionally, the limitations inherent to iEMG recordings were discussed, including the lack of systematic studies on optimal electrode configurations for HD-iEMG, challenges in automating decomposition for both HD-iEMG and HD-sEMG, and the limited reliability of current MU-based interfacing methods. These challenges underscore the ongoing need for advancements in EMG recording and analysis techniques to improve the accuracy and robustness of neuromuscular signal interpretation.

The next chapter introduces a novel method for decomposing invasive HD signals, establishing a new standard of accuracy and effectiveness in automatic decomposition by improving both the number of decomposed MUs and the accuracy of the decomposition process.

*Everything that is obvious hides
within itself a mystery*

Florenskij, Pavel

3

Blind Source Separation of Invasive Neurophysiological Time Series

NOTE: Aside from minimal alterations to fit the format and flow of the thesis as a cohesive single document, the content of this chapter has been published in the following journal article:

Grisson, A., Clarke, A. K., Muceli, S., Ibáñez, J., Kundu, A., Farina, D. (2024). A Particle Swarm Optimised Independence Estimator for Blind Source Separation of Neurophysiological Time Series. IEEE Transactions on Biomedical Engineering. doi: <https://doi.org/10.1109/TBME.2024.3446806> (Under licence CC-BY)

In Chapter 2, challenges in decomposing invasive HD neurophysiological signals were explored, with limitations of traditional methods highlighted. Specifically, template-matching approaches have struggled to provide an automated solution for multi-channel signal decomposition, often requiring substantial manual effort from expert operators. BSS methods were introduced as a solution to this issue but were shown to only decompose a limited number of the MUs active in the signal. This chapter identifies a gap in current research and presents a novel BSS decomposition method, validated across a range of invasive neurophysiological signals. The chapter concludes by demonstrating the extensive information that can be extracted from novel HD micro-electrode intramuscular arrays using the proposed decomposition method and addresses the remaining limitations that need to be overcome to further enhance the information retrievable from HD-iEMG signals.

3.1 Introduction

In vivo neurophysiological signals recorded by invasive probes, such as HD-iEMG and intracortical probe recordings, generally represent the summed electrical activity of many individual spiking neural sources (Stark & Abeles, 2007). The automated and semi-automated extraction of these sources from the multi-unit time series without any *a priori* knowledge of their spike timings or the shapes of their AP waveforms is the domain of BSS algorithms (Sawada et al., 2019). Such neurophysiological signal decomposition, also known as spike sorting, has been a core component of many neuroscientific research workflows for decades, in many cases supplanting tedious manual sorting (Rey et al., 2015). In recent years, there has been a consistent trend towards multivariance in neurophysiological time series, driven by dramatic advances in the miniaturisation of recording hardware (Jun et al., 2017; Putzeys et al., 2019).

Modern HD invasive probes can have hundreds of sensing electrodes, arranged in high density grids to achieve impressive spatial resolutions (J. E. Chung et al., 2022). Unprecedented volumes of data have resulted in correspondingly unprecedented yields of decomposed sources (Pachitariu et al., 2023), but have also introduced new challenges in error correction and reproducibility (Magland et al., 2020), whilst worsening known problems related to the effects of signal non-stationarity (Buccino

et al., 2022). In many settings, manual spike sorting continues to outperform automated methods in both source yield and accuracy (Mayer et al., 2023), adding an additional workload to studies that is generally unmanageable in the HD setting.

Spike sorting BSS algorithms can be broadly divided by whether they use lower-order or higher-order statistics to separate sources (Takahashi et al., 2003). Lower-order methods find correlations between segmented AP waveforms and follow a pipeline of detection and sorting (Rey et al., 2015). The timestamp of a waveform is first found through thresholding and temporal alignment, then the timestamp is assigned to a source by clustering (Pachitariu et al., 2023), usually after a step of linear feature extraction, such as principal components analysis or wavelet convolution (Buccino et al., 2022; Lee et al., 2020). This order of operations is reversed in higher-order methods; metrics of statistical independence are first used to find the separated source time series (Hyvärinen & Oja, 2000), which are then thresholded to obtain the spike timestamps (Leibig et al., 2016; Negro et al., 2016). Higher-order statistical methods, such as ICA, both manage AP waveform superpositions better and are generally more resistant to non-stationarities in the data (Farina & Holobar, 2016; Leibig et al., 2016), as they do not require AP alignment and consider the entire time series simultaneously. Higher-order statistical methods have been largely ignored in the spike sorting literature. The exception is recording modalities such as surface EMG, which require high-order statistical methods for successful BSS. This is most likely due to their comparatively heavy computational loads. In recent years, this has been mitigated by the widespread adoption of parallel computing architectures such as graphics processing units.

Despite these advancements, current methodologies in decomposing invasive neurophysiological recordings are not specialized towards solving the BSS problem in spiking distributions, which are highly sparse and skewed. The primary issue with current methods is their inability to effectively distinguish between spike sources due to the similarity in waveforms. This similarity leads to overlapping signals that current contrast functions and non-linear metrics are not optimized to resolve. The nature of these distributions suggests a different optimal form for the contrast function, the non-linearity used as a metric of statistical independence. In the successful FastICA algorithm, on which most higher order BSS algorithms for spike sorting are based (Buccino et al., 2018; M. Chen & Zhou, 2024; M. Chen et al., 2018; Jäckel et al., 2012; Leibig et al., 2016; Muceli et al., 2022), this

contrast function is designed to be robust to outliers in the source. However, for spiking sources it is precisely these outliers that are interesting. This motivates the design of new contrast functions, in particular adaptive forms that can balance the requirements of fine source separation and optimisation stability. In this study, we propose to find the best form of this contrast function for each source using a particle swarm optimization (PSO) methodology.

By integrating an adaptive contrast function as the metric of statistical independence, our method directly addresses the limitations of current SoA techniques. This approach enhances the accuracy of spike sorting by adapting to the unique characteristics of spiking distributions. Thus, our study bridges the gap in the literature by offering an optimized solution for BSS of neurophysiological time series, demonstrating significant improvements over existing methodologies.

In summary, in this study we contribute the following:

1. We explore in detail how the design of the non-linearity in projection pursuit ICA affects the successful separation of invasive neurophysiological recordings, whose sources often have very similar AP waveforms.
2. Using these insights, we propose an adaptive contrast function which can be modified for each source to maximise the chances of a successful separation vector optimisation. We incorporate this into Swarm-Contrastive Decomposition (SCD), a novel automated BSS algorithm which uses particle swarm optimisation to find the best non-linearity for each source.
3. We demonstrate SoA performance of SCD on a range of experimentally-collected intramuscular EMG datasets, before going on to show potential utility in the intracortical domain.

3.2 Theory and Algorithm

3.2.1 Blind Source Separation in Neurophysiological Signal

The signal detected during neurophysiological recordings is a volume conducted electrical action potential from the activation of one or more cells (Rutkove, 2007). The volume conductor has an attenuating effect on the magnitude of the potential recorded, meaning that sensing electrodes that are further away from the source will read a lower voltage than one closer (Stegeman et al., 1997). In the context of a HD microelectrode array, and assuming each sensing electrode has the same active area, the detected activity from a source will be much more prominent in some channels than others. Thus, if we disregard non-stationarities by assuming a fixed anatomical geometry and no movement in the recording array, each source will contribute an associated spatiotemporal AP waveform to the signal on every activation (Merletti & Farina, 2016). For EMG, the waveform is not the output of a single cell, but instead represents the synchronised activity of the set of muscle fibers controlled by a single MN (Merletti & Muceli, 2019), called a MU, and is called a MUAP. The objective of BSS in spiking time series is to identify the timestamps of all source activations and to assign each timestamp to the correct source.

The objective of BSS is thus to stably find the separation matrix $\mathbf{W}$, which is the approximate inverse of $\tilde{\mathbf{H}}$. The columns of $\mathbf{W}$ are the separation vectors w_j , recovering an estimate of the j th source when applied to the extended observation matrix.

3.2.2 An Adaptable Contrast Function

The selection of the contrast function is important for the stability of numerical optimisation methods. One family of non-linearities which has an intuitive theoretical appeal are the cumulants. Gaussian distributions have no higher order cumulants as they are entirely specified by κ_1 and κ_2 , respectively the mean and variance. So by optimising the separation vector to maximise a higher order cumulant, most commonly the kurtosis κ_4 , nongaussianity is also maximised and therefore

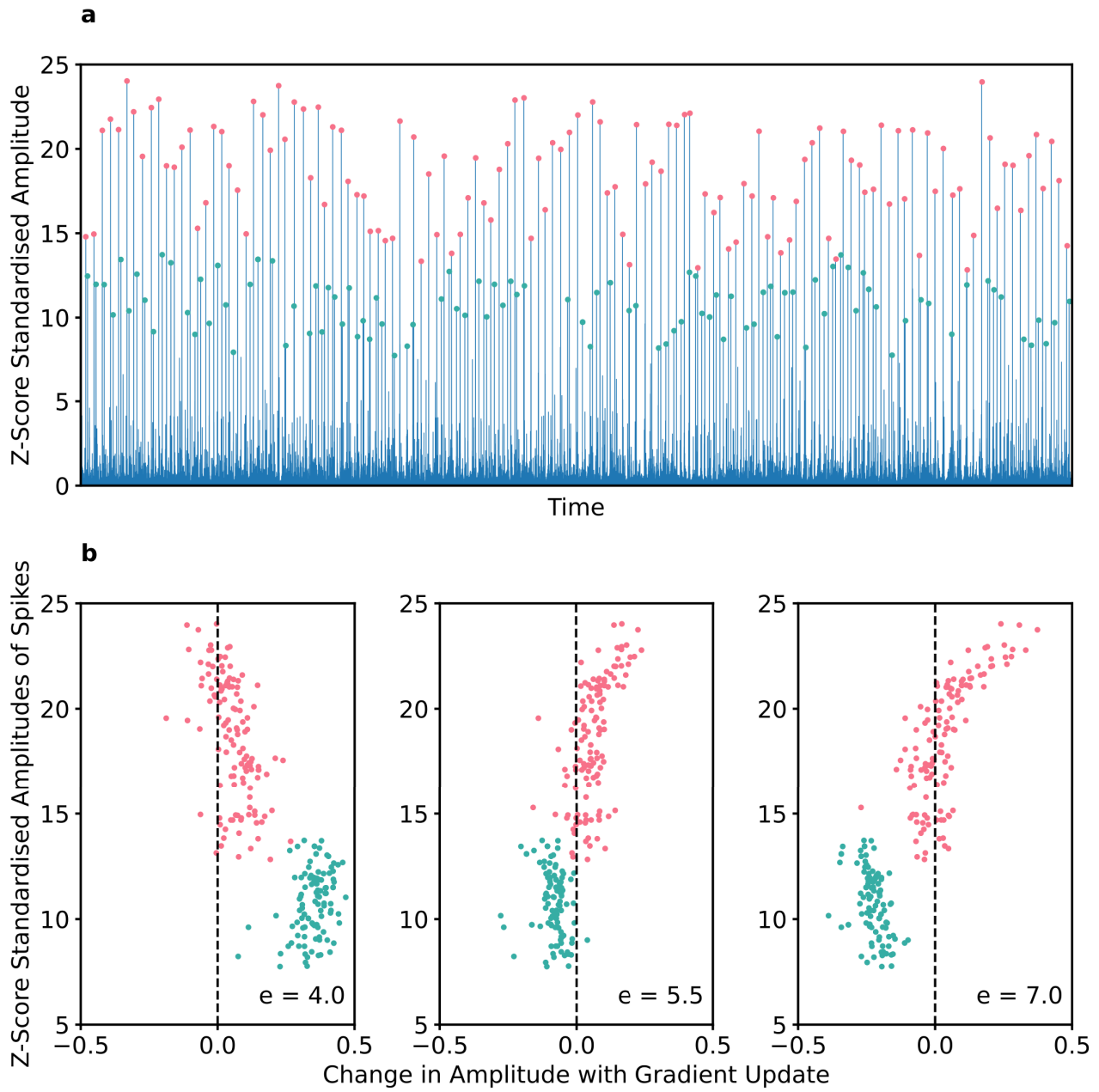


Figure 3.1: **a** The Z-score standardised source activity estimated by applying a partially-optimised separation vector to whitened intramuscular EMG signal. The activity features two sources (pink and green) which have very similar action potential waveforms and hence are both being highlighted by the separation vector. **b** Superimposed spike triggered averages of the two sources. **c** The change in spike amplitude after a gradient step versus the current spike amplitude for three different values of e in $f(s_j) = E\{\text{sign}(s_j)|s_j|^e\}$. When e is too small at 4.0, between-source variance is suppressed by the separation vector. When e is too high at 7.0, in-source variance is enhanced by the separation vector and the optimisation begins to collapse to a single spike. Stable source separation begins only when e is at an optimal value at 5.5, suppressing the spikes from the green source, whilst maintaining those from the pink source.

the likelihood that the source is independent. In practice, as the cumulant order increases, so does its sensitivity to outliers (Hyvärinen et al., 2001d). This can be a problem in many signal domains, motivating the use of more robust cumulant estimators such as the hyperbolic tangent. However, for spiking sources, outliers are precisely the samples of interest. The cumulant order can therefore be seen as a method of tuning the selectivity of w_j to the spikes in a signal and so is extremely important for accurate decomposition.

We propose to adaptively find the contrast function that has the optimal level of selectivity. Cumulants have the disadvantage of being specified only by the integer order, so instead we propose a continuous and strictly asymmetric polynomial approximate:

$$f(s_j) = E\{sign(s_j)|s_j|^e\} \quad (3.1)$$

where $sign(.)$ is a function that operates on each element in a vector, returning 1 when an element is positive and -1 when negative. An asymmetric function is chosen to match the distribution asymmetry of spiking sources, whose samples are mostly close to zero with rare excursions into highly positive values during an activation.

As an example for why tuning the contrast function is important in spike sorting in particular, consider an observation matrix consisting of additive noise and two active sources with very similar AP waveforms. When the value of e in equation 3.1 is low, its value will be dominated by all spikes in the signal, and w_j will therefore optimise to enhance all spikes, failing to separate the two sources. When e is optimal, it will be dominated by a smaller pool of outliers, and w_j will enhance one source and suppress the other. At higher values of e , the outlier pool will decrease further, and the within-source variance in the enhanced source's waveforms caused by the additive noise will start to dominate, until eventually w_j collapses down to enhancing only a single spike. This means that as e increases, the algorithm becomes overly sensitive to noise, leading it to focus solely on the most prominent waveform feature within a source. Consequently, the enhancement process no longer effectively separates different sources but zeroes in on the largest amplitude spike within a single source, collapsing the enhancement process to a single point of interest. In short, there exists an optimal value of e for each source, where w_n is enhancing between-source waveform variance, but

not enhancing within-source waveform variance.

Figure 3.1 uses experimental HD-iEMG to illustrate this effect. For lower values of e , between-source variance for sources with similar AP waveforms (refer to panel **b** to visualise the similarity between the sources) is suppressed and sources are incorrectly merged, whereas for higher values of e the in-source variance is enhanced and the source begins to collapse. The main issue is that the optimal value of e is unknown *a priori* and likely to vary between sources and data types. However, in the spike sorting literature there exists a number of efficient methods for validating and comparing the quality of decomposition automatically, i.e., the experiments are expensive, but the validation is cheap. This motivates the use of an efficient search algorithm that quickly finds the optimal e to build the most selective separation vector (Huang et al., 2023; Yuan et al., 2023).

3.2.3 Particle Swarm Optimisation of Contrast Functions

Particle swarm optimisation is a population-based stochastic optimisation methodology (Kennedy & Eberhart, 1995). A number of candidate solution particles are evaluated, with the particle positions then updated on each step based on their personal best solution, the global best solution across particles and an inertia term which encourages exploration (Shi & Eberhart, 1998). We propose to use this methodology to optimise the exponent e of equation 3.1, using as the objective function a metric of source quality $Q(s_j)$ after a projection pursuit ICA run. J particles are created, each represented by a duplicate of a randomly initialised separation vector and an initial value of e (refer to Section 3.3.4). On each step an ICA run is conducted and then the j -th particle's current value of e , e_j^0 is updated to its new value e_j^1 by:

$$e_j^1 = e_j^0 + v_j^1 \quad (3.2)$$

where v_j^1 is the particle's new velocity and is calculated from the old velocity v_j^0 by:

$$v_j^1 = wv_j^0 + c_1r_1(pBest_j - e_j^0) + c_2r_2(gBest - e_j^0) \quad (3.3)$$

with $pBest_j$ being the personal best of the j -th particle, i.e. the value of e which returns the highest $Q(s_j)$. Likewise, the global best, $gBest$, is the value of e that returns the best quality metric value so far. The inertia coefficient w , cognitive coefficient c_1 and social coefficient c_2 are hyperparameters which tune the balance of the particle update towards its current trajectory, its personal best and the global best, determining exploration-exploitation trade-offs. Finally, r_1 and r_2 are samples from a normal distribution of mean 0 and standard deviations σ_1 and σ_2 .

An important feature of the algorithm, which we call Swarm-Contrastive Decomposition (SCD), is that on each PSO step, the separation vector for all particles is reinitialised to be the spike triggered average of the spike timestamps from the best source from that round of ICA, as estimated by a quality metric Q . This helps counter the fact that small changes in e can lead to different sources being found by their respective ICA runs, without heavily constraining the optimisation. SCD is run until the global best stops improving for a set number of steps, at which point a final review of the best source is conducted, with the time stamps of the acceptable sources saved and the contribution of the source to the observation matrix estimated and peeled off. The pipeline of the algorithm is presented in Figure 3.2.

3.2.4 Source Quality Metrics

A number of source quality metrics exist for automatically assessing the success of a decomposition operation. Selecting a sensitive and accurate metric is vitally important for the success of the SCD algorithm, with different metrics being the most appropriate for different data types. In this study, we use a robust coefficient of variance calculated on the interspike intervals for the HD-iEMG data and a pseudo-silhouette score calculated post-thresholding for the intracortical data. A peak finding algorithm is first used to filter the source samples, followed by a two class k-medoid clustering algorithm to select the peaks which are likely spikes and add them to a timestamp set.

MU firing rates are quite regular in case of sustained contractions at a fixed target force (Martinez-Valdes et al., 2017), as in our experiments, meaning a good metric of source quality is the CoV of the interspike intervals. The interspike intervals are first calculated on the timestamp set, with intervals

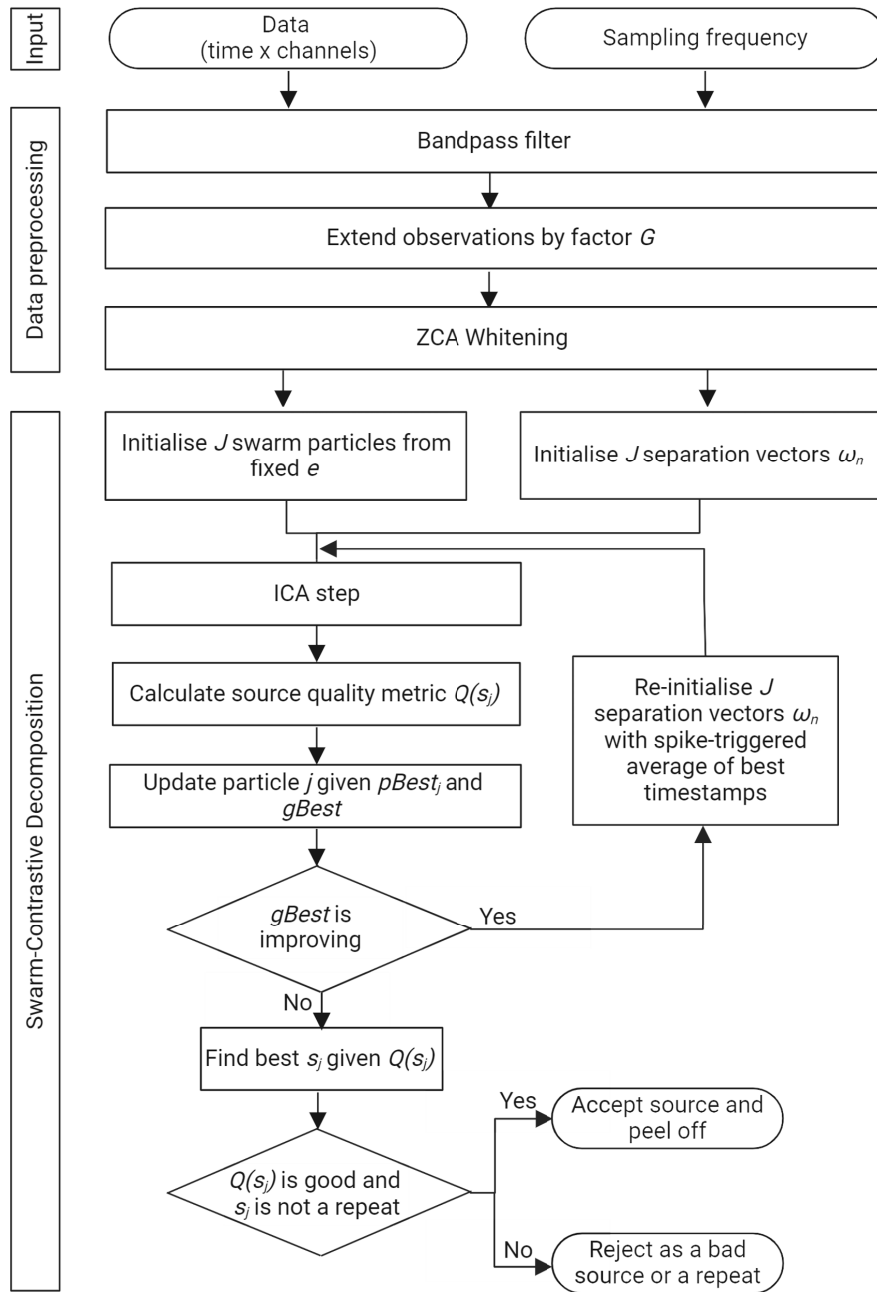


Figure 3.2: The neural data is preprocessed through filtering, extension, and ZCA whitening. Particles representing candidate sources and their separation vectors are initialized. An iterative procedure alternates between ICA to estimate sources and a PSO scheme to update the separation vectors based on a quality metric Q that assesses source validity. In each PSO iteration, particle vectors are updated using their $pBest$ and the $gBest$. This process terminates when the global best solution ceases to improve. The final accepted sources meeting a quality threshold are identified as valid sources, their spike times are extracted, and their contributions are removed from the signal matrix.

five times larger than the median discarded. Rather than then estimating the CoV directly, we instead opted for a bootstrap version which penalises sources with less spikes, which can bias the CoV. We create 1000 timestamp sets by sampling from the original timestamp set with replacement, and then

calculate the CoV on each set, the standard deviation of the intervals divided by the mean. We then take the 75th quantile of the 1000 CoV values, which we will refer to as the Q_{CoV} .

In intracortical data, there is no similar expectation of regularity in the firing statistics. Instead, we use the values of the two k-medoids and the sample assignments to calculate a pseudo-silhouette score:

$$Q_{SIL} = \frac{1}{D} \sum_{d=1}^D \frac{|\alpha_d - \bar{\mu}_{out}| - |\alpha_d - \bar{\mu}_{in}|}{\max(|\alpha_d - \bar{\mu}_{out}|, |\alpha_d - \bar{\mu}_{in}|)} \quad (3.4)$$

where D is the number of timestamps, α_d the source's value at the d -th timestamp. $\bar{\mu}_{in}$ and $\bar{\mu}_{out}$ are respectively the values of the medoid that the d -th timestamp is and is not assigned to.

3.3 Materials and Methods

3.3.1 Experimental Datasets

The effectiveness and reliability of the proposed SCD algorithm were validated using a diverse range of invasive HD-iEMG and intracortical recordings.

The HD-iEMG signals were recorded from either the TA or the forearm muscles. In all HD-iEMG experiments, multi-channel intramuscular electrodes designed for acute recordings were used (Muceli et al., 2015, 2022). The EMG signals were recorded with a multi-channel amplifier (OT-Bioelettronica, Torino, Italy), sampled at 10240 Hz, high pass filtered at 100 Hz, and finally A/D converted with 12-bit resolution for the TA sets and with 16-bit resolution for the forearm data. The EMG signals were acquired in unipolar derivation. A reference electrode was placed on the ankle (TA) or the wrist (forearm). In all experiments, the participants were instructed to sustain an isometric contraction (ankle dorsiflexion for the TA, and extension of the little finger for the forearm). The relative forces were determined in all cases as percentages of the MVC. A visual feedback of the exerted force and the target was provided to the subject. All experimental procedures adhered to the ethical guidelines set by Imperial College London (FM) and by the Ethical Committee of the University Medical Centre

of Göttingen (TA1 and TA2). The studies were performed according to the Declaration of Helsinki, with an informed consent form signed by all participants before each experiment.

The TA recordings were made using two thin-film intramuscular electrodes, with 16 platinum detection points ($140\ \mu\text{m} \times 40\ \mu\text{m}$) placed on a linear array with 1 mm interelectrode distance (Muceli et al., 2015), inserted in the TA muscle of two participants (TA1 and TA2), approximately 2 cm apart in the longitudinal direction. The subjects were seated in a Biodex System 3 (Biodex Medical Systems Inc., NY, USA) with the right leg and foot constrained to the equipment. The subjects were instructed to perform three 30 second isometric contractions at 10, 20, and 30 %MVC. A central segment of 10 s was then used for algorithm validation. To study the robustness of the algorithm to different noise conditions, we generated signals with [30, 25, 20, 15, 10, 5, 0] dB of Additive White Gaussian Noise (AWGN) to the original reconstructed signals (Mishra et al., 2019).

For the forearm muscles, data were recorded from three thin-film arrays with 40 platinum electrodes ($140\ \mu\text{m} \times 40\ \mu\text{m}$) linearly distributed over a length of 2 cm, with 0.5 mm interelectrode distance (Muceli et al., 2022), inserted in the forearm muscles of one participant (FM). To capture the precise activity of the fingers in extension, the muscles targeted were: the extensor pollicis longus (EPL), the EDC, and the flexor digitorum superficialis (FDS). The experimental protocol was designed following MRI scans of the forearm, and the insertions were performed with the guidance of a portable ultrasound (Butterfly iQ+) (Figure 3.3 a). The forearm and hand of the participant were constrained in an instrumented platform to measure isometric forces from each finger individually, in flexion and in extension (Figure 3.3 b). The data acquisition framework featured ten calibrated load cells (TAL 220, 10 kg) mounted on a platform and tailored to accommodate the individual size of the participant. The force data were concurrently recorded with the EMG data through 10 auxiliary connections with the Quattrocento to ensure perfect synchronization between the physiological signals and the behavior. Within this framework, the participant was asked to follow a trapezoidal force trajectory at 15% MVC whilst extending the little finger. As with the TA recordings, a central segment of 10 s was then used for algorithm validation.

In addition to HD-iEMG signals, we tested the proposed algorithm on a publicly-available dataset of invasive intracortical recordings (Horváth et al., 2015). The dataset contains signals recorded

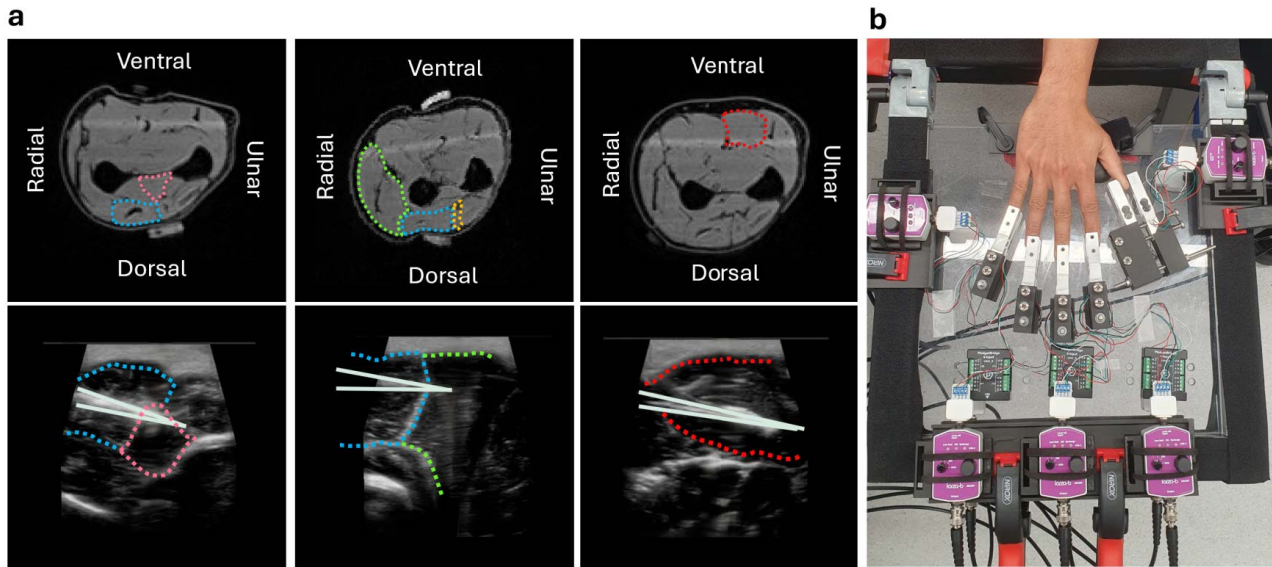


Figure 3.3: **a** MRI scans of the forearm. The boundaries of the muscles targeted are highlighted in color. In the first MRI image, a marker is visible, used to determine the precise relative positioning of the slice image within the forearm's anatomical structure. Ultrasound images of the muscles during the insertion of the thin-film electrodes. The boundary of the guiding needle is highlighted in white. The boundaries of the relevant muscles are in color (EPL: Pink, EDC: Blue, extensor carpi radialis brevis (ECRB): green, FDS: red). **b** Platform built for the forearm experimental protocol.

from two or three levels of the neocortex of 20 anaesthetised rats using a single-shank silicon probe with a 32x4 dense array. The signals were sampled at 20 kHz and filtered with a 250 Hz high-pass cut-off. Each recording had also been decomposed into units using the Kilosort2 MATLAB package (Pachitariu et al., 2016), followed by further manual curation, which gave a validated ground truth that we could compare SCD against. We selected the first 30 s of the recordings from the 12 rats with data from three anatomical levels, giving 36 time series to decompose.

3.3.2 Validation Methodology

We initially planned to validate the intramuscular against an existing set of spike timestamps (Muceli et al., 2022), obtained by a skilled manual operator using the EMGLAB software (McGill et al., 2005). However, we found that SCD discovered a number of units that were not in this set. These units were not detected by manual decomposition of the expert operator but, when their spike trains were shown to the operator, they were confirmed as valid sources. These sources were likely initially missed due to two reasons. Firstly, manual decomposition of signal from HD probes places an

extremely high burden on the manual operator, which increases the risk of missing units. Secondly, some of the units found only by SCD were extremely small and missed by the automatic thresholding method in EMGLAB, despite having spiking temporal statistics and a clear spike triggered average (PTP 0.12 mV), with propagation of the AP across channels (Fig. 3.4). To compensate for this deficit in the ground truth, we elected to present the results by SCD in two modes. In SCDa, the algorithm was run in a fully automated mode, with no *post hoc* correction. In SCDm, we went on to load the output timestamps from SCDa into EMGLAB and perform a spike-by-spike validation of each unit, before searching for any units in the residual signal that may have been missed, as in normal EMGLAB operation. This means that whenever SCDm has a higher yield than SCDa, it is because SCDa missed some units which were then found manually by an operator in EMGLAB. We also compared SCDa to the current SoA method for automated HD-iEMG decomposition (Negro et al., 2016), i.e. cBSS. This algorithm performs FastICA with a fixed contrast function set to $e = 3$ to find a source, before conducting an additional stage of optimisation, where the separation vector is recalculated using the spike triggered average and then reapplied to the signal in a loop until the CoV stops decreasing (Holobar & Zazula, 2007a).

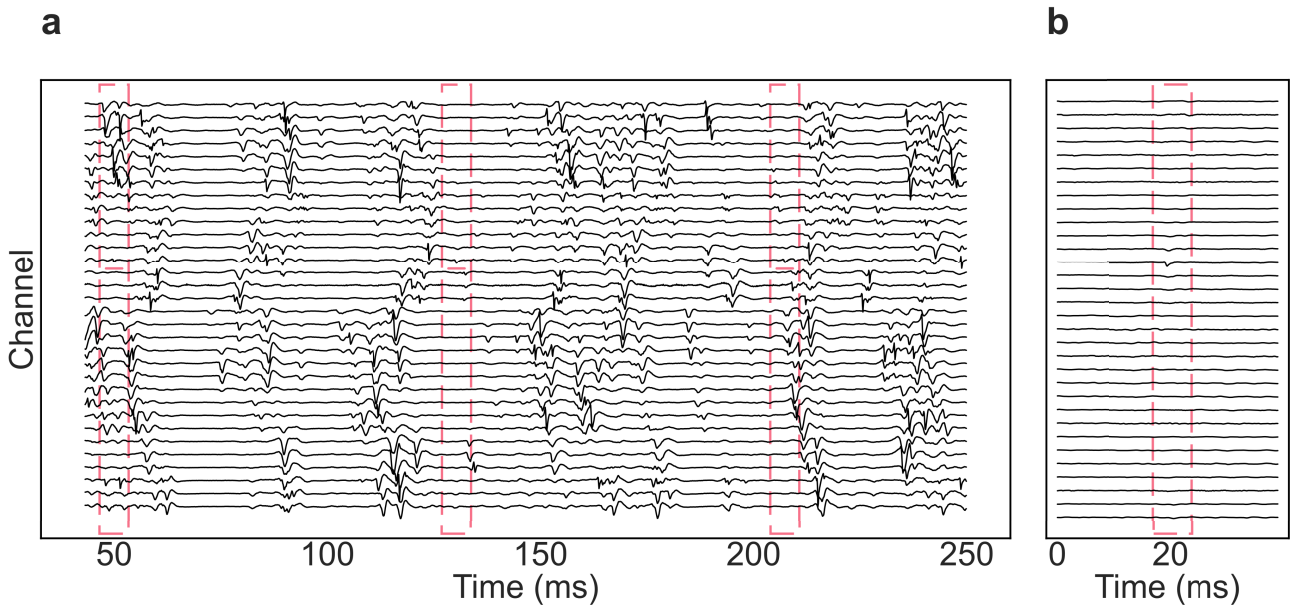


Figure 3.4: **a** A section of HD-iEMG signal from the tibialis anterior of subject 1. Highlighted in the red boxes are the spikes from a unit found by SCD, but missed by EMGLAB's automated thresholding and by the manual operator, due to a low peak-to-peak amplitude (0.12 mV). **b** Spike triggered average of the same unit. The waveform is clearly visible, which, combined with the clearly repeating nature of the timestamps, can allow us to be confident that this is indeed a real unit.

Table 3.1: Yield and per-source rate of agreement (median and interquartile ranges) results for the intramuscular EMG decompositions.

ID	Muscle	Force Level (%MVC)	Source Yield				RoAs (%)		
			cBSS	SCDa	Manual Only (EMGLAB)	SCDm (SCDa→ EMGLAB)	cBSS (all units / common with SCDa)	SCDa (common with cBSS)	SCDa (all units)
TA1	TA	10	10	48	53	57	95.5 (95.1-96.6)	95.6 (95.3-99.6)	96.1 (94.4-99.3)
	TA	20	13	66	57	71	98.4 (97.0-99.2)	100.0 (99.2-100.0)	99.1 (96.6-100.0)
	TA	30	11	64	60	82	98.5 (96.7-100.0)	99.7 (98.7-100.0)	95.9 (91.6-99.2)
TA2	TA	10	11	17	14	17	100.0 (100.0-100.0)	100.0 (100.0-100.0)	100.0 (99.3-100.0)
	TA	20	13	16	20	21	99.0 (97.3-100.0)	100.0 (98.5-100.0)	100.0 (98.5-100.0)
	TA	30	12	28	27	30	99.6 (99.1-100.0)	100.0 (99.0-100.0)	98.8 (96.8.0-100.0)
FM	EDC	15	4	10	-	13	98.6 (95.2-100.0)	99.0 (96.6-99.5)	95.5 (92.8-98.4)
	EPL	15	8	10	-	10	98.8 (97.8-99.4)	100.0 (99.1-100.0)	100.0 (99.1-100.0)
	FD	15	11	25	-	30	100.0 (99.6-100.0)	100.0 (99.6-100.0)	98.8 (95.3-100.0)

3.3.3 Assessment Metrics

For source acceptance, after a full set of PSO iterations, the Q_{SIL} is first calculated. For both HD-iEMG and intracortical data, a source was rejected if the Q_{SIL} was below a threshold τ_{SIL} of 0.85. Of the sources that pass this check, HD-iEMG sources are accepted if the Q_{CoV} is below a threshold τ_{CoV} and the firing rate Q_{FR} is below a threshold $\tau_{FR<}$, whilst for intracortical sources only the firing rate is calculated, which must be more than $\tau_{FR>}$ (refer to Section 3.3.4).

We quantitatively assessed the accuracy of the automatic decomposition methods by determining their RoA with the ground truth (Holobar et al., 2010; Muceli et al., 2022). This evaluation was undertaken for each MU, wherein the RoA was computed as the fraction of correctly identified discharges relative to the sum total of all discharges, both correctly and incorrectly identified.

The unit yield of decomposition was defined as the number of units that matched with a ground truth unit to an RoA of at least 30%, which was the case for all units.

3.3.4 Hyperparameters and Training

The hardware used for running the decomposition was an AMD Rome processor with an Nvidia RTX 6000 GPU. The *PyTorch* machine learning package was used to build the algorithm (Paszke et al., 2019). The optimal hyperparameters for the particle swarm update and source acceptance were determined by grid search, and were as follows: a standard deviation of 0.1 for both σ_1 and σ_2 ; respectively a value of 0.3 and 0.15 for c_1 and c_2 and finally an inertia of 1 which is then decayed by 0.1 with each PSO step. The number of particles J was set to 6 and associated starting contrast function e exponents set to [2, 3, 4, 5, 6, 7]. The same methodology was used to set the source acceptance thresholds τ_{SIL} and τ_{CoV} , set to 0.85 and 0.4 respectively, whilst $\tau_{FR<}$ and $\tau_{FR>}$ was set to 35 Hz and 1 Hz respectively based on the literature (Laine et al., 2015; Negro et al., 2016). For the optimisation of the separation vector, we employed stochastic gradient descent with a learning rate of 0.1 and a momentum of 0.9. Within the main decomposition loop, the maximum number of iterations was limited to 1000. If no new acceptable sources were found consecutively for 30 iterations, the termination criterion would be triggered. A source was considered a repeat if it had a maximum rate of agreement of 30% with the previously found sources. In such cases, the timestamps of the repeated source would be used in the peel-off process again, but not then saved to the library of timestamp sets. The computational time taken for the decomposition of 10 s of the 30 %MVC signal of TA1 was 128 hours for the "Manual Only" approach and 32 minutes for SCDA. The parameters described are generalizable to all datasets, ensuring consistent performance across different types of neural recordings. The key to this generalizability lies in the adapted contrast function, which automatically adjusts to the specific characteristics of each dataset.

3.4 Results

3.4.1 Intramuscular Recording Decomposition

The proposed algorithm, SCDA, significantly outperformed the current SoA automated BSS algorithm, cBSS (Negro et al., 2016), for HD-iEMG decomposition in both source yield and timestamp

accuracy as measured by the rate of agreement with the manual decomposition (Table 3.1). In fact, in a first for HD-iEMG we are aware of in the literature, SCDa actually outperforms manual decomposition in terms of MU yield on four of the six recordings for which manual decomposition timestamps were available. The bulk of the sources found by SCDa and not manual decomposition had very small peak-to-peak amplitudes and were not detected by EMGLAB's automated thresholding procedure, as can be seen in Figure 3.4. The semi-automated approach SCDm, when a manual operator instead decomposes the HD-iEMG using SCDa-generated timestamps as a reference, gives a higher number of sources than both the automated and manual methods. The median and interquartile range (IQR) of the Q_{CoV} and Q_{FR} for the 231 manually decomposed units were 12.3 (IQR 10.6-14.3) and 12.3 (IQR 10.8-13.6) respectively. For the 47 extra units found from SCDa and successively cleaned in EMGLAB we report a median Q_{CoV} of 12.7 (IQR 11.2-14.5) and median Q_{FR} of 13.6 (IQR 12.4-14.9), further confirming their physiological characteristics. Consequently, we use the SCDm yield and timestamps as gold standards with which to compare the automated methods.

Across the HD-iEMG recordings TA1, TA2 and FM, SCDa found 85.8% of the total sources found by SCDm, whilst cBSS found 28.1%. All 93 sources found using cBSS were also found by SCDa, with a further 191 sources found by SCDa. None of the sources found by either SCDa or cBSS were subsequently found to not be sources on manual inspection. Per-source decomposition accuracy of the automated algorithms was also assessed, by calculating the rate of agreement between their output timestamps and the manually-curated timestamps from SCDm. The median RoA of SCDa across all HD-iEMG recordings was 98.8% (IQR 96.1%-100.0%), whilst for only those sources matched with cBSS it was 100.0% (IQR 99.5%-100.0%) versus 98.8% (IQR 98.5%-99.6%) for cBSS, a highly significant difference as measured by Wilcoxon signed-rank test ($p < 0.0001$).

3.4.2 Intracortical Recording Decomposition

In the rat intracortical recordings SCDa found 1062 sources, 449 of which were matched with the manually-curated Kilosort2 decomposition (Fig. 3.5), with a median RoA of 96.7% (IQR 92.8%-98.5%). Figure 3.6 shows an example raster from an SCDa decomposition from depth two of rat six, alongside some waveform examples and principal component projections. SCDa was able to find even very

low peak-to-peak amplitude AP waveforms. Another advantage is that the separation vector acts to align the AP waveforms temporally, meaning no further alignment is needed after thresholding, as is needed in lower-order spike sorting methods like KiloSort (Pachitariu et al., 2023).

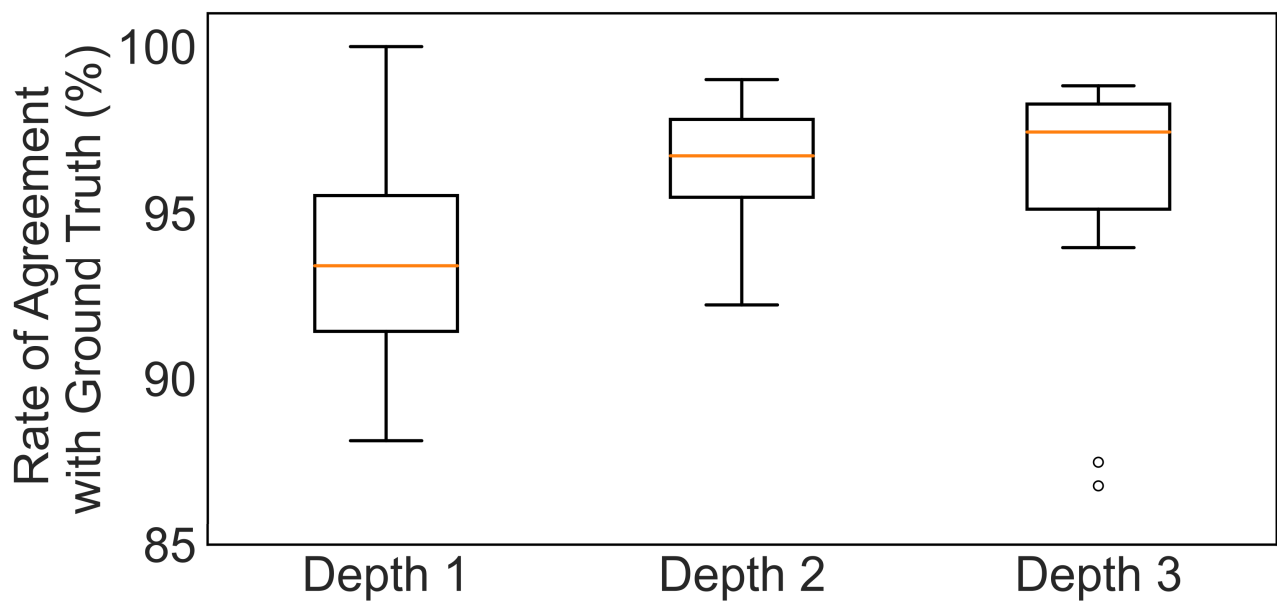


Figure 3.5: Distribution box plot of the median rates of agreement calculated between SCDa and KiloSort2 across all intracortical recordings.

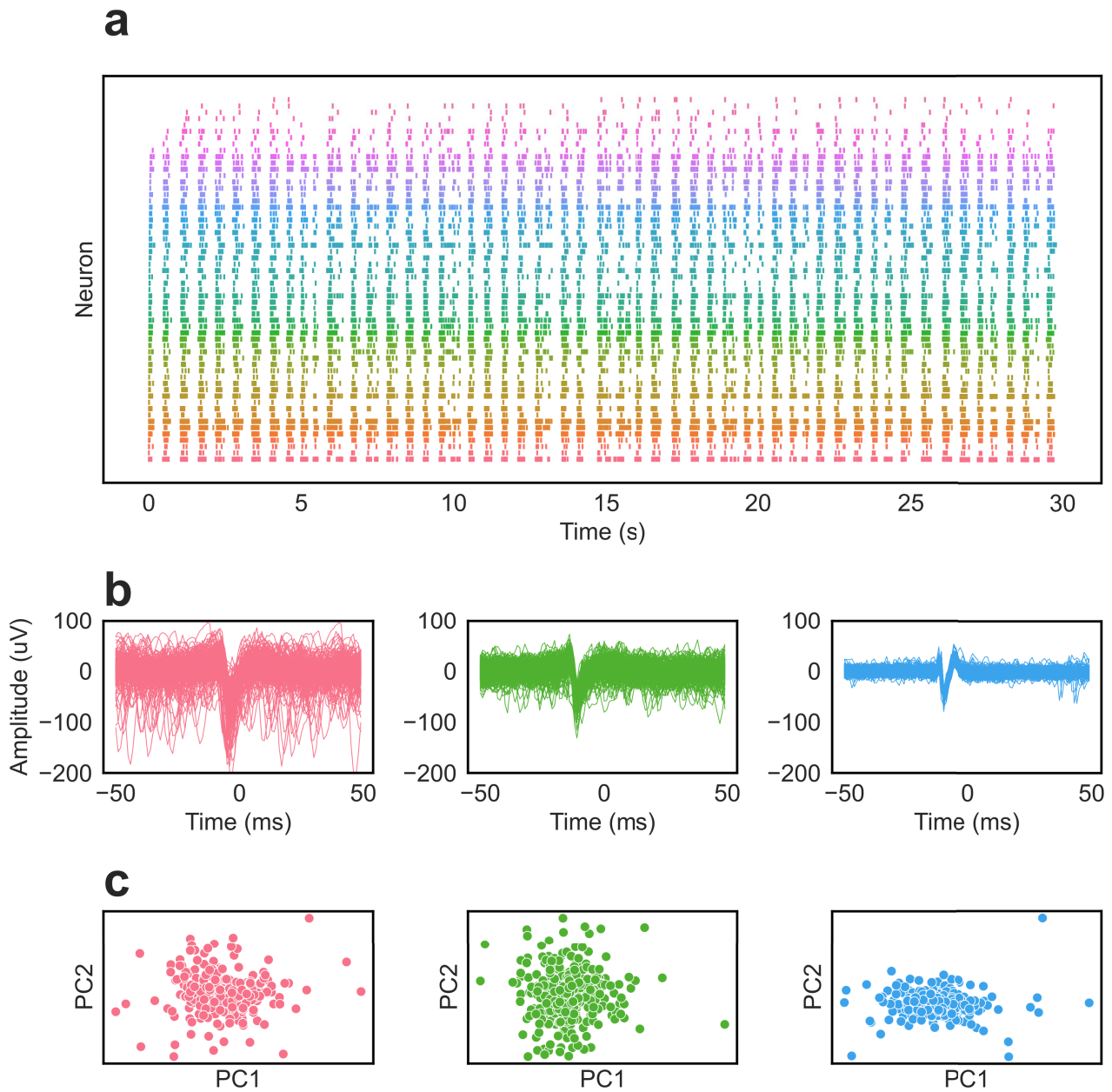


Figure 3.6: **a** Example raster plot of all sources after running SCDa. **b** Spike-triggered waveforms of three found sources with low peak-to-peak amplitudes (≤ 0.30 mV). **c** Projection of the action potential waveforms in principal component space for each of the above sources. One cluster is visible, confirming the correct feature was extracted.

3.4.3 Effect of Particle Swarm Optimisation

Once it was confirmed that SCDa was performing well across the data types included in this study, we further examined the effect of using PSO to discover the best ICA contrast function for each source. Figure 3.7 shows how the PSO algorithm was able to quickly converge on an optimal contrast

exponent e for each source, which varied significantly at the population level between data types (one-way ANOVA, $p < 0.0001$).

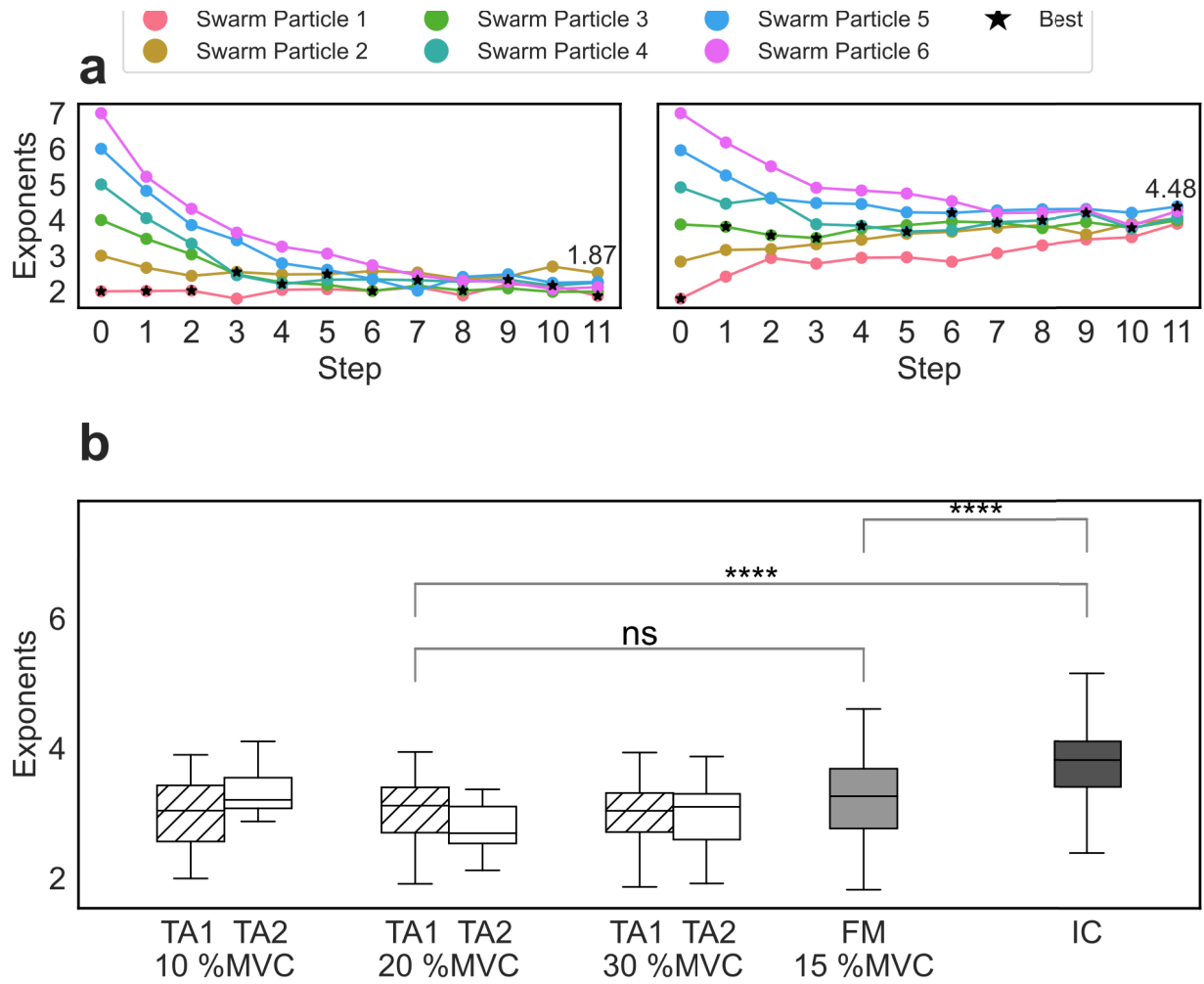


Figure 3.7: **a** Convergence of swarm particles for two sources during projection pursuit ICA on HD-iEMG data. Particle swarm optimisation quickly finds the optimal exponent value e in the contrast function $f(s_j) = E\{sign(s_j)|s_j|^e\}$ for each source. **b** Box and whisker plots of the final best value of e across the different data types, showing the optimal e can be significantly different.

A further ablation study was performed using TA1 and TA2, where the PSO component was removed and the cubic contrast function of $E\{s_j^3\}$ used instead (Negro et al., 2016), the results of which are shown in table 3.2. SCDa had a significantly higher yield than the fixed projection pursuit ICA as assessed by paired t-test ($p = 0.01$), proving its utility in the decomposition of invasive neurophysiological data.

Table 3.2: **Yields** and *MEDIAN RATE OF AGREEMENTS (IQR)* for TA1 and TA2 of a fixed cubic contrast (as in (Negro et al., 2016)) versus SCDa.

ID	Force Level (%MVC)	Fixed Cubic	SCDa
TA1	10	42 , 95.9 (93.8-97.9)	48 , 96.1 (94.4-99.3)
TA1	20	52 , 98.8 (97.2-99.5)	66 , 99.1 (96.6-100.0)
TA1	30	53 , 97.0 (92.8-98.5)	64 , 95.9 (91.6-99.2)
TA2	10	15 , 100.0 (99.3-100.0)	17 , 100.0 (99.3-100.0)
TA2	20	9 , 100.0 (99.2-100.0)	16 , 100.0 (98.5-100.0)
TA2	30	26 , 99.1 (96.4-100.0)	28 , 98.8 (96.8-100.0)

Table 3.3: The effect of additive white noise on SCDa **yields** and *MEDIAN RATE OF AGREEMENTS (IQR)* compared to the ground truth.

SNR dB	10 %MVC	20 %MVC	30 %MVC
30	44 , 100.0 (99.0-100.0)	44 , 99.3 (98.5-100.0)	47 , 100.0 (98.8-100.0)
25	42 , 100.0 (97.4-100.0)	49 , 99.3 (98.5-100.0)	45 , 100.0 (97.9-100.0)
20	36 , 100.0 (96.6-100.0)	45 , 99.3 (97.1-100)	46 , 99.1 (94.3-100.0)
15	31 , 100.0 (96.5-100.0)	33 , 99.2 (95.4-100.0)	38 , 98.7 (93.7-100.0)
10	25 , 98.9 (96.6-100.0)	23 , 97.1 (92.6-99.3)	19 , 97.9 (91.1-99.7)
5	11 , 97.7 (96.3-100.0)	11 , 97.0 (94.4-99.6)	9 , 97.2 (94.5-99.3)
0	1 , 96.9	3 , 97.0 (95.3-98.0)	1 , 95.8

3.4.4 Noise Sensitivity Analysis

Finally, in Table 3.3, we report the source yield for the dataset when a range of additive normally distributed noise was added to the reconstructed signals to give specific SNR as calculated by Welch's

method. The yield is relatively stable until 15 dB, when it decreases quickly, eventually collapsing at 0 dB. The RoA is consistently high across different levels of additive noise, most likely because these are only the largest amplitude AP waveforms that can be found.

3.5 Conclusion

This study presented a re-evaluation of the non-linear contrast function used to estimate independence of a source in projection pursuit ICA, specifically when those sources are the sparse time series that make up multichannel invasive recordings. We showed that there exists an optimal level of non-linearity when optimising a separation vector: too low and the vector will fail to separate similar sources; too high and the optimisation collapses as the within-source variance begins to dominate the selectivity of the vector. We then demonstrated that a particle swarm optimisation method can quickly find this optimal non-linearity. The resultant BSS algorithm, Swarm-Contrastive Decomposition, extracts large volumes of neural sources in a variety of invasive data, including SoA performance in the intramuscular EMG domain, even outperforming manual source separation in some recordings.

A high-accuracy and fully automated decomposition pipeline offers a realistic method by which HD-iEMG recordings can be processed. Consequently, it paves the way for population-level muscle data investigations, refining neural interfaces and analytical methodologies for a deeper understanding of neural movement control. Regarding potential limitations, like most BSS algorithms, the *post hoc* assessment of sources is limited by the accuracy of the quality metric. This motivates planned work integrating better methods of automatically assessing neurophysiological sources. Whilst it was not a focus of the study, SCD showed promise in intracortical data as well; we hope to validate this more robustly in a future study. The source code is available at <https://github.com/AgneGris/swarm-contrastive-decomposition>.

While HD-iEMG technological advances are promising, fundamental questions remain about the optimal design of HD-iEMG recording configurations. Building on these findings, the next chapter undertakes a systematic investigation of electrode array configurations to maximize decomposition

performance. Specifically, it examines critical design parameters in large muscles such as the TA, including the optimal number of electrodes required for comprehensive coverage and the impact of density and IED spacing on signal quality and decomposition accuracy. This analysis will provide essential insights for optimizing HD-iEMG array designs, ultimately advancing our ability to extract reliable MU data from complex muscular structures.

The joy of learning is as indispensable in study as breathing is to running

Weil, Simone

4

Motor Unit Sampling from Intramuscular Micro-electrode Array Recordings

NOTE: Aside from minimal alterations to fit the format and flow of the thesis as a cohesive single document, the content of this chapter has been published in the following journal article:

Grisson, A., Ibáñez Pereda, J., Farina, D. (2025). Motor unit sampling from intramuscular micro-electrode array recordings, IEEE Transactions on Neural Systems and Rehabilitation Engineering. doi: <https://ieeexplore.ieee.org/document/10844937> (Under licence CC-BY)

Chapter 3 proposed a novel BSS decomposition method for HD-iEMG signals. Our proposed method maximizes the amount of information that can be extracted from HD-iEMG signals, allowing to study the population of neurons in an automatic and reliable way.

Although HD-iEMG has been shown to allow the accurate decoding of a larger number of MUs compared to HD-sEMG, the dependence of MU yield on the parameters of the micro-electrode arrays is still unexplored. In this chapter, we used recently developed HD-iEMG electrodes to record from hundreds of recording sites within a single muscle. This allowed us to investigate the impact of the number of electrodes, IED, and the number of muscle insertions on the ability to sample MUs within the muscle. Specifically, we recorded both HD-sEMG and HD-iEMG from the TA muscle of two healthy subjects at various contraction intensities (10%, 30%, and 70 %MVC). For the first time, we present intramuscular recordings with more than 140 electrodes inside a single muscle, achieved through multiple implants of HD micro-electrode arrays. Through systematic offline analyses of these recordings, we tested different electrode configurations to identify optimal setups to accurately capture MU activity. The results revealed that the density of electrodes in the micro-electrode arrays is the most critical factor to maximize the number of identified MUs and to ensure very high accuracy. Comparisons between intramuscular and surface recordings also confirmed that HD-iEMG consistently captures larger and more stable numbers of MUs between subjects and contraction levels. These results underscore the potential of HD-iEMG as a powerful tool for both clinical and research settings, particularly when precise MU decomposition is crucial.

4.1 Introduction

Rapid advancements in neural interface technologies are fostering the development of innovative systems capable of restoring sensory, communicative, and control capabilities for individuals with impairments. These technologies enable interactions between the nervous system and external devices.

Myoneural interfaces utilising electromyography (EMG) to interface muscles offer a reliable and accessible means to capture motor commands issued by the central nervous system (at Reality Labs

et al., 2024; Bergmeister et al., 2019). By interfacing directly at the muscle level, these systems access the MUs, comprising spinal motor neurons and their innervated muscle fibers. The activity of MUs has been extensively studied over the past decades using intramuscular needles or wire electrode recordings (Adrian & Bronk, 1929; Farina & Gandevia, 2024; Muceli et al., 2022).

To overcome the limited MU sampling offered by traditional needle or wire electrodes, HD-sEMG has been shown to enable the decoding of a significantly larger number of MUs (Farina & Enoka, 2023; Zheng et al., 2022). This has paved the way for the development of HD-iEMG technologies (Farina et al., 2016; Muceli et al., 2015), which combine the selectivity of traditional intramuscular EMG with the extensive spatial sampling of high-density surface recordings. Indeed, HD-iEMG overcomes several limitations of traditional non-invasive muscle recordings to decompose motor neuron activity, such as the filtering effects of the volume conductor, sensitivity to electrode positioning (Mesin et al., 2009), amplitude cancellation (Keenan et al., 2005), and cross-talk (Farina, Merletti, Indino, et al., 2002), which often lead to reduced accuracy in estimating the neural drive to muscle. By providing greater spatial sampling and access to a larger pool of motor neurons, HD-iEMG enables a more precise and direct measurement of muscle activity. This advancement facilitates enhanced analysis of the multiple neural inputs that the motor neurons receive from different regions of the nervous system, offering a deeper understanding of the neural information underlying volitional motor control.

Recent advancements in intramuscular electrode technology have enabled EMG recordings with highly dense configurations (Muceli et al., 2015, 2022), allowing multi-channel recordings from dozens of detection sites within the muscle. Current HD-iEMG electrode designs have largely been informed by theoretical models, with limited empirical studies investigating the critical factors for optimal configuration in extracting MU information. As a result, the critical design parameters that maximise the ability of HD-iEMG to decode the neural signals driving muscle activity remain insufficiently explored.

In this study, we conducted experiments using newly developed HD-iEMG electrodes (Muceli et al., 2022) to record muscle activity from the Tibialis Anterior muscle of two healthy participants at 10 %, 30 %, and 70 % of the MVC during ankle dorsiflexion. We simulated different electrode configurations

offline by selecting subsets of electrodes and analysing the number of decomposed units and the quality of the decomposition as outcome measures. This approach allowed us to evaluate the impact of key parameters, such as IED, the number of recording sites, and the use of multiple HD-iEMG micro-electrode arrays within the same muscle, to maximise the decomposition potential of HD-iEMG.

In summary, this study made the following contributions:

1. We conducted, for the first time, intramuscular recordings with over 140 recording sites from a single muscle, marking the first instance of truly HD-iEMG;
2. We directly compared surface and intramuscular recordings from the same individuals under identical experimental conditions, assessing their respective capabilities in identifying MU populations;
3. We analysed the relative importance of IED and number of recording sites on MU sampling in intramuscular EMG.

4.2 Methods

4.2.1 EMG Model and Decomposition

A key challenge in EMG signal analysis is the deconvolution of the composite signals to isolate individual MUAPs and their firing sequences (D. Stashuk, 2001).

Decomposition strategies for HD-EMG signals can be broadly categorised based on whether they utilise classic spike sorting methods (template matching) or BSS. Template matching approaches correlate the observed MUAP waveforms with predefined templates (Zhou & Rymer, 2004). While effective under low-force conditions, these methods face challenges at medium to high-force levels (Kleine et al., 2007) due to the increased recruitment of MUs. A large number of active MUs determines a high probability of MUAP superimpositions over time, making it difficult to separate individual MUAPs (Zhou & Rymer, 2004).

In contrast, BSS techniques, such as the CKC (Holobar & Zazula, 2007a) and its variants (Holobar & Zazula, 2007b; Ning et al., 2014), rely solely on the statistical properties of the MU discharge patterns for decomposition, ignoring filter kernel information (Holobar et al., 2012).

Initially developed for HD-sEMG signal decomposition, these methods utilise contrast functions G , such as $G(s_j) = \frac{1}{6} s_j^3$, to maximise source independence (or *sparseness*) while being robust to outliers (Negro et al., 2016).

Initially, BSS methods were developed and applied to HD-sEMG, as these techniques require a large number of recording channels. However, the advancement of HD-iEMG technologies has now enabled the use of BSS for intramuscular recordings as well. Recently, a novel BSS-based algorithm, SCD, was proposed specifically for HD-iEMG (Grison et al., 2024). SCD utilises a particle swarm optimisation approach to adaptively traverse a polynomial family of nonlinearities. These nonlinearities are designed to approximate the asymmetric cumulants of the sources, which are statistical measures capturing the asymmetry and higher-order dependencies in the data.

The key innovation of SCD lies in its ability to tailor the nonlinearity to each source individually, allowing it to adapt more precisely to the characteristics of the data. By doing so, SCD can search the solution space more efficiently and effectively. It can also leverage data points that would typically be considered outliers, turning them into useful information for the decomposition process.

SCD has demonstrated superior accuracy and performance compared to other convolutive BSS techniques, for HD-iEMG data. In some instances, it has even surpassed the accuracy of manual decomposition methods, considered the gold standard in many applications (Grison et al., 2024). Given these advantages, in this study we employed SCD for the signal decomposition of HD-iEMG data. For the decomposition of HD-sEMG data, we used the more commonly applied cBSS technique (Negro et al., 2016). In this way, we utilised the best algorithm for each type of data, acknowledging that SCD has not yet been validated for HD-sEMG data, hence our choice to use the validated cBSS method.

4.2.2 Data

The HD-iEMG signals were recorded using multi-channel micro-electrode arrays designed for acute recordings (Muceli et al., 2015, 2022). Each micro-electrode array comprised 40 platinum detection points ($140\ \mu\text{m} \times 40\ \mu\text{m}$) arranged in two linear arrays of 20 electrodes each, spaced by 1 mm. The two sides of the filament were shifted by 0.5 mm, resulting in a linear array with a 0.5 mm IED (Muceli et al., 2022).

The HD-iEMG and the HD-sEMG signals were recorded using a multi-channel amplifier (OT-Bioelettronica, Torino, Italy), sampled at 10,240 Hz, high-pass filtered at 10 Hz, and analog-to-digital converted with 16-bit resolution. The EMG signals were acquired in a monopolar derivation configuration, with a reference electrode placed on the ankle.

All experimental procedures adhered to the ethical guidelines set by Imperial College London (ICREC Project ID 19IC5640) and were performed in accordance with the Declaration of Helsinki, with informed consent obtained from all participants prior to each experiment.

Experiments were performed on the TA muscle of two healthy men, aged 30 and 39 years. The participants had no history of neurological or musculoskeletal disorders. Although the sample size was small, the complexity of the recordings, involving multiple insertions into the same muscle, limited the feasibility of including a larger subject pool. Three intramuscular micro-electrode arrays were inserted into the TA of Subject 1 (S1), and four intramuscular micro-electrode arrays were inserted into the TA of Subject 2 (S2), approximately 3 cm apart in the longitudinal direction. An additional HD-iEMG array was inserted in the TA of S2 because of poor recording quality of one of the previously inserted arrays that had nearly half of the recording sites outside the muscle. Following insertion, subjects were asked about any discomfort caused by the HD-iEMG arrays at rest and during muscle contractions, and they reported none. Two 64-channel surface grids (4 mm IED, 13x5 configuration) were placed on the belly of the muscle, next to the intramuscular insertions for both subjects. Subjects were seated with their right leg and foot constrained to a dynamometer and instructed to sustain an isometric ankle dorsiflexion during all experiments. The participants completed an MVC trial after the insertion of the intramuscular electrodes, which helped secure the

electrode and provided a reference for measuring relative forces. The relative forces were determined as percentages of the MVC, with visual feedback provided to the subjects regarding the exerted force and target. The subjects performed trapezoidal contractions at 10, 30, and 70 %MVC ramping up and down at a rate of 10 %MVC/s. The force was sustained for 20 seconds at 10% and 30 %MVC, and for 10 seconds at 70 %MVC. These force levels were selected to encompass a range from low to medium to high intensity muscle contractions. The isometric portion of the contractions, corresponding to the plateau phase, was used for the decomposition to adhere to the stationary assumption of the model. All results are reported based on the isometric segments of the recordings, except for the recruitment threshold analysis. For this analysis, the separation filters of the decomposed MUs were reapplied to the entire contraction to track the MUs during the ramp (Martinez-Valdes et al., 2017). After tracking, the discharge rates of each MU were recomputed during the plateau phase of the recording and utilized for the recruitment threshold analysis. Outliers, defined as values falling outside the interquartile range, were excluded as no manual cleaning was performed after tracking. The recruitment threshold was determined as the force level at which each MU began to fire regularly.

Figure 4.1 shows the setup of the electrodes for S1 (i) and for S2 (ii).

4.2.3 Experiments

Experiments were conducted to isolate specific variables and evaluate their impact on the yield and accuracy of MU identification from HD-iEMG recordings. Additionally, the yield and accuracy of decomposition from concurrently recorded HD-sEMG recordings are presented to provide a comparative analysis between the two types of recordings. The accuracy in decomposition was evaluated with the SIL measure, as defined in (Negro et al., 2016). This metric is linearly associated to the accuracy with which the series of MU discharge times is estimated. To compare the accuracy of decomposition between HD-iEMG and HD-sEMG, we selected the top ν units with the highest accuracy from the full set of HD-iEMG signals (i.e., 112 channels for S1 and 142 for S2), where ν represents the number of units decomposed from the corresponding HD-sEMG. For example, if the HD-sEMG decomposed 11 units at 10 %MVC for S1, we selected the top 11 units from the HD-iEMG

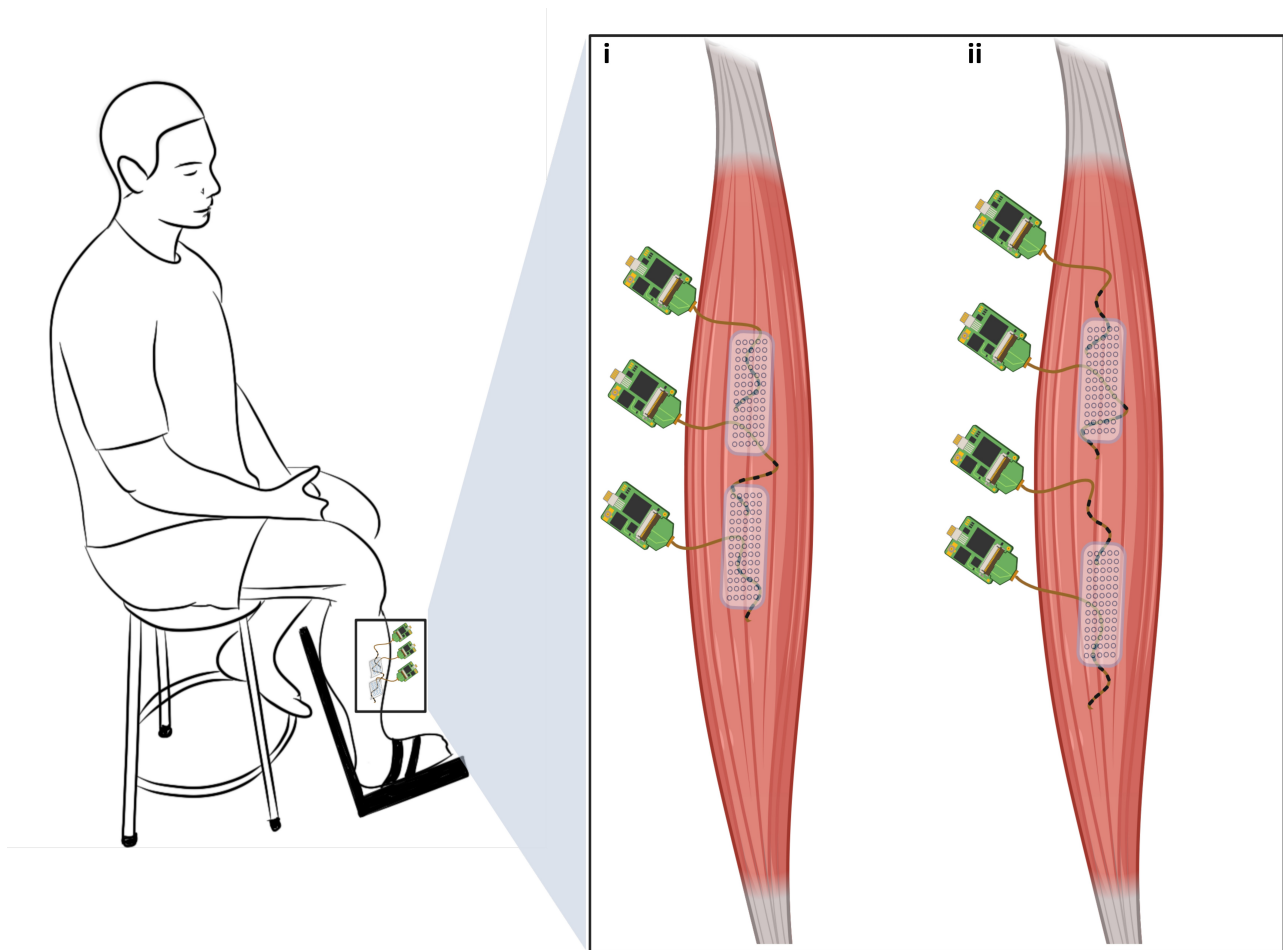


Figure 4.1: Schematic representation of the experimental setup. HD-iEMG and HD-sEMG signals (2 grids, 64 channels with 4 mm IED) were concurrently recorded while participants were seated in a chair and were producing ankle dorsiflexions. **i** Three HD-iEMG electrodes were inserted in the TA of S1. **ii** Four HD-iEMG electrodes were inserted in the TA of S2. Created with BioRender.com.

decomposition and compared the distributions of the SILs.

For all subsequent analyses, channels that presented flat signal profiles or baseline noise exceeding three standard deviations the mean noise level were excluded from the study. Notably, approximately 15 channels from the third micro-electrode array in S2 were identified as external to the target muscle area. Consequently, the effective channel count was adjusted to 112 from an initial 120 for S1, and 142 from an initial 160 for S2. For the HD-sEMG analyses, 2 channels from S1 and 17 channels from S2 were excluded.

4.2.3.1 Data Analysis 1

This analysis aimed to study the accuracy and the number of decomposed MUs based on different numbers of available channels and their locations within the muscle. A subset of channels, denoted as p , was subjected to combinatorial assessment. Permutations were derived from the binomial coefficient $\binom{p}{q}$ for each of $q = 1, 2, 3, \dots, p$. This means that for each value of q , different combinations of q channels were selected from the total p channels. This process was repeated $k = 100$ times for each value of q to ensure robustness and reliability of the results. For subject S1, $p = 112$, and for subject S2, $p = 142$. Figure 4.2a illustrates one such configuration for $q = 62$. This analysis was performed for all force levels (10, 30, 70 %MVC). Because the channels were always selected within the same micro-electrode arrays, changing the number of channels also corresponded to changing the density of electrodes.

4.2.3.2 Data Analysis 2

This analysis assessed the number of decomposed MUs by subsampling the channels for each electrode maintaining a fixed IED. Six configurations were tested per electrode. The electrode with 15 channels outside the muscle in S2 was excluded from this analysis. The configurations were as follows: IED 1 mm (**i**, **iv**), IED 2 mm (**ii**, **v**), IED 4 mm (**iii**, **vi**), as illustrated in Figure 4.2c. Channels identified as flat or noisy in Experiment 1 were also excluded from this analysis. This occasionally resulted in fewer channels than the expected theoretical number.

To evaluate whether a stochastic selection of channels could yield comparable results to a systematic channel reduction, we compared these results with random subsampling of the channels. This analysis was conducted separately for each array, selecting k permutations based on the minimum number of available channels across all electrodes. This resulted in $k=35$ for S1, and $k=36$ for S2. Due to computational constraints, this analysis was performed only for 30 %MVC.

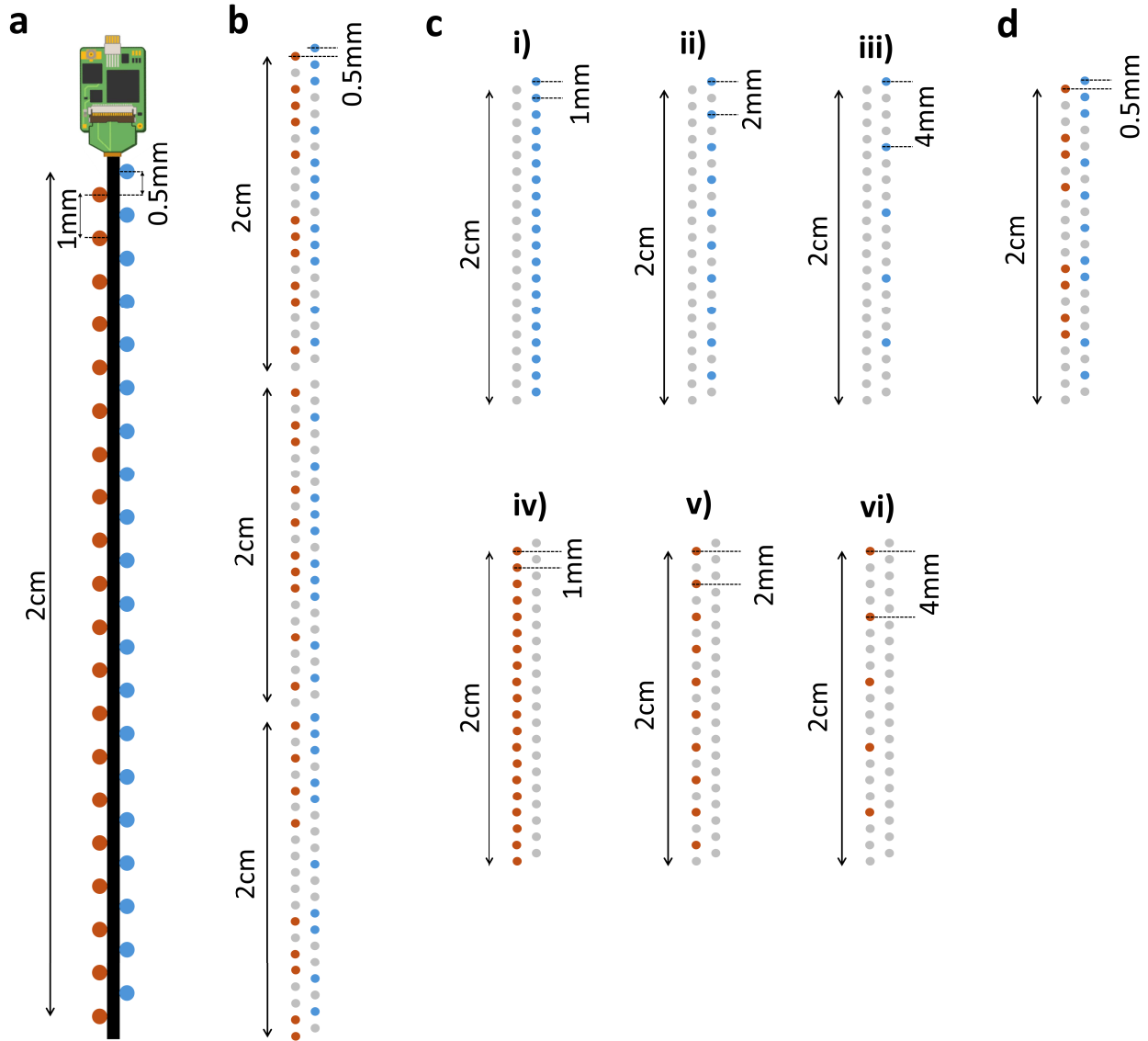


Figure 4.2: The electrode array configurations considered in this study. **a** Schematic of electrode. **b** Representative example of the configuration for number of channels $q = 62$, and random selection $k = 1$. **c** Per electrode channel subsampling keeping a fixed IED. In detail: IED 1 mm (**i**, **iv**), IED 2 mm (**ii**, **v**), IED 4 mm (**iii**, **vi**). **d** Schematic representation of the random subsampling implemented per electrode.

4.2.4 Hyperparameters

The decomposition process was executed on an AMD Rome processor paired with an Nvidia RTX 6000 GPU. In all experiments, only the selected channel configurations were varied. Signals were filtered with a high-pass filter with a cutoff frequency of 10 Hz. The low-pass filter cutoff was set at 4400 Hz for HD-iEMG and 500 Hz for HD-sEMG. Decompositions were conducted exclusively during isometric contractions, with the number of iterations capped at 250. For SCD applied to HD-iEMG, an extension factor of 20 was applied to the observations. The decomposition process was terminated if no new acceptable sources were identified for 20 consecutive iterations. For HD-sEMG decomposition, all parameters were chosen as specified in (Negro et al., 2016).

For all decompositions, the SIL cutoff was set at 0.85. In postprocessing, only units with a coefficient of variation of the interspike interval below 40 % and FRs lower than 30 Hz were retained for further analysis.

The level of agreement between the activities of MUs decoded from the surface and intramuscular recordings was assessed using the RoA. The RoA measures the fraction of commonly identified discharges relative to the total number of discharges, considering both common and not common firings.

4.3 Results

4.3.1 Characterization of the decomposed motor units

The number of MUs decomposed using HD-iEMG across the three force levels and the two subjects ranged between 24 and 53. With the HD-sEMG signals, the number of units decomposed was between 3 and 19. Table 4.1 summarizes the number of decomposed MUs from the maximum number of available channels per subject and contraction level. The distribution of discharge rates of all decomposed MUs is presented in Figure 4.3. Boxplots and density plots depict the distributions of MUs from HD-iEMG signals, while black circles indicate the values for the HD-sEMG signals. The

discharge rates showed a consistent increase with force levels (Fig. 4.3 a), which is an expected outcome reflecting the physiological relationship between force production and neural drive. The values of the HD-sEMG closely align with the distributions of the HD-iEMG units, showing that similar information was extracted with both methods. Figure 4.4 shows the relationship between the recruitment threshold and the discharge rates. As expected, the firing rates increased relative to the excitatory input (De Luca & Hostage, 2010).

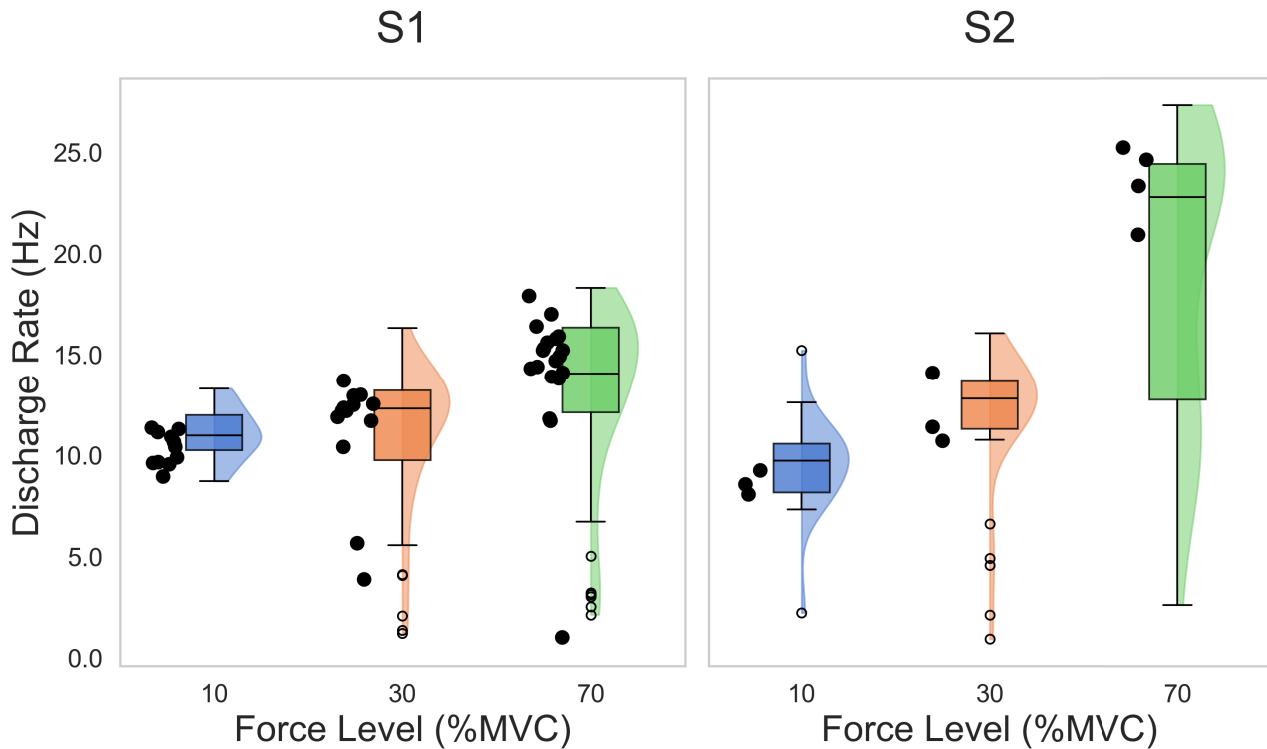


Figure 4.3: Distribution of MU discharge rates (Hz) measured at three force levels (10%, 30%, and 70 %MVC) in two subjects (S1 and S2). The data combines measurements from two recording techniques: HD-iEMG (represented by boxplots with overlaid density plots in blue, orange, and green) and HD-sEMG (shown as black circles). The figure demonstrates how discharge rates generally increase with force level, with wider distributions observed at higher force levels. Each data point represents an individual MU's discharge rate, with the vertical axis showing rates from approximately 0-25 Hz.

Additionally, Table 4.1 reports the number of matched units between the HD-iEMG and the HD-sEMG, and the RoA between them. This analysis provides a conservative estimate of the decomposition accuracy for the two recording types. Indeed, the RoA considers the total number of decomposition errors from the two methods. Importantly, we could find matched units between surface and intramuscular recordings in both subjects and at all contraction levels, with very high RoA, which provides a strong validation of the decomposition carried out with the two types of sig-

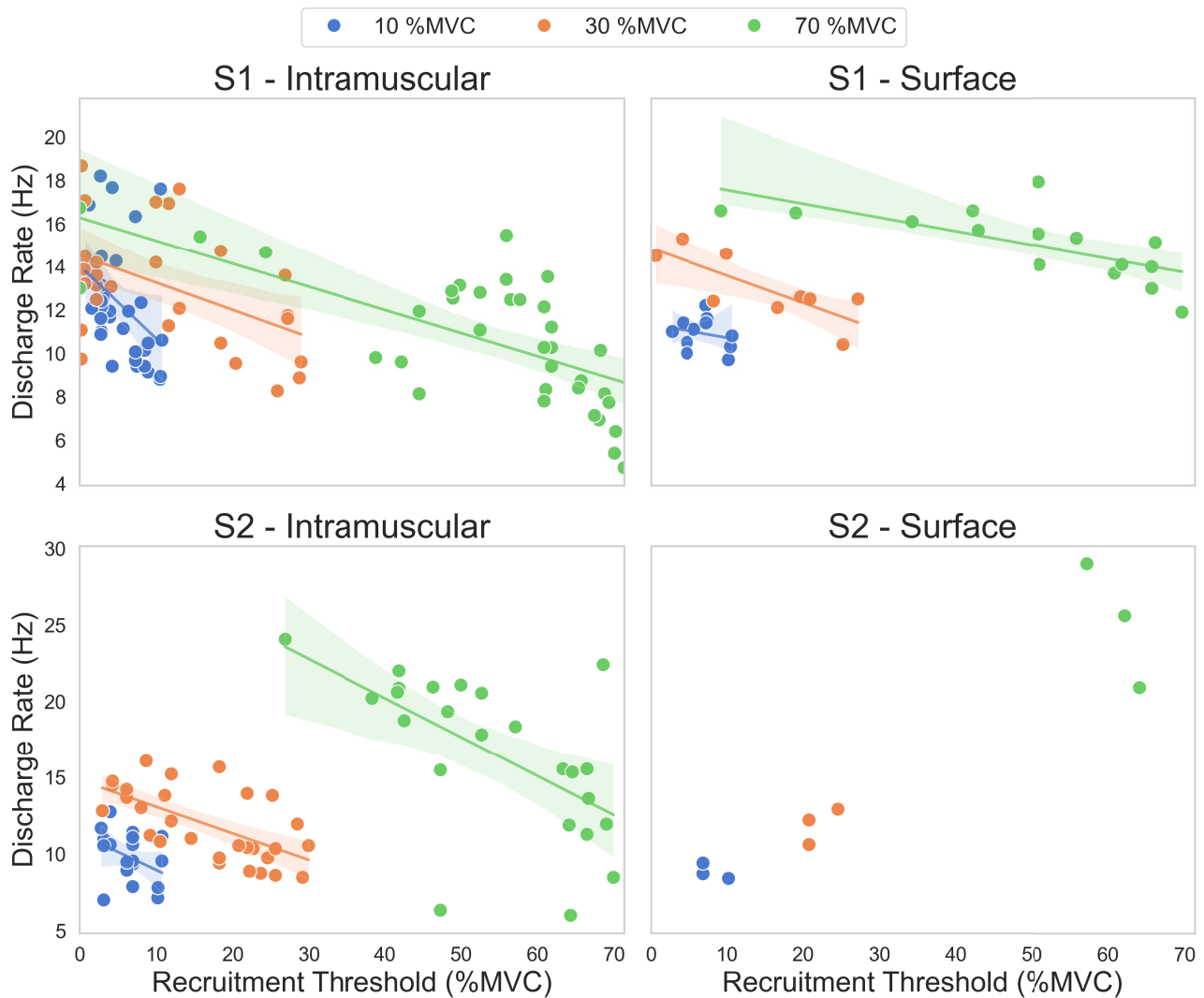


Figure 4.4: Recruitment thresholds and MU discharge rates across the three force levels and the two subjects, and compared between MUs decomposed from the HD-iEMG and HD-sEMG signals. Relationship between MU recruitment thresholds (%MVC) and discharge rates (Hz) at three force levels (10%, 30%, and 70 %MVC) for two subjects (S1 and S2). Data are shown separately for intramuscular recordings (HD-iEMG, left panels) and surface recordings (HD-sEMG, right panels). Linear regression lines with 95% confidence intervals (shaded areas) demonstrate the negative correlation between recruitment threshold and discharge rate, particularly visible in the intramuscular recordings. Different force levels are color-coded (blue: 10 %MVC, orange: 30 %MVC, green: 70 %MVC), showing distinct clusters of MU behaviour.

nals (Mambrito & De Luca, 1984; Negro et al., 2016). The median SIL value of the matched units between the HD-iEMG and HD-sEMG signals was 0.94 (interquartile range: 0.93-0.97) for the HD-iEMG signals and 0.95 (interquartile range: 0.93-0.97) for the HD-sEMG signals. The slightly higher median SIL for HD-sEMG units likely reflects the fact that the matched units are predominantly superficial and so farther from the intramuscular electrodes, making them more difficult to decompose in HD-iEMG recordings.

Table 4.1: Number of decomposed MUs from the HD-iEMG and the HD-sEMG signals for S1 and S2 at different force levels. For each subject and force level, the number of common MUs between the HD-iEMG and the HD-sEMG, together with the range of their RoA, are provided.

Subject	Force Level (%MVC)	No. of MUs (HD-iEMG)	No. of MUs (HD-sEMG)	No. of common MUs	RoA of common MUs
S1	10	40	11	4	89.3 - 100.0
	30	53	13	1	90.9
	70	54	19	3	87.9 - 96.4
S2	10	24	3	2	98.1 - 98.9
	30	45	3	1	97.4
	70	37	4	1	92.7

4.3.2 Number of Decomposed Motor Units as a Function of the Number of Channels

Figure 4.5 illustrates an example of concurrently recorded signals at 70 %MVC for S1. Panel **a** shows the HD-iEMG signals and panel **c** shows the HD-sEMG signals. The respective raster plots are presented in panels **b** (HD-iEMG) and **d** (HD-sEMG). The intramuscular signals provide higher spatial specificity, with individual action potentials typically being detectable across 6-8 channels. As a consequence, HD-iEMG allowed a decomposition of a larger set of units than HD-sEMG (54 units

vs 19 in this example).

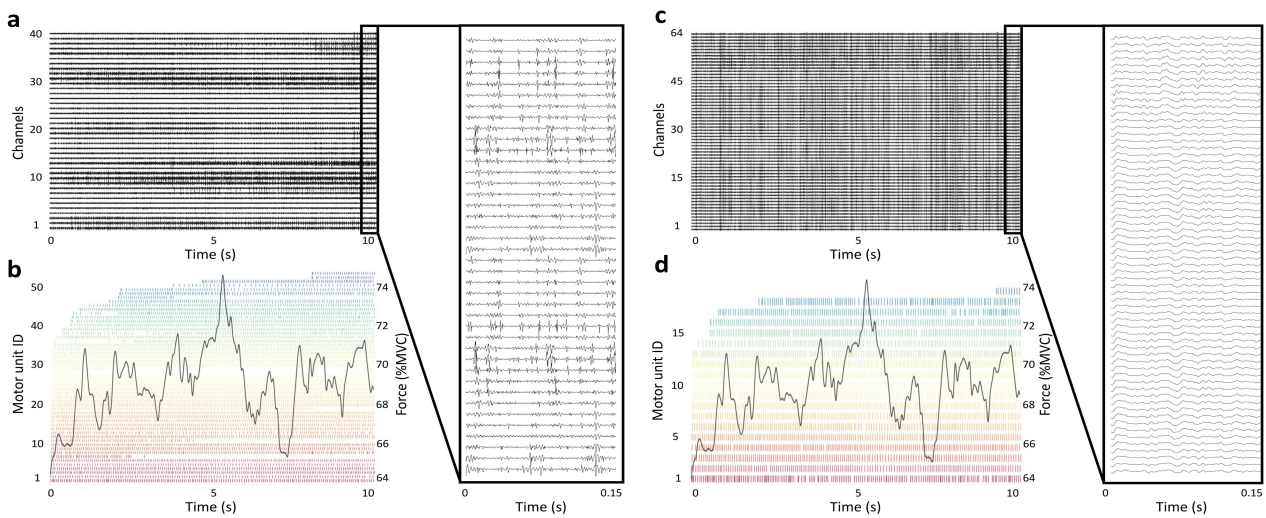


Figure 4.5: Example data and respective raster plots. **a** HD-iEMG (40 channels, 10 seconds), with a 150 ms zoom-in on the data **b** Raster plot from the HD-iEMG (120 channels) **c** HD-sEMG (64 channels, 10 seconds), with a 150 ms seconds zoom-in on the data **d** Raster plot from the HD-sEMG (192 channels)

Figure 4.6 displays the number of MUs decomposed as a function of the number of channels used. Results are presented separately for each subject and force level. Each data point represents the yield from a permutation of a channel subset (Figure 4.2b). The figure displays the relationship between the number of random channels and the number of identified MUs at 10 (blue), 30 (orange), and 70 %MVC (green). Each point on the graph represents one of the 100 permutations for each channel count. The solid lines on the graph indicate the average yield for a given channel count. The range of MUs decoded across different channel permutations was relatively broad. For example, in subject S1, using 80 channels, the number of decoded MUs varied between approximately 40 and 60. This substantial variation suggests that the precise location of the recording channels influences the decomposition yield, though it is not possible to determine the optimal placement of each recording site a priori.

The dashed lines in Figure 4.6 represent the yield of MUs decomposed by HD-sEMG decomposition. The number of units is projected on the channel axis for HD-iEMG to indicate the number of channels needed on average by intramuscular recordings to match the MU yield of HD-sEMG based on 126 (S1) and 111 (S2) channels. Overall, HD-iEMG allowed the decomposition of a relatively large number of MUs across subjects. Notably, a strong reduction in units decomposed was

observed when HD-sEMG data was used. For both subjects, decomposition of large sets of MUs (always above 20) was successfully achieved for all force levels using HD-iEMG.

4.3.3 Accuracy of Decomposition as a Function of the Number of Channels

Figure 4.7a displays the mean SIL value of the MUs decomposed from each permutation run, for the HD-iEMG. As expected, SIL levels were generally higher at lower force levels (Gazzoni et al., 2004) and for greater number of channels. The variability in SIL was greater with fewer channels, which is expected because fewer channels provide less information about each MU, making the decomposition process less reliable. In contrast, more channels provide a more robust filter, leading to a more accurate decomposition, as the same unit is observed across multiple channels. The SIL values began to plateau at around 80 channels for both subjects, suggesting that approximately 80 channels are necessary to achieve consistent and stable accuracy in decomposition.

The decomposition quality was generally high, with SIL levels above the commonly used threshold value of 0.9. Figure 4.7 b presents the distribution of the SIL values when comparing the accuracy of decomposition between surface and intramuscular recordings. In all cases, the SIL values for the HD-iEMG were higher than those from the HD-sEMG, and the difference increased with increasing contraction force.

4.3.4 Number of Motor Units and Accuracy of Decomposition as a Function of Channel Selection

Table 4.2 reports the mean and standard deviation of the number of MUs decomposed from the electrode configurations with fixed IED (refer to Figure 4.2c). The yields exhibited a negative correlation with IED, with a more marked impact of the number of MUs for higher contraction levels (only 1.8 MUs were decomposed at 70 %MVC level with 4 mm IED for S1, and no MUs were decomposed at that level for S2).

Figure 4.8 and Figure 4.9 present two key analyses at 30 %MVC. Figure 4.8 shows the mean and

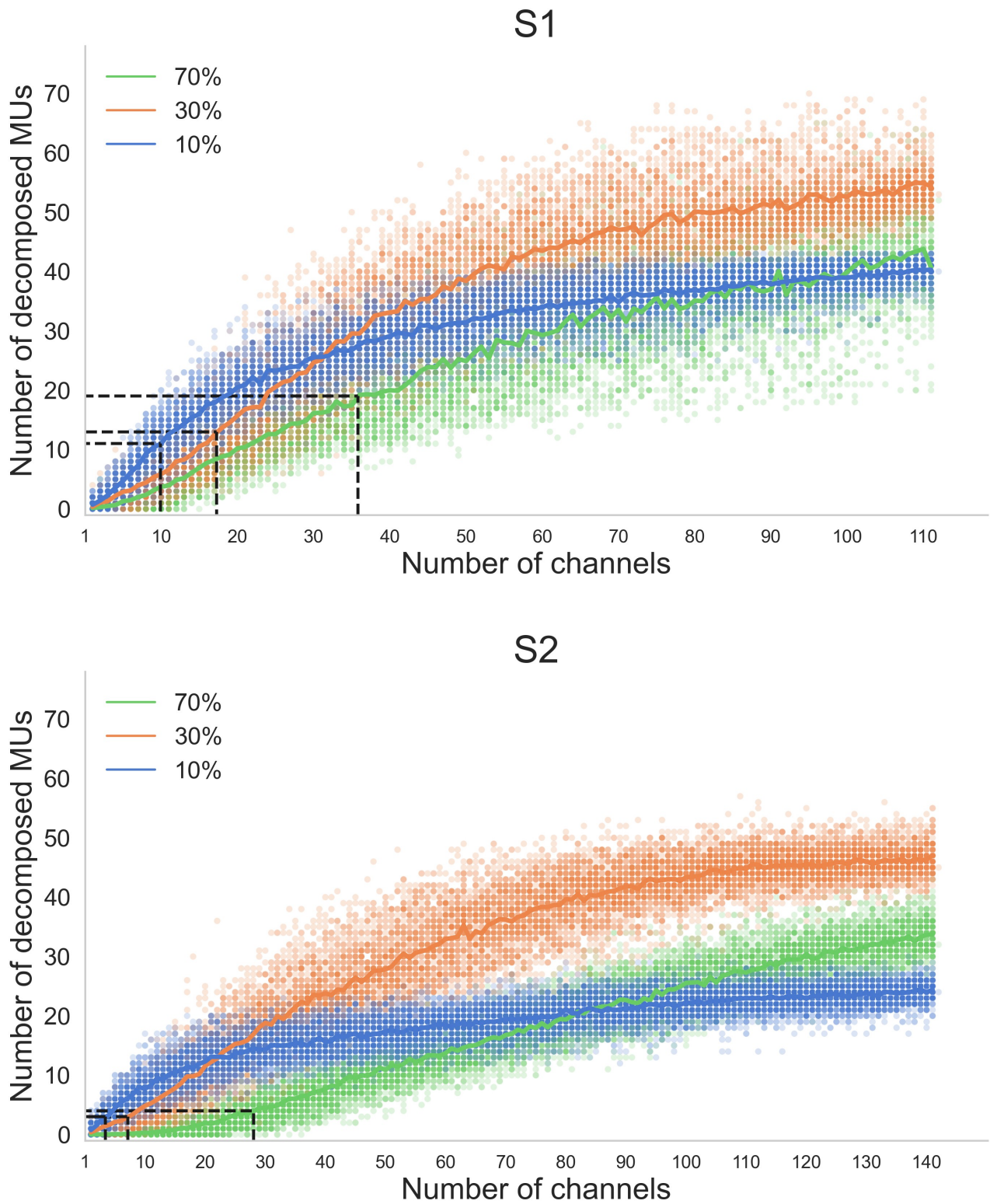


Figure 4.6: Effect of the number of random channels (a) on the number of identified MUUs at 10, 30, and 70 %MVC for S1 (top) and S2 (bottom) (b). Each point represents one of the 100 permutations for each channel count. The solid line indicates the mean yield of these permutations. Horizontal dashed lines show the MU yield from HD-sEMG decomposition, while vertical dashed lines denote the number of intramuscular channels needed to achieve the same yields.

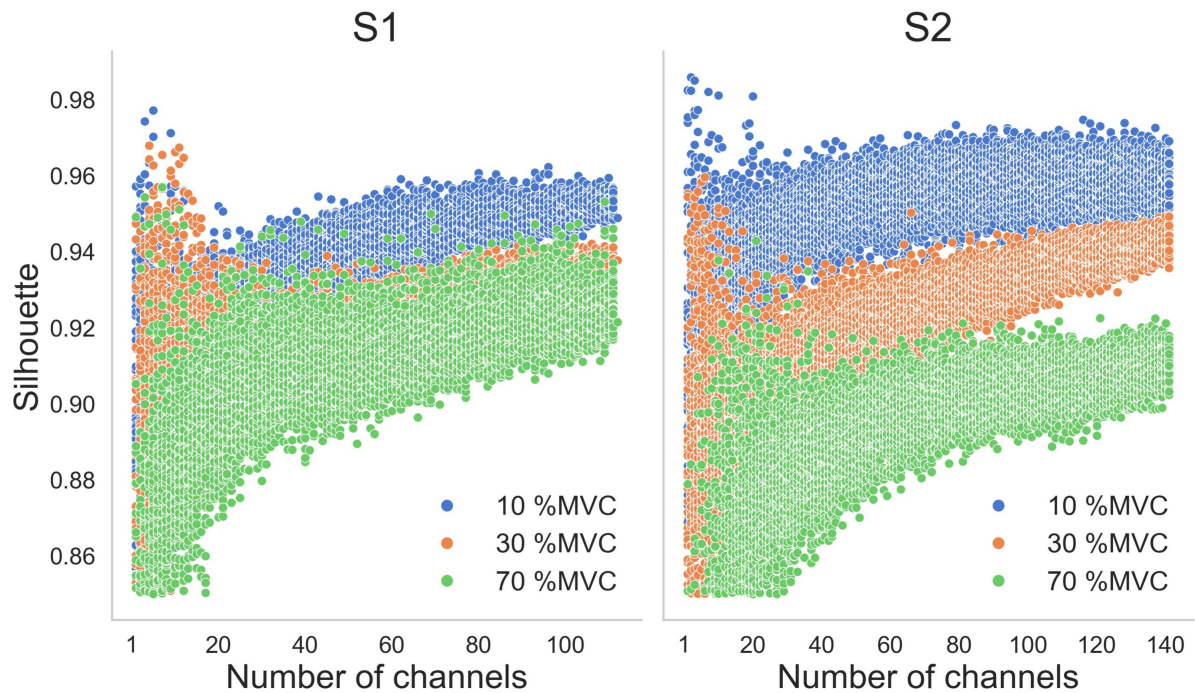
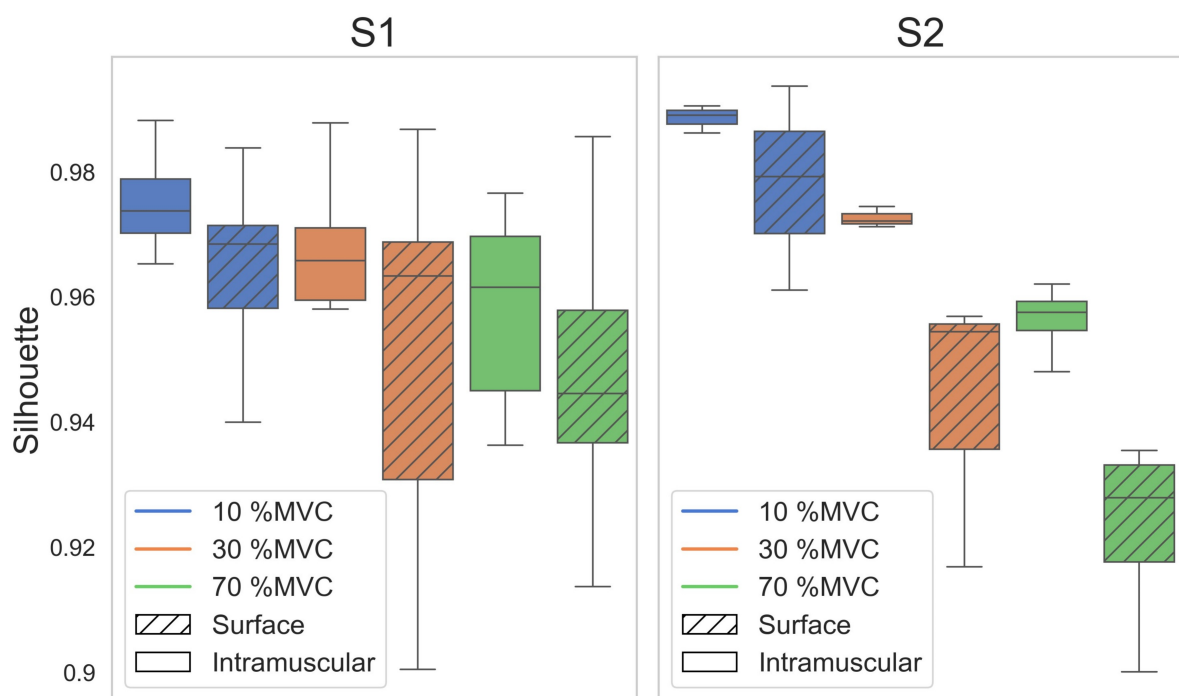
a**b**

Figure 4.7: Effect of channel number on the accuracy of decomposition. **a** Mean SIL value of each permutation run for the HD-iEMG, for the three force levels (10, 30, 70 %MVC) and the two subjects (S1, S2). **b** Distribution of SIL values for the intramuscular and surface EMGs, and for the three force levels (10, 30, 70 %MVC).

Table 4.2: Number of MUs decomposed from the electrode configurations presented in Figure 4.2c, averaged across IEDs.

Subject	Force Level (%MVC)	IED 1 mm	IED 2 mm	IED 4 mm
S1	10	13.0 ± 7.4	10.5 ± 6.3	5.5 ± 3.1
	30	15.5 ± 13.0	7.3 ± 1.7	3.0 ± 2.1
	70	12.3 ± 8.8	5.5 ± 2.2	1.8 ± 1.3
S2	10	10.7 ± 4.8	8.8 ± 3.8	6.3 ± 1.0
	30	19.8 ± 5.8	9.0 ± 3.6	2.0 ± 1.1
	70	6.5 ± 3.2	1.3 ± 1.03	0

standard deviation of the number of decomposed MUs, while Figure 4.9 displays the mean and standard deviation of the SIL values for those units. These results were obtained from various random subsampling configurations (refer to Figure 4.2d). The data are organised by electrode, with channels divided into three subsets of 40 channels each. In addition, scatter points in the design/figures represent the mean yield (Figure 4.8) and mean SIL (Figure 4.9) for the fixed IED configuration. The analysis reveals a clear trend: as the number of channels increases, the number of decomposed MUs increases, and the SIL values generally improve. The SIL values show greater variability when fewer than 10 channels are used, consistent with the findings from Figure 4.7a. Importantly, the number of MUs decomposed and their SIL values in the fixed IED configuration closely follow the trend observed in the random subsampling data, indicating that the number of channels, rather than their specific location in the muscle, is the primary factor driving decomposition performance. Note that this does not mean that all channel selections will lead to very similar number of decoded MUs. As commented in relation to Figure 4.6, there is a large variability of number of decoded MUs across permutations. Yet, on average, choosing the channels randomly along the micro-electrode array led to similar results. Since it is not possible to determine the channel position along the array that leads to the best performance a priori, maximising the number of channels, rather than optimising their locations, has the largest effect on the number of decoded units.

The number of common units identified across the intramuscular micro-electrode arrays and their RoA is presented in Figure 4.10. The common units are low for S1 across electrodes. For S2, the

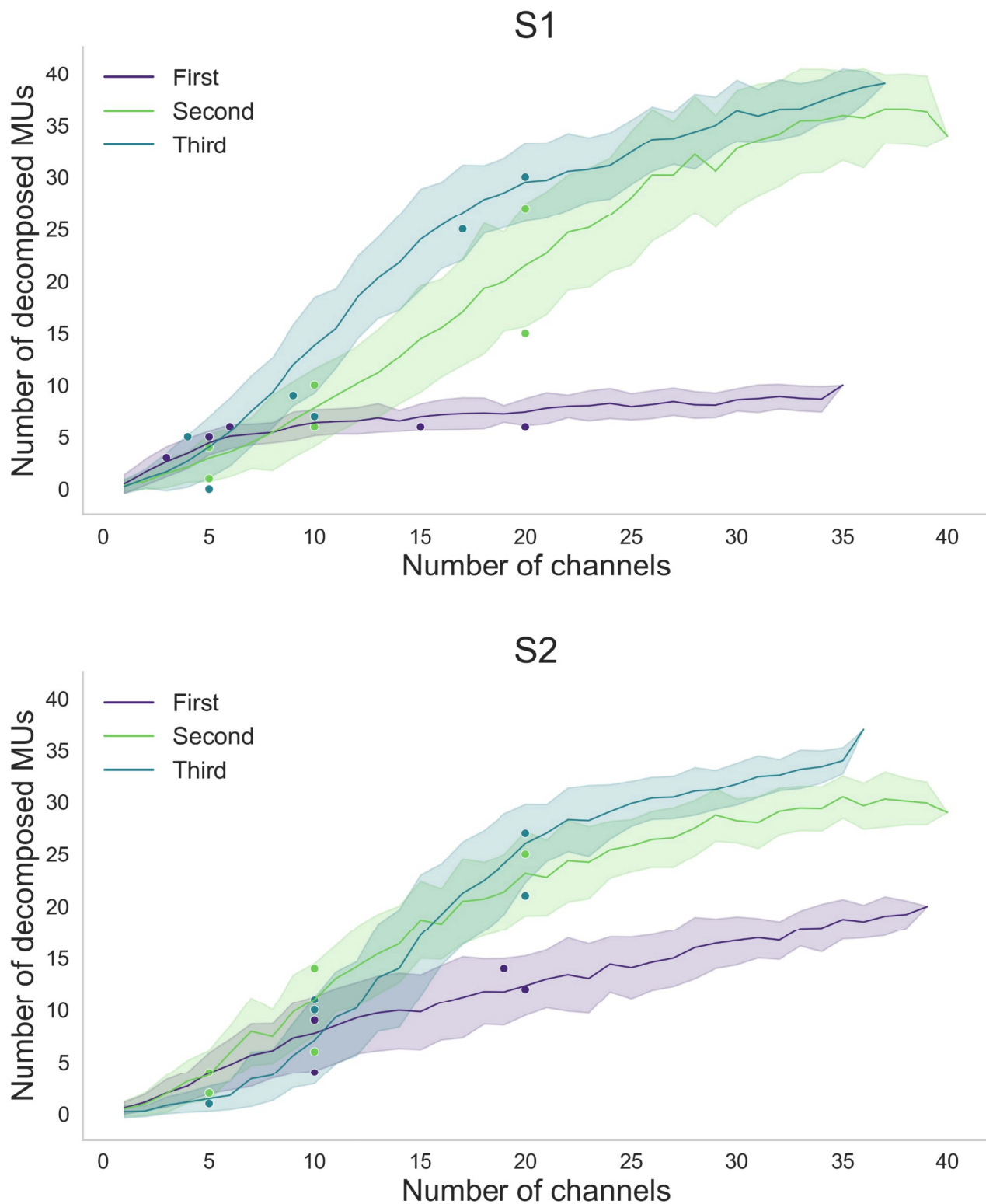


Figure 4.8: Effect of the channel location on the number of decomposed MUs for each channel configuration. Results are reported for S1 and S2 at 30 %MVC, per electrode. The solid line indicates the average number of MUs decomposed from the random selection of channels. The shaded area represents the standard deviation. The scatter points represent the number of MUs decomposed from the fixed IED configurations.

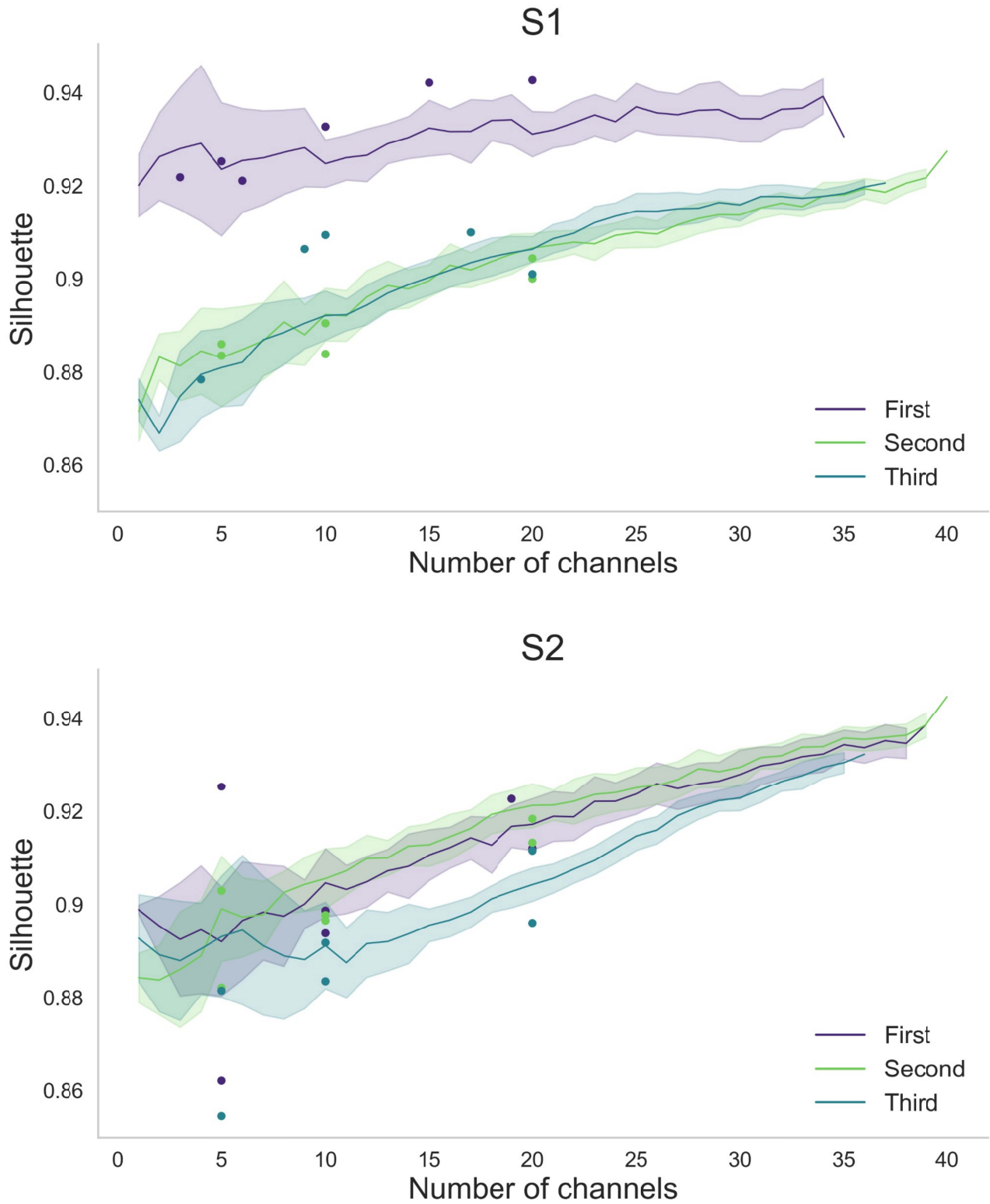


Figure 4.9: Effect of the channel location on the mean SIL for each channel configuration. Results are reported for S1 and S2 at 30 %MVC, per electrode. The solid line indicates the average SIL of the units decomposed from the random selection of channels. The shaded area represents the standard deviation. The scatter points represent the mean SIL decomposed from the fixed IED configurations.

numbers are generally low, except for 30 %MVC, where 18 units are shared between the first and second electrodes, and another set of 18 units is shared between the second and third electrodes.

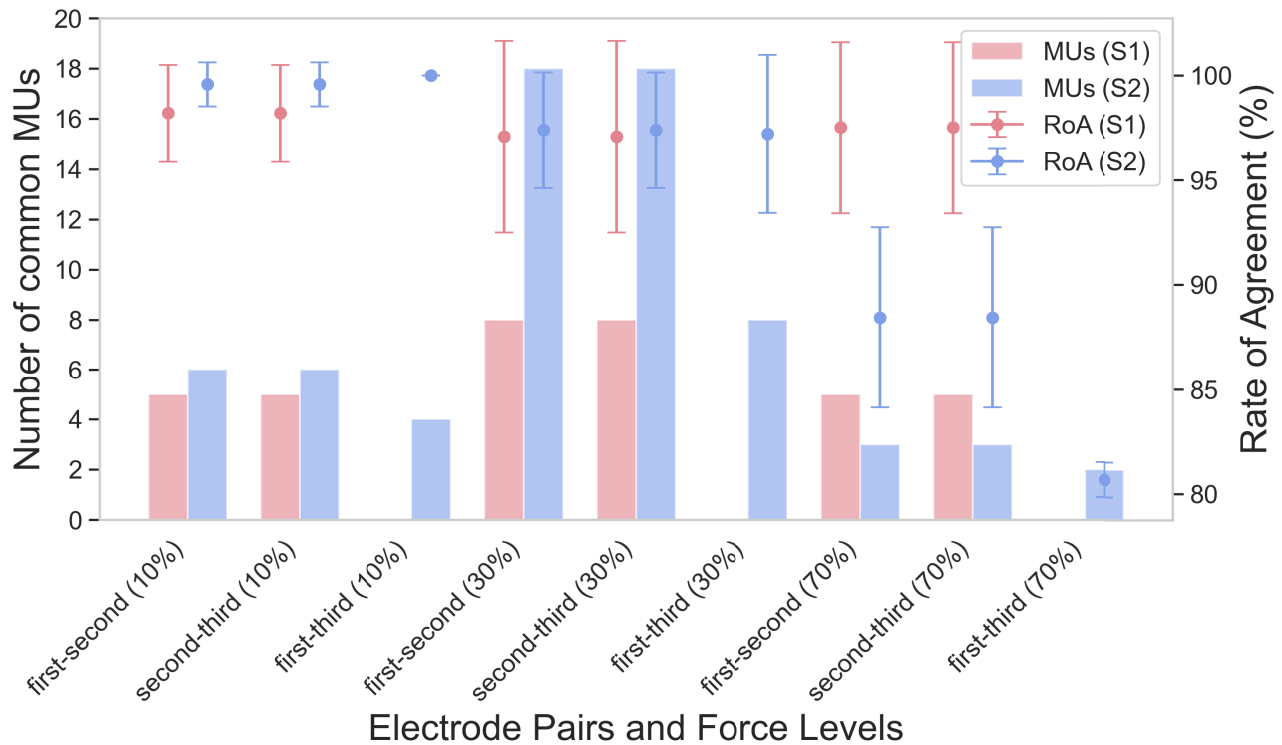


Figure 4.10: Counts of common MUs across intramuscular electrodes for S1 and S2, and the three force levels. The error bars indicate the RoA (mean and std) between the common units.

4.4 Discussion

This study systematically investigated the impact of parameters of HD-iEMG micro-electrode arrays on the number of identified MUs and the accuracy of their decomposition. This is the first study to record muscle activities using >140 electrode sites inside the muscle simultaneously.

The analysis of MUs extracted from both HD-iEMG and HD-sEMG recordings demonstrated physiologically consistent discharge rates (Figure 4.3). The discharge rate distribution for the MUs identified with HD-sEMG was similar to that for MUs decomposed from HD-iEMG, particularly for subject S1. Therefore, despite the lower yield of decomposed units from HD-sEMG, the extracted population appeared to be representative of the broader MU pool. The MUs derived from the HD-iEMG showed the expected inverse relationship between recruitment threshold and discharge rate,

with earlier-recruited units exhibiting higher firing frequencies compared to later-recruited units (De Luca & Hostage, 2010). The recruitment pattern of the HD-iEMG MUs revealed predominantly uniform unit recruitment throughout the contractions, with the exception of 70 %MVC in subject S2, where early-recruited units were underrepresented in the decomposition. This likely occurred because smaller action potentials from early-recruited units were masked by larger action potentials from later-recruited units. For subject S1, the recruitment-discharge rate relationship remained consistent between HD-iEMG and HD-sEMG recordings. However, the limited number of decomposed units from the HD-sEMG decomposed from subject S2 precluded meaningful analysis on the recruitment pattern.

Our findings show that increasing the number of channels results in the identification of a greater number of MUs, consistent with previous analogous research conducted with surface electrodes (Caillet, Avrillon, et al., 2023). The result is not obvious since the channels were always selected within the same micro-electrode array and thus always covered the same portion of muscle section. Therefore, the only variable analysed was electrode density rather than different areas of muscle investigated. The increase in number of detected units with increasing number of channels may be partly due to the sampling of motor MUs with more channels but more likely it was determined by a better identification of separation filters with a greater electrode density.

Furthermore, we examined whether the selection of channel locations, given a fixed density, is critical for MU decomposition. The results indicated that the yields and accuracy from random channel subsampling closely matched those from fixed IED configurations on average. This indicates that the accuracy is not significantly impacted by whether the channels are selected randomly or arranged in a closely spaced configuration, as long as they are selected from the same array. This confirms that the density of channels is the most critical factor when optimising for the yield of MUs. Moreover, given the lack of convergence in the observed trends, using larger sets of channels with even shorter IEDs than in this study (i.e., <0.5 mm) could potentially lead to even better performance.

We also compared the results between HD-sEMG grids and HD-iEMG. The number of MUs decomposed from HD-sEMG was considerably lower than from HD-iEMG. Additionally, the results (in terms of yield and SIL) were variable across subjects at the surface level, while they were more

consistent at the intramuscular level. Similar number of MUs were obtained with the two subjects in all conditions with HD-iEMG, indicating lower intra-subject variability compared to HD-sEMG. This higher variability in HD-sEMG is likely due to its greater sensitivity to subject-specific factors, such as volume conduction effects and electrode shifts (Araujo et al., 2000; Hug, 2011; Oskouei et al., 2013). The accuracy of decomposition was also lower in the HD-sEMG setting compared to the HD-iEMG (Figure 4.7). This can be attributed to the superior quality of HD-iEMG recordings, which, due to their spatial selectivity, can record units in 6-8 channels. Intramuscular recordings thus result in a much greater temporal and spatial sparseness than surface recordings, and this is a significant factor in determining differences between the two methods.

In agreement with recent findings for surface recordings (Caillet, Avrillon, et al., 2023), our findings show that denser arrays allow better spatial sampling, which improves the identification of MUs, especially at higher force levels. The number of MUs plateaued at 10 % and 30 %MVC in S1 (Fig 4.6), but continued to rise at 70 %MVC. A similar trend was observed in S2. This suggests that increasing the density of recording points could be particularly advantageous for decomposing data acquired at higher force levels.

As shown in Figure 4.8, the number of channels, rather than their specific placement within the muscle, was the primary factor influencing the yield of decomposed MUs. Additionally, Figure 4.9 supports the claim that decomposition accuracy improves with increased channel count, as evidenced by consistent SIL trends across all electrodes and both subjects, with the exception of the first electrode in S1. This electrode deviated from the overall trend also in the MU yield. Indeed, unlike the other electrodes, it produced fewer decomposed MUs overall, which likely explains the differing SIL trend observed (Figure 4.8).

The number of common surface/intramuscular units across modalities was very small, which can be attributed to the different regions of the muscle recorded with the surface and intramuscular electrodes. Additionally, the number of common units across intramuscular arrays was also low, except for S2 at 30 %MVC. The limited overlap of units across intramuscular electrodes is likely attributable to the high spatial selectivity of intramuscular recordings, which inherently reduces the likelihood of sampling the same muscle fibers from different electrode insertions. The higher number

of common units for S2 at 30 %MVC may explain the slightly lower overall number of MUs found for S2 (refer to Table 4.1). In the future, the ability to re-target electrodes after identifying common MUs across electrodes could facilitate the recording of unique MUs, thereby expanding the pool of identified MUs by multiple arrays. The TA comprises roughly 445 MUs (R. M. Enoka & Fuglevand, 2001). Given the limited overlap of MUs identified between HD-sEMG and HD-iEMG (as well as among different intramuscular electrodes), combining data from both modalities could significantly expand the pool of motor neurons detected, allowing for a more comprehensive estimate of the neural drive to the muscles.

4.5 Conclusions

The superior characteristics of HD-iEMG make it an attractive technology for investigating neuromechanical aspects of movement and developing neural interfaces. Compared to HD-sEMG, the technique offers greater consistency in results, yields a higher number of decomposed MUs, and achieves improved decomposition accuracy. Future developments in intramuscular technologies could further enhance these advantages by increasing both the number and density of channels within the electrode array. While our focus in this study was on a large muscle like the TA, which provides access to a large pool of MUs, further studies are necessary to determine whether these findings extend to muscles with different anatomical characteristics, such as differences in muscle size, fiber pinnation angles, and distribution of end-of-fiber regions.

The relatively lower performance of HD-sEMG decomposition may be attributed to two factors: the inherent limitations of surface signals, as discussed in Chapter 2, and potential shortcomings in current decomposition algorithms. To address the latter, the next chapter presents an adaptation of SCD for non-invasive signals, demonstrating improved decomposition performance in terms of both MU yield and accuracy.

*What could an entirely rational
being speak of with another
entirely rational being?*

Levinas, Emmanuel

5

Blind Source Separation of Non-Invasive Neurophysiological Time Series

NOTE: Aside from minimal alterations to fit the format and flow of the thesis as a cohesive single document, the content of this chapter has been published in the following article:

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Chapter 2 detailed the fundamental differences between surface and intramuscular recordings, particularly highlighting the limitations of sEMG due to the low-pass filtering effect of the volume conductor separating the muscle fibers from the recording points. Despite these limitations, the non-invasive nature of HD-sEMG has established it as a valuable tool for investigating motor control. Specifically, the decomposition of HD-sEMG signals into constituent MU discharge patterns provides crucial insights into MN recruitment strategies and firing behavior during movement. However, current algorithms, while very effective under certain conditions, face significant challenges in complex scenarios, as their accuracy and MU yield are highly dependent on anatomical differences among individuals. This can limit the number of decomposed MUs, as seen in Chapter 4. To address this issue, we recently introduced SCD, which dynamically adjusts the separation function based on the distribution of the data and prevents convergence to the same source. Initially applied to HD-iEMG signals, SCD is here adapted for HD-sEMG signals. In this chapter, we demonstrated its ability to address key challenges faced by existing methods, particularly in identifying low-amplitude MUAPs and effectively handling complex decomposition scenarios, like high-interference signals. We extensively validated SCD using simulated and experimental HD-sEMG recordings and compared it with current SoA decomposition methods under varying conditions, including different excitation levels, noise intensities, force profiles, sexes, and muscle groups. The proposed method consistently outperformed existing techniques in both the quantity of decoded MUs and the precision of their firing time identification. For instance, under certain experimental conditions, SCD detected more than three times as many MUs compared to previous methods, while also significantly improving accuracy. Even though HD-iEMG is still the gold standard method in detecting a high number of MUs, these advancements represent a major step forward in non-invasive EMG technology for studying MU activity in complex scenarios.

5.1 Introduction

The nervous system regulates muscle force by transmitting signals from alpha MNs in the spinal cord to muscle fibers. The collective activity of these MNs, known as the neural drive to the muscle, determines the overall muscle activation and force production (De Luca & Erim, 1994; Farina & Negro,

2015). Each MN's discharge triggers an action potential in each muscle fiber it innervates (Kandel et al., 2000). The sum of the action potentials of the fibres innervated by a single motor neuron is the MUAP. The sum of the MUAPs trains from all active MUs generates the EMG signal, which represents the electrical activity of the muscle during a contraction (De Luca, 1979). Therefore, the EMG signal comprises both a neural component — the MN discharge times — and a peripheral/muscle component — the MUAP waveforms (D. Stashuk, 2001). EMG decomposition aims to separate these two components, enabling the precise identification of the discharge times of each MN.

For nearly a century, the primary method for investigating MUs has been through invasive EMG techniques, involving the decomposition of recordings from needle or wire electrodes under highly controlled conditions (Adrian & Bronk, 1929; Farina & Gandevia, 2024). These recordings have a high degree of selectivity, typically allowing the decoding of only a few MUs at a time. While high selectivity has been the primary condition to allow for the identification of individual motor unit activity from multi-unit recordings, this property also limits the number of detectable MUs. As a result, much of our current understanding of human MN function is derived from a narrow set of MUs per subject. While these studies have been critical in establishing foundational principles, they do not provide a broad view across to larger motor unit samples or more varied conditions. sEMG is less selective compared to invasive EMG and therefore traditionally lacked the precision required to isolate individual MUs.

The challenge of decomposing poorly selective sEMG recordings has been partially addressed by increasing the number of recording sites (electrodes) and applying BSS techniques. Increasing electrode counts led to HD-sEMG (Merletti & Muceli, 2019) and provided multiple observation points for the MU activities. Most BSS methods use the principles of ICA to separate sources, i.e., the MUs, from the observed mixtures. This is achieved through the use of a contrast function, a nonlinear measure of sparsity of the signals, that is applied to the estimated spike trains in an iterative fashion (Farina & Holobar, 2016).

Because the HD-sEMG signals are convolutive mixtures of the MN spike trains, decomposition of these signals require cBSS. In cBSS, spike trains are extracted by optimizing a contrast function that maximizes an approximation of the skewness or kurtosis of the sources.

While BSS of HD-sEMG has deeply improved the study of MUs using non-invasive recordings, several challenges remain in its practical application. One major issue is that the number of successfully decomposed MUs can vary greatly across conditions, muscles, and individuals (Del Vecchio et al., 2020). This variability stems from the differences across conditions in the distribution of MUAPs at the skin surface, which is an inherent limitation of surface recordings. Indeed, in some scenarios, HD-sEMG decomposition may not be feasible at all due to these challenges. As a result, non-invasive MU investigations often focus on a narrow set of muscles, specific experimental subjects, and controlled recording conditions (de Oliveira et al., 2022).

The underlying reason for poor HD-sEMG decomposition is that, in certain cases, the MUAPs from different MUs exhibit highly similar waveforms in both space and time, making it difficult for conventional contrast functions used in BSS to isolate them. This similarity limits the effectiveness of traditional decomposition algorithms. A promising direction for overcoming this limitation is the development of methods that enhance the differences between MUAPs by implementing novel strategies for blindly determining the optimal contrast function during decomposition. These approaches could improve the differentiation of similar MUAPs, leading to an increased number of decomposed MUs, improved classification accuracy, and broader applicability of sEMG decomposition in more challenging scenarios.

In a recent study (Grison et al., 2024), we introduced SCD for decomposing multi-channel intramuscular EMG recordings. The method optimizes the contrast function dynamically for each source, increasing the separation between sources. Here, we use the concepts of SCD and we adapt it to the decoding of surface recordings. By dynamically adapting the contrast function, we ensure that it is specifically tailored to the unique characteristics of the source being decomposed, significantly enhancing source separation performance. The adaptability of the contrast function distinguishes SCD from other SoA methods, which typically fix the contrast function to polynomial approximations of the kurtosis or the skewness (Holobar & Zazula, 2003; Negro et al., 2016). Moreover, SCD implements a peel-off strategy for sequential source removal, which allows the algorithm to detect smaller or more subtle sources that would have otherwise been overshadowed by the larger, more dominant ones.

The proposed method was extensively validated with both simulated signals and experimental recordings across multiple conditions, including varying excitation levels, noise intensities, MUAP overlap, muscle groups, and sexes. In all analyses, SCD was compared against current cBSS approaches, and specifically the method proposed by Negro et al. (2016), which is a current representative SoA decomposition approach. Henceforth, in the following we will refer to cBSS as to this particular method, representing one of the several solutions proposed to address the general convolutive demixing problem. The results demonstrated a substantial increase in performance of SCD in all conditions tested, proving the effectiveness of the approach in increasing both the number and accuracy of the decomposed MUs. These findings indicated that, while the new method does not fully eliminate all challenges, SCD marks a significant advancement in HD-sEMG decomposition. By increasing the yield and accuracy of decomposition, it broadens the recording conditions and experimental scenarios where reliable MU activity can be extracted, ultimately increasing the practical utility and robustness of surface EMG for a wide variety of applications.

5.2 Methods

5.2.1 Decomposition

We propose the use of the SCD decomposition algorithm (Grison et al., 2024) and compare it with a representative state of the art approach for cBSS decomposition (Negro et al., 2016).

Both algorithms rely on ICA to separate sources by maximizing a statistical measure of non-Gaussianity or sparsity of the estimated sources (Hyvärinen & Oja, 2000). In BSS of EMG signals, the main characteristics used for decomposition is sparseness (Farina & Holobar, 2016) since motor unit discharge times are not fully independent. The non-linear function used to assess sparseness is called the contrast function, G . Similar to the Gaussianity property, mixing sources results in a signal less sparse than the individual sources. Thus, sparse sources can be identified by finding projections that maximise sparseness, making the choice of contrast function critical for the stability of the numerical optimization process. Cumulants are a widely used class of non-linearities for this purpose. By op-

timizing the separation vector to maximize higher-order cumulants, such as skewness or kurtosis, non-Gaussianity as well as sparseness are amplified, increasing the likelihood of isolating sparse sources.

In the cBSS methods used for comparison (Negro et al., 2016), the contrast function is defined to maximize an approximation of the third cumulant, i.e., the skewness, of the sources, utilizing $G_{CBSS}(s) = \frac{1}{6}s^3$. In contrast, our approach proposes to maximize an adaptively tuned higher-order cumulant for each source. Specifically, $G_{SCD}(s) = E\{\text{sign}(s)|s|^e\}$, where e represents the exponent of the polynomial function, and E is the expectation operator. The core hypothesis is that each source requires a different level of selectivity to be effectively separated from the mixture. Sources that are more similar to one another may require stronger discriminants, whereas others may require less stringent criteria. This adaptivity is the main difference between cBSS and the algorithm SCD.

In SCD, a candidate separation vector $\mathbf{w}$ was randomly initialized from a zero-mean normal distribution with a standard deviation of 1. The candidate vector was then repeated for the number of initialised particles to produce the initial separation vectors $\mathbf{w}$. On each step, an ICA run was conducted on each separation vector independently for a maximum of 1000 iterations, with an early termination criterion applied if, after 20 iterations, $G_{SCD}(s)$ ceased to increase. After each ICA step, a peak-finding algorithm detected the source samples as calculated with the updated separation vectors, followed by a two-class k -medoid clustering applied to the estimated sources to identify their peaks as potential MU spikes. Source quality was evaluated using a fitness function based on the coefficient of variation of the interspike intervals, where the candidate with the lowest coefficient of variation was selected as the best source. The coefficient of variation was chosen as an appropriate metric due to the regularity of MU firing in isometric contractions. When regularity of the firings could not be assumed, like in the ballistic contractions, the SIL was used instead to assess source quality.

After each ICA update, the optimal exponent for the contrast function was chosen from the current pool of candidates and updated by moving toward the exponent that yielded the highest-quality source. The separation vectors were then reinitialised with the spike-triggered-average of the highest-quality source. Optimization was halted after 10 updates of the optimal exponent coef-

ficient. To assess the final source quality, the SIL measure (Negro et al., 2016) was used to evaluate the clustering separation between the decomposed source and the background noise. If the source passed this evaluation ($SIL > 0.85$) and had not been identified in previous iterations, the MUAPs were subtracted—i.e., peeled off—from the signal by creating a template motor unit waveform and removing it at each identified motor unit firing time. This subtraction prevented further convergence to the same source in subsequent decomposition iterations. However, from the estimated discharge times, MUs exhibiting discharge properties outside the predefined thresholds—specifically, a coefficient of variation above 35% or a FR outside the 2-35 Hz range (Martinez-Valdes et al., 2017)—were excluded from further analysis. Figure 5.1 presents a schematic of SCD.

To assess the importance of the two core features of SCD — the adaptive contrast function and the peel-off procedure — two targeted ablations were conducted. The first ablation focused on addressing repeated convergence to the same source. Three strategies were tested: (1) initializing the separation vectors $\mathbf{w}$ with the activity index, a proxy for global pulse train activity (Holobar & Zazula, 2007b), and eliminating the peel-off process, while preventing previously accepted MU firing timings from being reinitialized by excluding them from the activity index; (2) combining the activity index with a source deflation method to enforce orthogonality between separation vectors; and (3) comparing with the proposed SCD.

The second ablation maintained the peel-off method across all configurations and examined the effect of different exponents in the contrast function. These were compared against the performance of SCD with the dynamically adapted exponents. The decompositions were run on an Intel(R) Core(TM) i7-10700K CPU with an Nvidia RTX 3080 GPU.

5.2.2 Simulations

HD-sEMG data was simulated using NeuroMotion (Ma et al., 2024a), an advanced EMG simulator designed to produce physiological electric potentials during voluntary forearm movements. NeuroMotion operates through three modules. The first, an upper-limb musculoskeletal model developed using the OpenSim API (Delp et al., 2007), defines and visualizes movements while estimating muscle fibre lengths and muscle activation levels. These estimates are then input into BioMime (Ma et

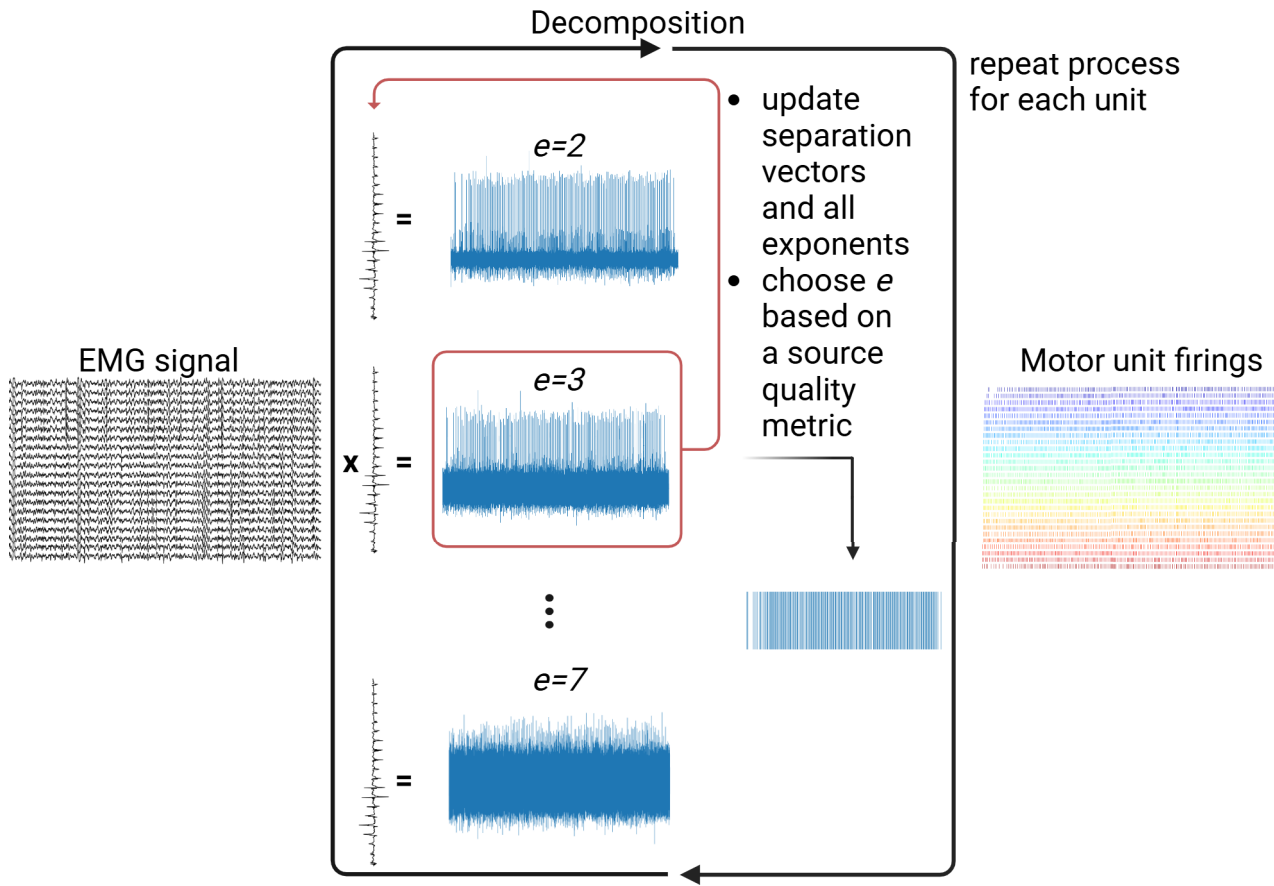


Figure 5.1: Schematic of Swarm-Contrastive Decomposition. A set of separation vectors are initialised to the same vector, with zero-mean and unitary standard deviation. Each separation vector is associated to a specific particle, the value of the exponent in the contrast function. This value tunes the sensitivity of the contrast function to outliers. Each separation vector is updated independently given a measure of sparsity of the produced source vector. The exponents are updated based on the optimal value with a particle swarm optimisation algorithm. The final output are the peaks of the source vector, which represent the firing times of the current motor unit. The process is repeated in an iterative fashion until all motor units are decomposed.

al., 2024), an Artificial Intelligence-based volume conductor model that generates MUAPs based on parameters like fibre number, depth, angular position, innervation zone, and conduction velocity, derived from a myoelectric digital-twin model (Maksymenko et al., 2023). The final module, a MU pool model, converts neural inputs into spike train simulations, completing the muscle activation simulation (Fuglevand et al., 1993).

An isometric and isotonic index finger contraction from the FDS was simulated with NeuroMotion. The simulated signals were generated for an electrode band comprising a 10×32 electrode grid, positioned around the proximal third of the forearm. Figure 5.2 a displays a schematic of the simulated setup. A pool of 100 MNs was simulated. The innervated muscle fibers were randomly and

uniformly distributed throughout the muscle volume, with innervation zones normally distributed around $50\% \pm 10\%$ of the total fiber length. An exponential function was employed to model the recruitment thresholds and number of innervated fibers for each MN, resulting in a higher proportion of small, low-threshold MNs compared to larger, high-threshold ones. Conduction velocities were sampled from a normal distribution (mean $4.0 \pm 0.5 \text{ ms}^{-1}$, truncated between 3 and 4.5 ms^{-1} to avoid non-physiological parameters) and sorted based on the number of innervated fibers. Motor neurons began firing at a baseline rate of 8 pps once the excitation level surpassed their recruitment threshold (Fuglevand et al., 1993). The discharge rate increased linearly by 3 pps for every 10% increase in excitation (Fuglevand et al., 1993; Keenan et al., 2006). Maximum discharge rates varied from 35 pps for the first recruited MN to 25 pps for higher-threshold MNs (Fuglevand et al., 1993). The final MN was recruited at 50% of the maximum excitation level (Fuglevand et al., 1993). The variability in discharge times followed a Gaussian random process with a coefficient of variation of 0.2 (Fuglevand et al., 1993; Keenan et al., 2006). While the myoelectric digital-twin model (Maksymenko et al., 2023) provided the muscle geometry based on magnetic resonance imaging, NeuroMotion estimated other MU parameters (position, length, depth, angle, innervation zone, and conduction velocity) based on physiological ranges. These distributions were randomized over 10 bootstrapping iterations to capture the full variability observed in real data ($N=10$). Three distinct analyses were performed on different types of simulated data to investigate the characteristics of the proposed algorithm. The first analysis examined the effect of excitation level on the decomposition process by gradually increasing the excitation from 10% to 100% of MVC in 10% increments, forming a dataset of 100 recordings, given by 10 bootstrapping iterations for each of the 10 force levels. For each MVC level, each contraction lasted 30 s. Throughout this analysis, the SNR was maintained at 25 dB (zero-mean white Gaussian noise with standard deviation based on the amplitude of the originally simulated noiseless EMG signals across time and channels) to isolate the effect of the excitation level.

The second analysis investigated the impact of the noise level on MU decomposition. In these simulations, the excitation level was fixed at 30 %MVC during 30-second isometric and isotonic contractions, while the noise level was varied from 10 to 30 dB, with 5-dB increments for ten bootstrapping iterations ($N=10$). This approach allowed for the examination of the algorithm performance un-

der different levels of signal contamination, simulating more challenging real-world conditions. The last analysis focused on ballistic contractions (high synchronization level), where the force increased rapidly from 0 to 40 %MVC. Thirty bursts of isometric muscle activity were simulated for ten bootstrapping iterations ($N=10$), each lasting 1 second and separated by 3-second intervals. These conditions mimicked sudden, explosive muscle contractions, enabling the evaluation of the algorithm performance under high levels of synchronised MU activity. The noise level in this experiment was maintained at 25 dB. It is noteworthy that while the contraction was dynamic in terms of force, the posture and muscle geometry remained constant throughout the simulation.

5.2.3 Experimental Data

All reported experiments adhered to the ethical guidelines set by Imperial College London (ICREC Project ID 19IC5640). All procedures were conducted in accordance with the Declaration of Helsinki, with informed consent obtained from the participants prior to each experiment. Three experiments were conducted, with the first two focusing on the TA muscle of healthy male and female participants respectively, and the last one focusing on the forearm of healthy female participants. In the TA experiments, participants were seated with their right leg and foot secured to a dynamometer and they were instructed to sustain an isometric and isotonic ankle dorsiflexion. Figure 5.2 **b** displays a schematic of the experimental setup. Similar to the simulated conditions, subjects in the forearm experiments were instructed to perform isometric index finger flexion (Figure 5.2, **a**). The force levels were set as percentages of the participants' MVC, with visual feedback provided for both exerted force and target. EMG signals were recorded in monopolar derivation, with a reference electrode placed on the ankle (TA), and wrist (forearm), and bandpass-filtered between 20 and 500 Hz for the signals collected at the surface, and between 20 and 4400 Hz for the intramuscular signals. All signals and force data were recorded concurrently using the Quattrocento multi-channel amplifier (OT-Bioelettronica, Torino, Italy), featuring a high common mode rejection ratio >95 dB, high-pass filtered at 10 Hz, and digitized at 16-bit resolution.

5.2.3.1 Pilot 1: Two-source Validation (TA)

Two healthy men, aged 39 and 30, were recruited (N=2). The protocol included 20-second isometric and isotonic contractions at 10, 20, and 30 %MVC, a 15-second contraction at 40 %MVC, and 10-second contractions at 50, 60, and 70 %MVC. Three 40-channel HD-iEMG micro-electrode arrays (Muceli et al., 2015, 2022) were implanted in the TA, oriented longitudinally and spaced approximately 3-cm apart. The electrodes, made of platinum, are arranged on two sides of a wider filament, with each side consisting of 20 electrodes spaced 1 mm apart. The two sides are offset by 0.5 mm (Muceli et al., 2022). Two 64-channel HD-sEMG grids (4 mm inter-electrode distance, 13x5 electrode configuration) were placed on the skin surface above the intramuscular detection sites. Intramuscular and surface EMG signals were concurrently sampled at 10,240 Hz. The concurrent recording of intramuscular and surface EMG signals allowed for the use of the two-source validation methods for an objective and rigorous assessment of decomposition accuracy (Farina, Merletti, & Enoka, 2014; Mambrito & De Luca, 1984).

5.2.3.2 Pilot 2: Sex Differences in Motor Unit Yield (TA)

Two healthy women, aged 23 and 24, were recruited (N=2). The protocol included 20-second isometric and isotonic contractions at 5 and 10 %MVC. One 256-channel HD-sEMG grid (4 mm inter-electrode distance, 32x8 electrode configuration) was placed on the TA. HD-sEMG signals were sampled at 2,048 Hz. The measurements on female individuals allowed to compare the algorithms in conditions that are challenging for surface EMG decomposition since it has been reported that decomposition yield and accuracy decreases in female individuals (Del Vecchio et al., 2020; Lulic-Kuryllo & Inglis, 2022; Taylor et al., 2022).

5.2.3.3 Pilot 3: Motor Unit Yield in Complex Muscle Groups (forearm)

Two healthy women, aged 26 and 30, were recruited for this study (N=2). The protocol included 20-second isometric and isotonic index finger flexions at 15 %MVC. Three 64-channel HD-sEMG grids (8 mm inter-electrode distance, 13x5 electrode configuration) were placed around the proximal third of

the forearm of each participant. HD-sEMG signals were sampled at 2,048 Hz. The measurements on the forearm allowed to analyze the performance of the algorithms in more complex muscle groups, where the number of MUs decomposed is usually limited to less than ten (Del Vecchio et al., 2020).

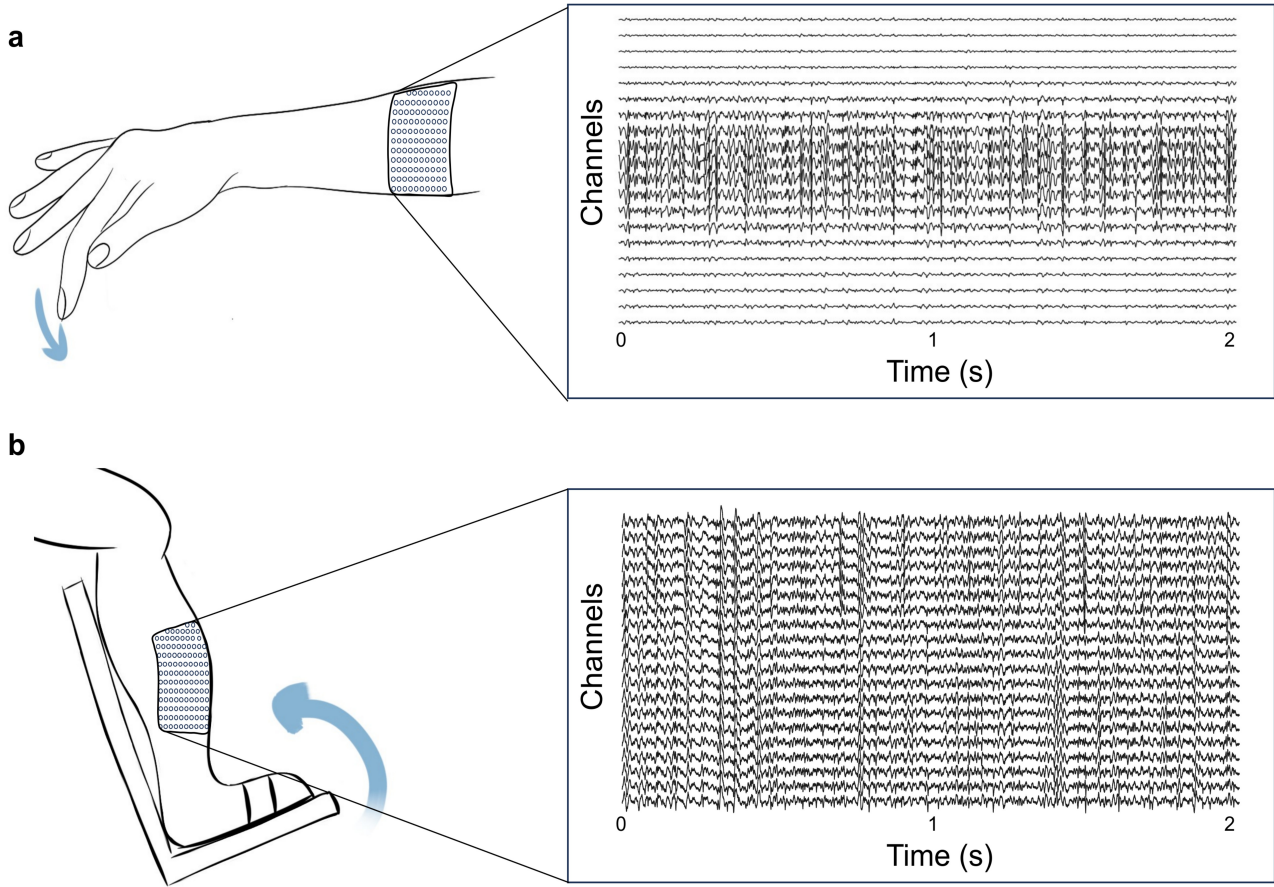


Figure 5.2: Schematics of the HD-sEMG data used in the analysis. **a** Simulated HD-sEMG data from forearm muscles during index finger flexion, with the electrode grid positioned over the proximal third of the forearm. The same setup was applied for the experimental recordings from the forearm. Representative data (simulations) is shown for a 30 %MVC contraction. **b** Experimental HD-sEMG data were also recorded from the TA muscle during ankle dorsiflexion. Representative data for a 20 %MVC contraction is displayed.

5.2.4 Metrics for Accuracy

The level of agreement between the discharge times of MUs as decoded from the decomposition of the simulated HD-sEMG and the ground truth was assessed using the RoA. The RoA measures the fraction of commonly identified discharges relative to the total number of discharges, considering both common and not-common discharge times.

Several methods have been proposed to assess the accuracy of decomposition of experimental HD-sEMG data (Holobar et al., 2014; Holobar et al., 2010; Hu et al., 2013), with the two-source validation being the most reliable (Farina, Merletti, & Enoka, 2014; Mambrito & De Luca, 1984). This approach involves recording both HD-sEMG and HD-iEMG signals concurrently, decomposing them independently, and comparing the discharge times from the two decompositions to determine the RoA, as defined above. This approach operates on the principle that similar results from two independent algorithms applied to different signals are likely correct, as the probability of identical errors is low. The RoA thus reflects the relative performance of the algorithms without bias toward either method (Farina, Merletti, & Enoka, 2014). Here, when available, we utilized the concurrent recording of the HD-iEMG micro-electrode arrays to validate the surface decomposition. The HD-iEMG data was decomposed using SCD, as described in (Grison et al., 2024). When the concurrent recording of the HD-iEMG signals was not available, the number of MUs decoded by SCD and cBSS was compared, and the RoA between the commonly identified MUs (by SCD and cBSS) was also analyzed.

For all analyses, the RoA s reported in percentages as the mean and standard deviation. Additionally, the number of MUs is reported as the mean and standard deviation across bootstrapping iterations.

5.2.5 Statistical Analysis

All statistical analyses were conducted in Python 3.10 using the SciPy library (Virtanen et al., 2020) and the Pingouin statistical package. A significance threshold of $p < 0.05$ was applied to all tests.

Two-way repeated measures ANOVAs were performed to assess the effects of independent factors (MVC levels, noise levels, the exponent used in the contrast function, and the method for preventing repeated source convergence) on the dependent variable (i.e., the number of decomposed MUs), including their interaction effects. Post-hoc pairwise comparisons with Bonferroni corrections were conducted to identify specific differences between factors. To compare MU characteristics (MUAP peak-to-peak amplitudes, conduction velocities, number of fibers, depth, and exponents of the contrast function), independent t-tests were performed when normality was assumed (as determined by the Shapiro–Wilk test, $p < 0.05$); otherwise, Mann–Whitney U tests were used. For comparing

the RoA of MUs decomposed from HD-iEMG recordings and commonly identified by both SCD and cBSS, Wilcoxon signed-rank tests were applied due to non-normal distributions of the RoAs.

5.3 Results

5.3.1 Simulations: Excitation Level

Figure 5.3 summarizes the results of MU decomposition using SCD and cBSS across varying excitation levels in the simulated data. Panel **a** illustrates the mean number of decomposed MUs over 10 bootstrapping iterations. Across all excitation levels and bootstrapping iterations, on average, cBSS identified 13.9 ± 2.7 MUs per contraction, while SCD identified on average nearly a double number of MUs (25.9 ± 5.8 MUs). The two-way repeated measures ANOVA revealed significant main effects of the decomposition algorithm ($p < 0.001$) and MVC level ($p < 0.001$), as well as a significant interaction between the algorithms and the MVC levels ($p < 0.001$). Post-hoc analyses showed that SCD resulted in significantly higher number of decomposed MUs than cBSS ($p < 0.001$). Of the MUs identified by cBSS, 98% were also detected by SCD, demonstrating that SCD primarily expanded the total count of detected units, rather than identifying significantly different units from those already captured by cBSS. Figure 5.3 **b** reports the final exponent distribution of the automatically selected contrast functions for all identified sources with SCD. The RoA of all the MUs decomposed by SCD was $98.90 \pm 2.33\%$, compared to $98.02 \pm 2.69\%$ for cBSS. Figure 5.3 **c** shows the RoA for the MUs identified by both methods. In these units, SCD showed a significant increase in RoA ($p < 0.001$). The effect size, as measured by a Rank-Biserial Correlation of 0.99, indicates a substantial and practically meaningful improvement in RoA.

A comparison between the MUs which were detected by both SCD and cBSS (*common*) and those identified only by SCD (*unique*) is presented across various MU properties. These properties include peak-to-peak amplitudes of the MUAP in the channel with the highest amplitude (Figure 5.3 **d**), MU conduction velocities (Figure 5.3 **e**), the number of fibers innervated per MU (Figure 5.3 **f**), and the depth of the MUs (Figure 5.3 **g**). These results show that the MUs uniquely identified by SCD

tended to exhibit lower peak-to-peak amplitudes, suggesting that these MUs were either located farther from the electrodes, or deeper within the muscle. However, Figure 5.3 **g** shows that, while significant, the difference in distance from the electrodes between common and unique units was relatively small. Moreover, the SCD-unique MUs typically had slower conduction velocities and a smaller number of innervated fibers, indicating they were smaller MUs. The computational time required to run one decomposition was approximately 2 minutes for cBSS and 12 minutes for SCD.

5.3.1.1 Ablations

Figure 5.4 **a** displays the mean number of decomposed MUs across bootstrapping iterations, stratified by force level, for the three methods employed to mitigate repeated convergence to the same source during the ICA optimization. On average, across force levels and bootstrapping iterations, the activity index method identified 8.4 ± 2.6 MUs, the deflation method 11.3 ± 1.7 MUs, and the peel-off method 25.9 ± 5.8 MUs. The two-way repeated measures ANOVA revealed significant main effects of the method used to prevent repeated convergence to the same source on the number of decomposed MUs. The analysis revealed significant main effects for both MVC ($p < 0.001$) and the method used ($p < 0.001$), as well as a significant interaction between the two independent factors ($p < 0.001$). Post-hoc pairwise comparisons revealed significant differences between the three methods in their ability to decompose MUs. The peel-off method consistently outperformed both the activity and deflation methods with highly significant differences ($p < 0.001$ for peel-off vs. activity; $p < 0.001$ for peel-off vs. deflation). These results demonstrate that the peel-off method identifies significantly more MUs than the other methods. Additionally, the deflation method also significantly outperformed the activity method ($p < 0.001$). These results indicate that both force level and method to prevent convergence on the same source significantly influence the number of decomposed MUs.

To further understand the interaction between the ablation method and the way in which contrast functions are selected, we compared the number of units decomposed using the best ablation method in the previous test (peel-off) and four ways of defining the contrast functions for the separation: fixed contrast functions with exponents of orders 2, 2.4, or 3, and using SCD to optimize the selection of the contrast function used for each decomposed source (Figure 5.4 **b**). The fixed values were

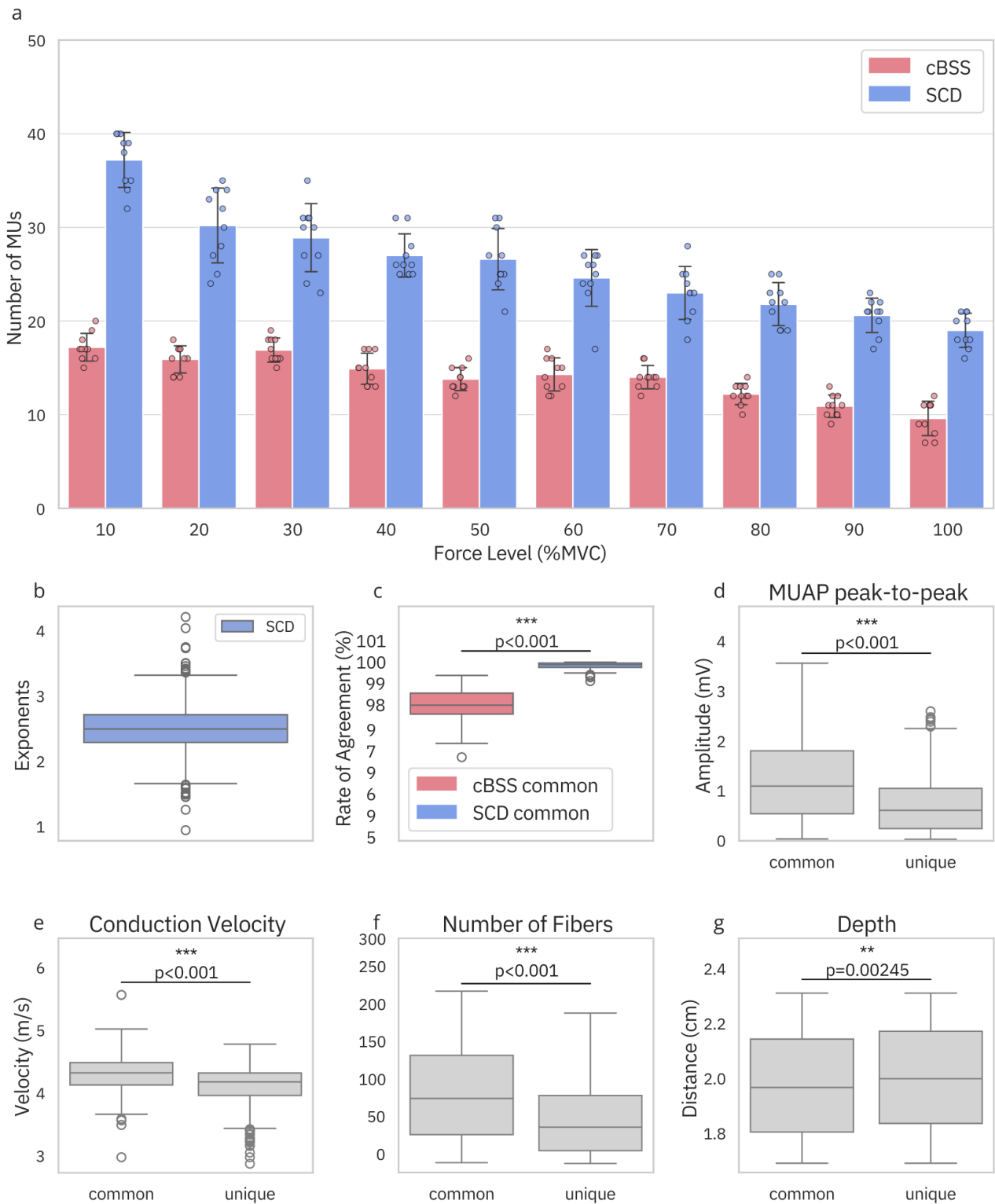


Figure 5.3: **a** Mean number of MUs per bootstrap iteration for SCD (blue) and cBSS (pink). The error bars indicate the standard deviation across bootstrapping iterations. Individual data points from each bootstrapping iteration are overlaid on the bar chart. SCD decomposed significantly more MUs than cBSS ($p < 0.001$). **b** Distribution of exponents for SCD. **c** Distribution of the RoA between the automatic methods and the simulated ground truth for the MUs commonly identified by SCD and cBSS. **d-g** Distributions of the peak-to-peak MUAP amplitudes (**d**), conduction velocity (**e**), number of fibers innervated (**f**), and depth (measured with respect to the skin, higher is deeper) (**g**) for MUs common to both SCD and cBSS, and those uniquely identified by SCD.

selected to look at the median value of the adapted swarm exponents ($e = 2.4$) and the closest integer lower ($e = 2$) and upper ($e = 3$) bounds. On average, across all force levels and bootstrapping iterations, the method with $e = 2$ identified 10.9 ± 4.6 MUs, the method with $e = 2.4$ identified 21.1 ± 3.5 , the method with $e = 3$ identified 6.4 ± 3.4 MUs, and the method with the adaptive exponent identified 25.9 ± 5.8 MUs. The two-way repeated measures ANOVA revealed significant main effects of both MVC level ($p < 0.001$) and exponents used in the contrast functions ($p < 0.001$) on the number of decomposed MUs, as well as a significant interaction between the two factors ($p < 0.001$). Post-hoc pairwise comparisons revealed significant differences between all four methods. The swarm method resulted in significantly higher number of decomposed MUs compared to $e = 2$ ($p < 0.001$), $e = 2.4$ ($p < 0.001$) and $e = 3$ ($p < 0.001$).

These results reflect an important improvement in the decomposition when an adaptive method to select contrast functions is combined with the peel-off method for iterative source removal. Using either of these two configurations alone with either fixed contrast functions or other possible source removal methods resulted in drastic reductions in the number of decomposed MUs.

5.3.2 Simulations: Noise Level

Figure 5.5 presents the mean number of decomposed MUs across bootstrap iterations at a fixed excitation level of 30 %MVC (panel a) and the RoA of the identified MUs compared to the simulated ground truth. The two-way repeated measures ANOVA revealed significant main effects of both noise level ($p < 0.001$) and decomposition algorithm ($p < 0.001$) on the number of decomposed MUs, as well as a significant interaction between the two factors ($p < 0.001$). Post-hoc pairwise comparisons revealed significant differences between all noise levels, with higher noise levels resulting in fewer decomposed MUs. Additionally, the SCD algorithm identified significantly more MUs than the cBSS algorithm across all noise levels ($p < 0.001$). These results indicate that both noise level and decomposition algorithm significantly influence the number of decomposed MUs, with SCD demonstrating superior performance. On average across all noise levels and bootstrapping iterations, cBSS identified 11.9 ± 6.9 MUs, while SCD detected 19.8 ± 13.5 MUs. Approximately 97% (11.5 ± 7.2) of the MUs detected by cBSS were also detected by SCD. The RoA for the MUs identified by SCD was

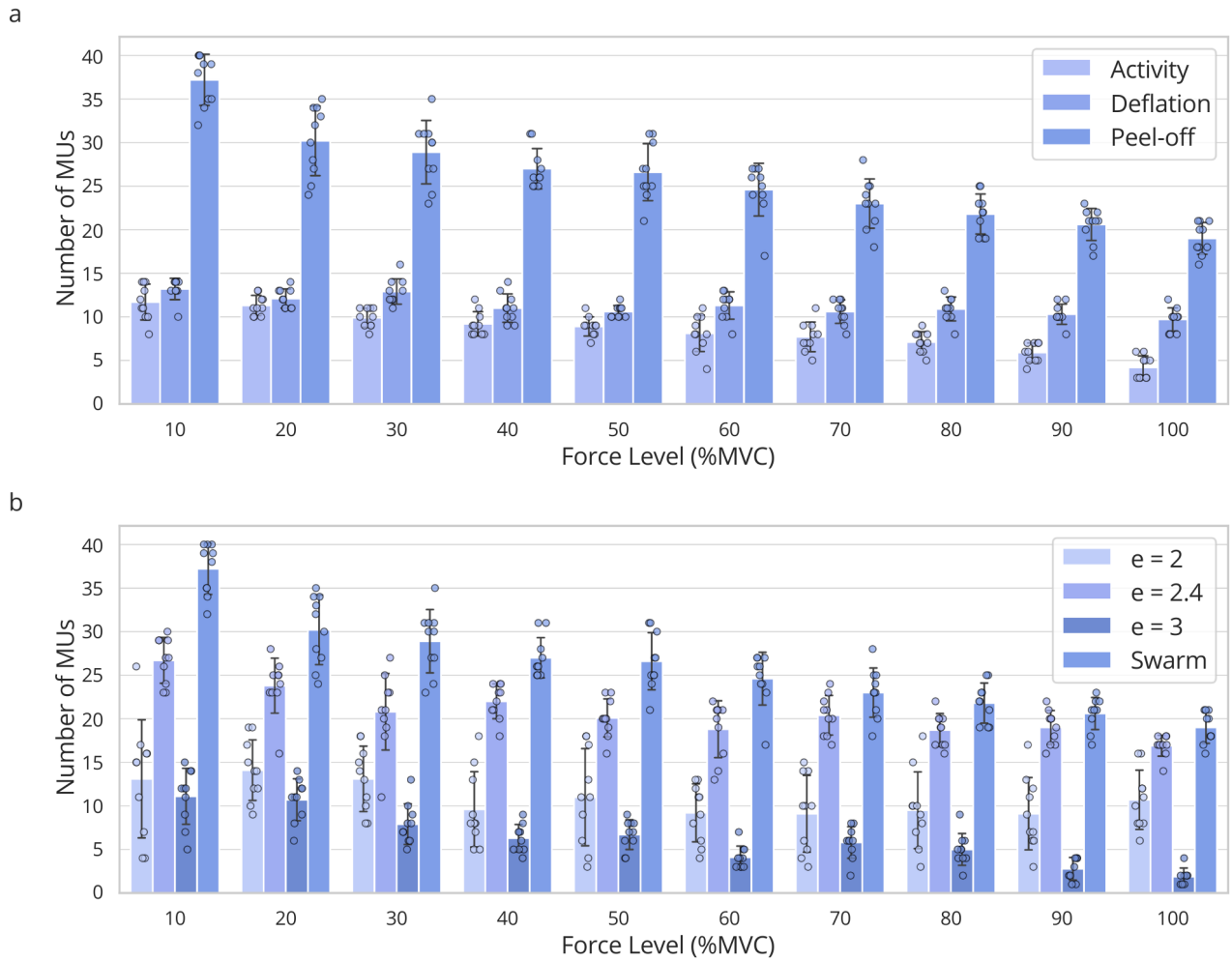


Figure 5.4: **a** Effect of the method used to prevent source convergence on the number of MUs found. The bars represent the mean of the bootstrapped samples, while the error bars reflect the standard deviation across these bootstrapping iterations. Individual data points from each bootstrapping iteration are overlaid on the bar chart. Three methods are reported: 1) activity (use the activity index to initialize the separation vectors), 2) deflation (activity index to initialize the separation vectors and orthogonalize the separation vectors), 3) peel-off (remove found sources from the EMG). The peel-off method decomposed significantly more MUs than the other two methods ($p < 0.001$). **b** Effect of the exponent of the contrast function on the number of MUs found. Peel-off approach was used for all the three methods reported: 1) exponent fixed at 2, 2) exponent fixed at 2.4, 3) exponent fixed at 3, 4) exponents starting at [2,3,4,5,6,7] and updated with particle swarm optimisation. The decomposition with the swarm update found significantly more MUs than fixing the exponent values ($p < 0.001$).

99.1 $\pm$ 1.8%, compared to 98.2 $\pm$ 2.6% for cBSS. The RoA of the MUs commonly identified by both SCD and cBSS (Figure 5.5 **b**) was significantly higher for SCD compared to cBSS ($p < 0.001$).

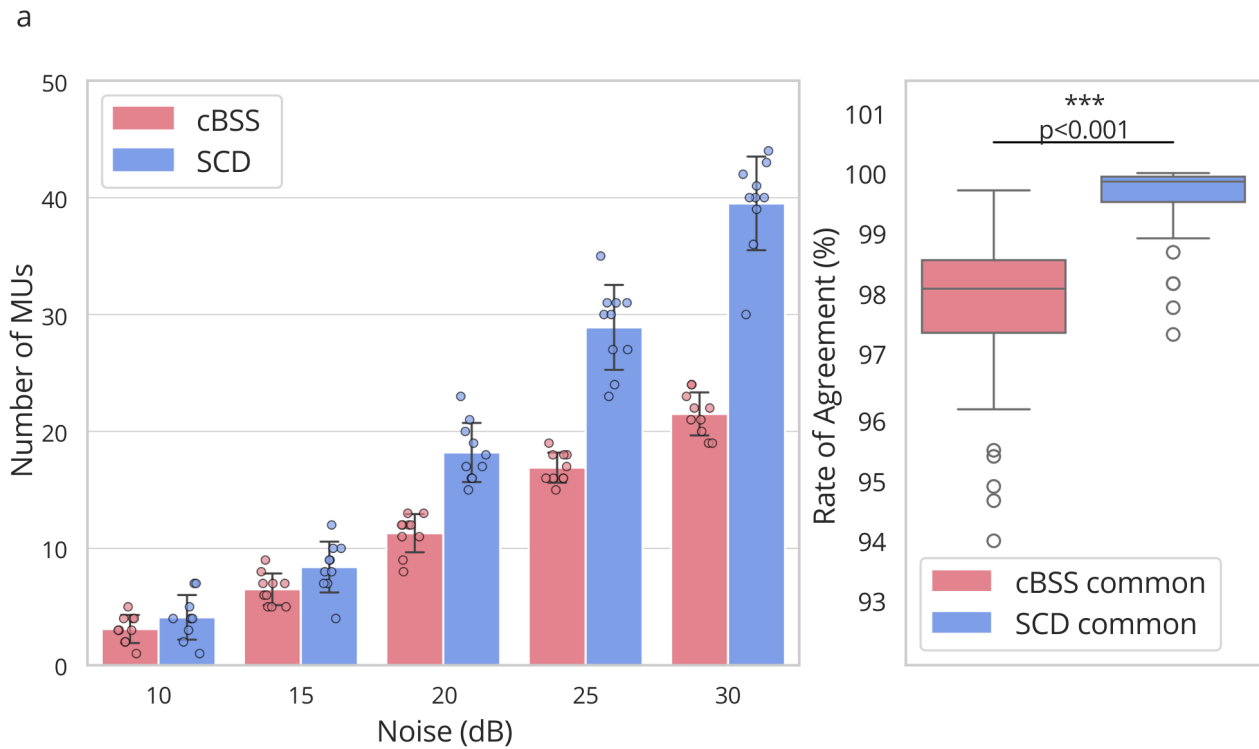


Figure 5.5: Effect of noise on the number of MUs found and the RoA with the ground truth for 30 %MVC force level. **a** Number of MUs against noise level, for SCD and cBSS. The bars represent the mean of the bootstrapped samples, while the error bars reflect the standard deviation across these bootstrapping iterations. Individual data points from each bootstrapping iteration are overlaid on the bar chart. SCD decomposed significantly more MUs than cBSS ($p < 0.001$). **b** Distribution of the RoA between decomposed MUs and their simulated ground truth for the MUs commonly identified by SCD and cBSS.

5.3.3 Simulations: Ballistic Contractions

The decomposition performance was preserved in contractions simulated with ballistic force changes, where the overlap of the MUAPs over time was greater due to the rapid recruitment and de-recruitment of the MUs.

Figure 5.6 provides a representative example of the signal and the decomposed activity. Panel **a** presents the spike-triggered average of the MUAP of a decomposed unit. The estimated activity of the unit in one of the bursts of EMG is shown in panel **b**, which illustrates a clear separation between the source components (marked with red circles) and the background activity. A representative example of ten channels of the simulated EMG for the same one-second interval is depicted in panel **c**, reported for the specific spatial grid configuration (10x32).

Figure 5.7 presents the results of this analysis. Panel **a** shows the number of decomposed MUs for both cBSS and SCD, with SCD identifying approximately three times the number of MUs of cBSS. On average across the bootstrapping iterations, cBSS detected 10.5 ± 1.7 MUs, while SCD decoded 31.2 ± 4.3 , which were statistically different ($p < 0.001$). On average, 96% of the MUs identified with cBSS were also identified with SCD. The RoA of all the MUs decomposed by SCD was $97.5 \pm 3.6\%$, while cBSS achieved $97.1 \pm 2.4\%$. Out of the commonly identified MUs, SCD achieved a significantly higher RoA ($p < 0.001$). The distribution of RoA for the commonly detected MUs is illustrated in Figure 5.7 **b**. Additionally, the MUs uniquely identified by SCD displayed significantly lower ($p < 0.001$) peak-to-peak MUAP amplitudes compared to those found by cBSS (panel **c**). This further demonstrates SCD's capability to decompose lower-amplitude MUs that are missed by cBSS.

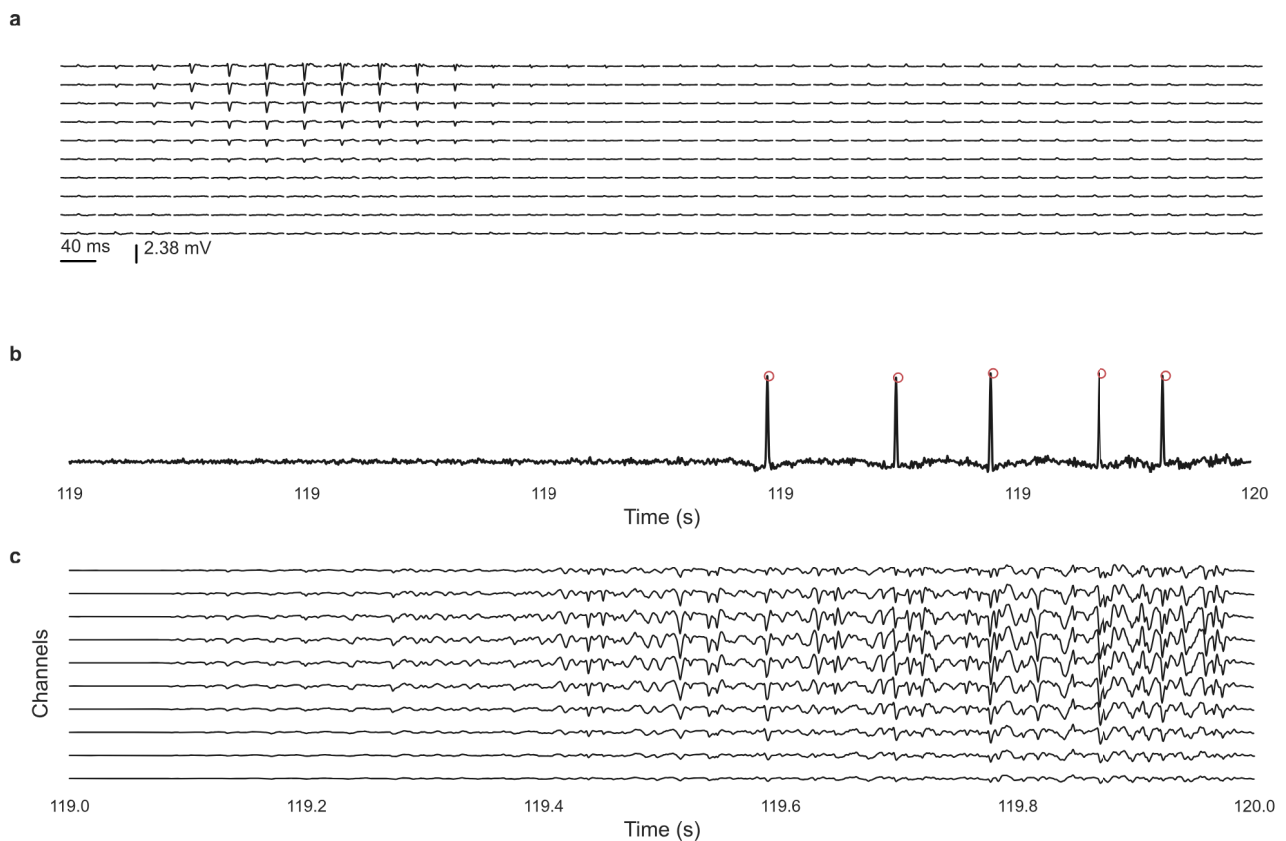


Figure 5.6: Representative example of a unit in a ballistic contraction decomposed with 100% accuracy. **a** Spatial distribution of the MUAP arranged in the 10x32 electrode configuration. **b** A one-second zoom-in of the innervation pulse train of the full source. The discharge times of the clustered source are shown in red. **c** A one-second zoom-in of the EMG.

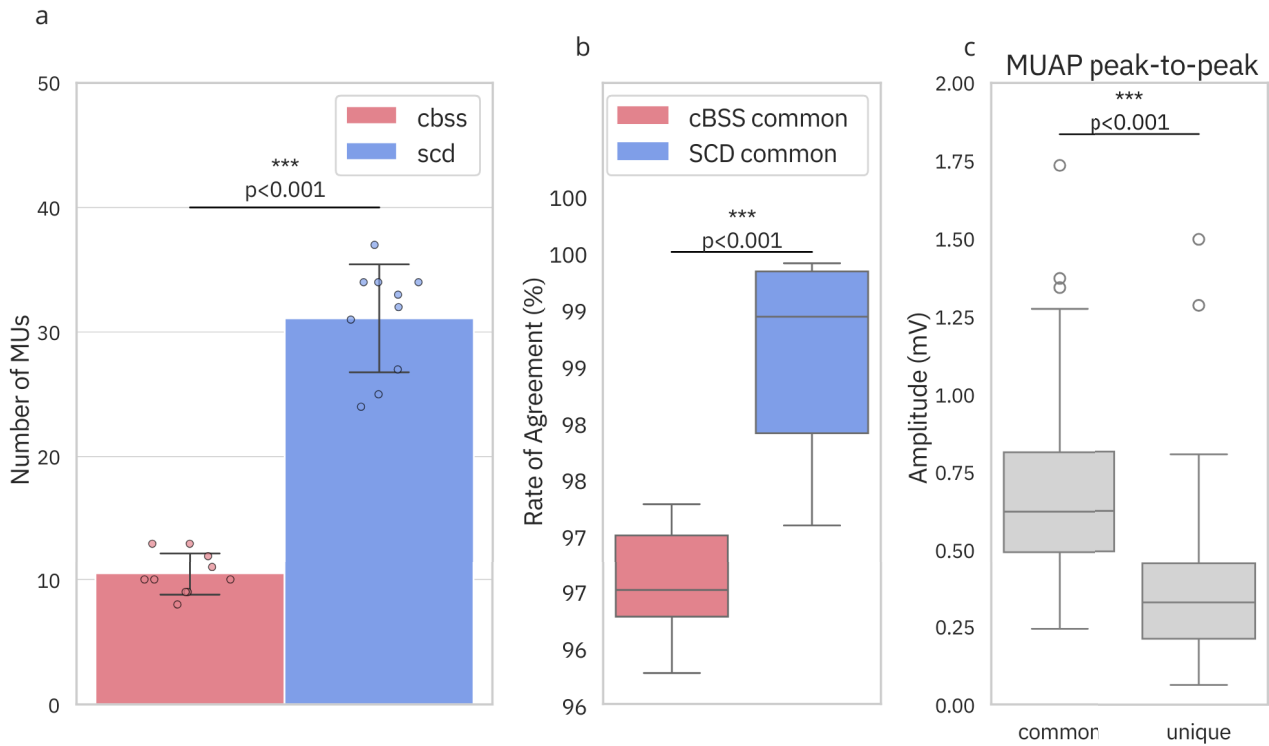


Figure 5.7: Effect of ballistic task on the number of MUs found and the RoA with the ground truth. **a** Number of MUs found for SCD and cBSS. The bars represent the mean of the bootstrapped samples, while the error bars reflect the standard deviation across these bootstrapping iterations. Individual data points from each bootstrapping iteration are overlaid on the bar chart. SCD decomposed significantly more MUs than cBSS ($p < 0.001$). **b** Distribution of the RoA between decomposed MUs and their simulated ground truth for the MUs commonly detected by SCD and cBSS. **c** Distributions of the peak-to-peak MUAP amplitudes for MUs common to both SCD and cBSS, and those uniquely identified by SCD.

5.3.4 Pilot 1: Two-source Validation

This experiment aimed to compare the HD-sEMG decomposition results between SCD and cBSS using experimental data. The outputs from both methods were validated by comparing them against the decomposition of concurrently recorded HD-iEMG signals, providing a reliable benchmark for assessing the accuracy of the surface decompositions.

Across the force levels, SCD identified 41.6 ± 12.1 MUs for subject 1, and 12.0 ± 5.3 for subject 2, a significant increase ($p < 0.001$) with respect to cBSS (13.7 ± 3.1 and 2.1 ± 0.7 respectively). Additionally, the number of matched MUs between HD-iEMG and HD-sEMG was greater for SCD than for cBSS (Figure 5.8 a). This was likely due to the fact that SCD could identify deeper or smaller sources that were not separable with cBSS but that were captured by the intramuscular multi-electrode arrays.

To assess decomposition accuracy of the two compared methods, the RoA for the MUs found by both cBSS and SCD, and matched with HD-iEMG, was calculated. The RoA for SCD was $97.3 \pm 3.6\%$, compared to $95.0 \pm 4.4\%$ for cBSS. Figure 5.8 **b** shows the distribution of the RoA for the commonly identified MUs, which shows a shift toward higher values for SCD (although there was only a trend for this shift; $p=0.08$). Additionally, when comparing the MUs identified by both cBSS and SCD, as well as those uniquely identified by SCD, it was observed that SCD successfully detected MUs with significantly lower peak-to-peak MUAP amplitudes ($p<0.001$), underscoring its ability to identify smaller MU activity that may have been overlooked by cBSS.

Additionally, the exponent for HD-sEMG was significantly higher ($p<0.001$) than that for HD-iEMG (Figure 5.8 **d**).

5.3.5 Pilot 2: Sex Differences in Motor Unit Yield (TA)

In this experiment, the performance of SCD and cBSS were compared when decomposing signals recorded from female subjects. Figure 5.9 shows the number of decomposed MUs per subject stratified by force level (panel **a**) and the RoA between the MUs identified by the two methods (panel **b**). On average across subjects and force levels, SCD identified 16.8 ± 3.8 MUs (20 and 14 for 5 %MVC, 20 and 13 at 10 %MVC for each subject respectively), while cBSS identified 10.0 ± 1.8 MUs (11 and 8 at 5 %MVC, and 12 and 8 at 10 %MVC for each subject respectively). Importantly, SCD identified all the MUs identified by cBSS, and additional ones. The RoA between SCD and cBSS was $97.2 \pm 2.4\%$ for 5 %MVC, and $95.9 \pm 3.6\%$ for 10 %MVC (Figure 5.9, **b**). The differences in the peak-to-peak amplitudes of the MUAPs between the units commonly found by both cBSS and SCD and those uniquely identified by SCD were not significant (panel **c**).

5.3.6 Pilot 3: Motor Unit Yield in Complex Muscle Groups (forearm)

In this experiment, the performance of SCD and cBSS were compared when decomposing signals recorded from the forearm muscles of female subjects. Figure 5.10 shows the number of decomposed MUs (panel **a**) and the RoA between the MUs identified by the two methods (panel **b**). For each

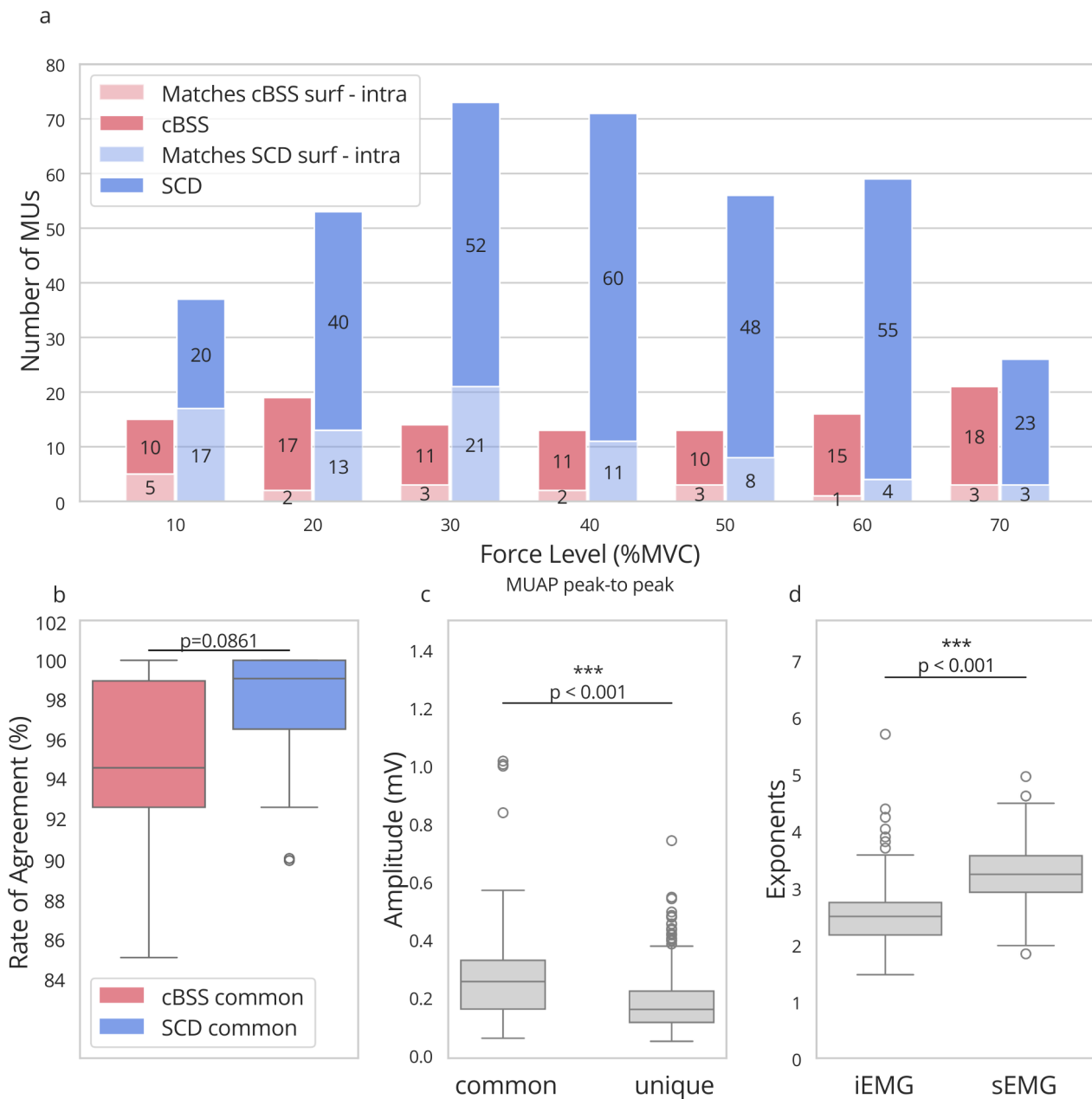


Figure 5.8: Effect of the decomposition algorithm used on experimental data. **a** Number of MUs found against the force level for SCD and cBSS. The number of matched MUs between the intramuscular and the surface recordings is reported in a lighter shade (light pink for cBSS and light blue for SCD). The results from the two subjects are pooled together. **b** Distribution of the RoA for the MUs that matched with the intramuscular recordings and common between cBSS and SCD. **c** Distribution of the MUAP peak-to-peak amplitudes for the MUs commonly identified by cBSS and SCD, and those uniquely found by SCD. **d** Distribution of the exponents for the intramuscular and the surface recordings.

subject, SCD identified 17 and 22 MUs, while cBSS identified 10 and 17 MUs respectively. Only four units that were identified by cBSS were not identified by SCD. Across the units with high level of agreement, the RoA between SCD and cBSS was $96.7 \pm 2.6\%$ (Figure 5.10 **b**). The differences in the

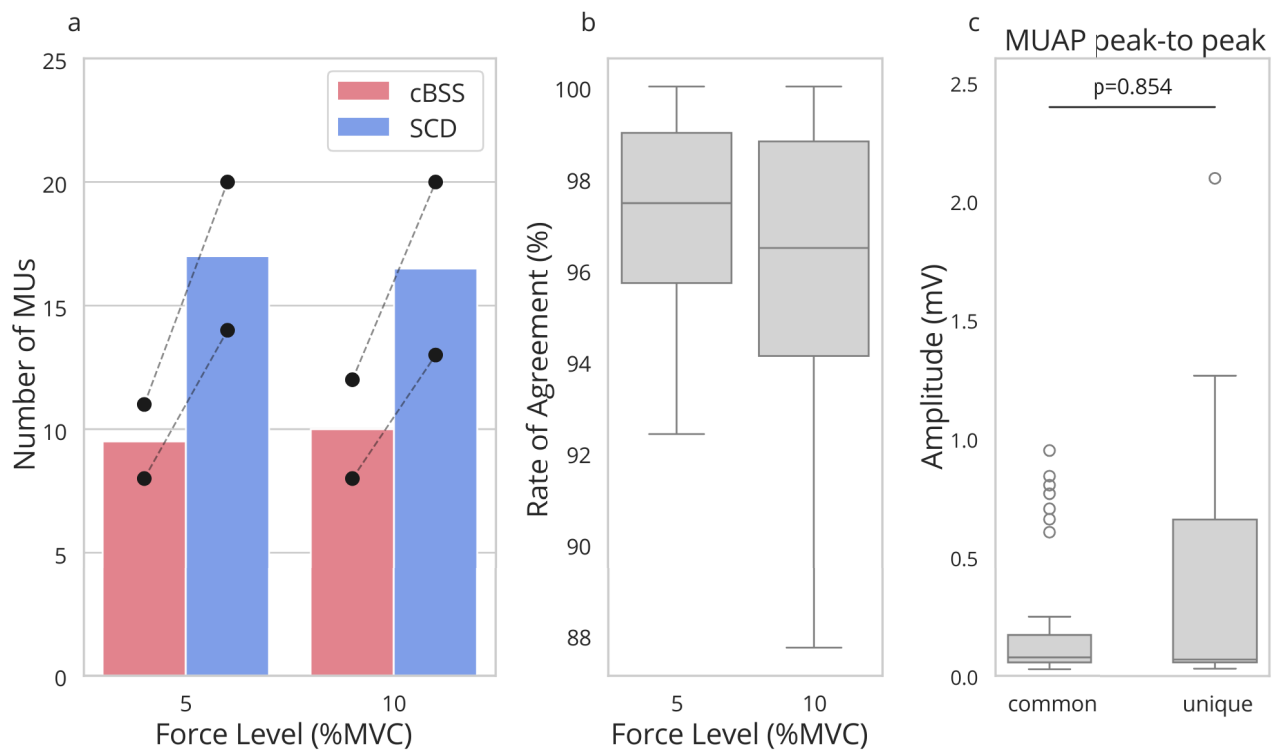


Figure 5.9: Effect of the decomposition algorithm used on experimental data. **a** Mean Number of MUs found against the force level for SCD and cBSS across the two participants. The black circles represent the value for each subject. The dashed lines connect the subjects across force levels. **b** Distribution of the RoA for the MUs that matched between cBSS and SCD. **c** Distribution of the MUAP peak-to-peak amplitudes for the MUs commonly identified by cBSS and SCD, and those uniquely found by SCD.

peak-to-peak amplitudes of the MUAPs between the units commonly found by both cBSS and SCD and those uniquely identified by SCD were not significant (panel c).

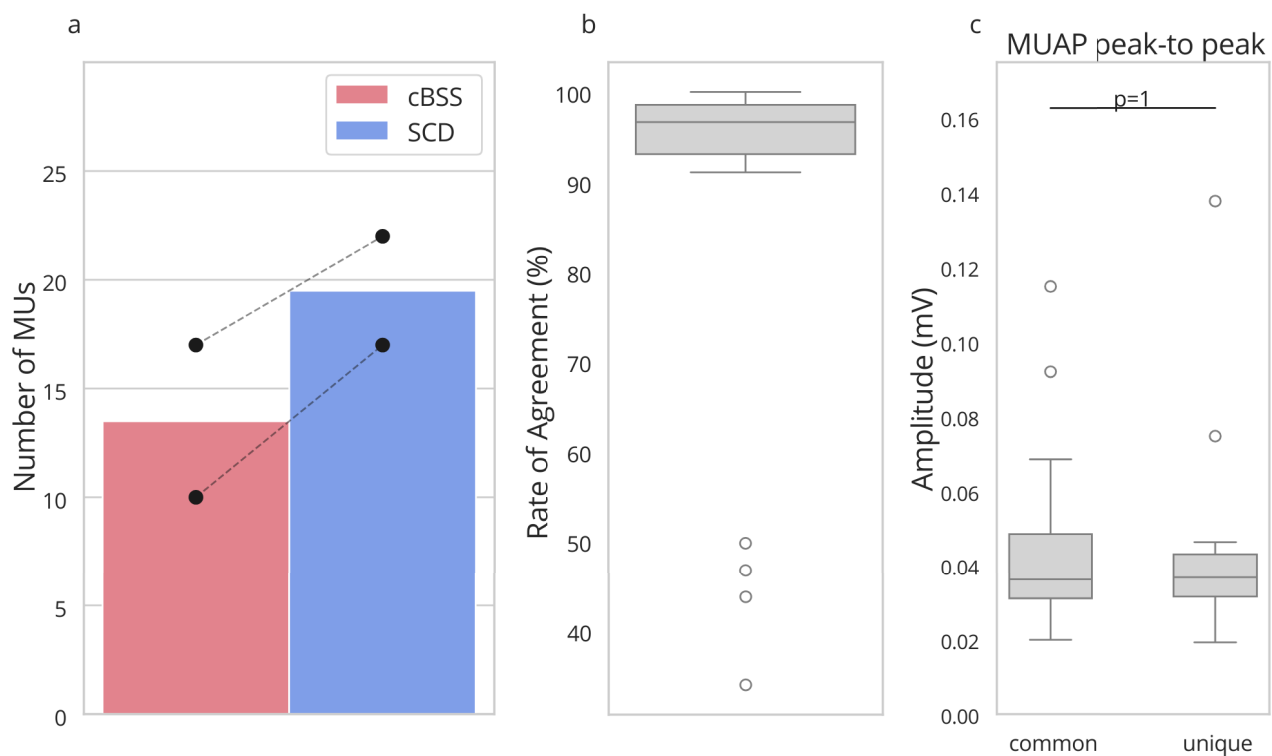


Figure 5.10: Effect of the decomposition algorithm used on experimental data recorded at the forearm of two female participants. **a** Mean number of MUs found for SCD and cBSS across the two participants. The black circles represent the value for each subject. The dashed lines connect the subjects across force levels. **b** Distribution of the RoA for the MUs that matched between cBSS and SCD. **c** Distribution of the MUAP peak-to-peak amplitudes for the MUs commonly identified by cBSS and SCD, and those uniquely found by SCD.

5.4 Discussion

We proposed and validated SCD for the decomposition of HD-sEMG signals, demonstrating its superior performance over SoA approaches. As demonstrated with both simulations and experimental results, SCD represents a major step forward in HD-sEMG decomposition, with broad implications for the study of the neural control of movement. The core strength of SCD lies in its dynamic adaptation of the contrast function via particle swarm optimization and the incorporation of a peel-off strategy for sequential source removal. These features enable SCD to address critical challenges in HD-sEMG decomposition, such as differentiating MUs with highly similar MUAPs and identifying MUs with low-energy MUAPs. Unlike conventional BSS algorithms that rely on fixed contrast functions (Holobar & Zazula, 2003; Negro et al., 2016), the ability of SCD to adjust its contrast function allows for a greater flexibility and improved handling of diverse signal characteristics.

SCD was validated using both simulated and experimental data. In simulation, SCD decomposed a number of MUs nearly double those identified by classic cBSS. The MUs identified by SCD had smaller MUAPs (Figure 5.3 **d** and Figure 5.8 **c**), and were therefore either deeper in the muscle tissue or smaller (Figure 5.3 **g**). Accordingly, the MUs identified by SCD had slower conduction velocities and fewer innervated fibers (Figure 5.3 **e, f**).

When further applied to simulated data during ballistic tasks (Figure 5.6 **c**), SCD demonstrated a 3-fold increase in the number of decomposed MUs (Figure 5.7 **a**), while reaching higher accuracy than the SoA. Furthermore, simulations also demonstrated that SCD identified a greater number of MUs than cBSS at varying levels of noise, despite the difference between methods decreased with an increase in noise (Figure 5.5, **a**).

The simulations also revealed that both the adaptive contrast function and the peel-off procedure significantly contributed to SCD's performance (Figure 5.4). Interestingly, we showed that fixing the exponent to the median value of the adaptively determined exponents during the swarm update yielded significantly fewer decomposed MUs compared to allowing the exponent to adapt (Figure 5.4**b**). This is because sources requiring higher or lower exponents would not be detected with a fixed $e = 2.4$, preventing the algorithm from removing their contributions and converging to alternative

sources. Moreover, the median exponent varied depending on the dataset, making it impractical to predefine an exponent value in advance; this determination would only be feasible post-hoc, following decomposition using the swarm update. By incrementally removing the contributions of higher amplitude MUAPs, SCD was able to converge to smaller, less prominent MUAPs that would otherwise remain undetected. Importantly, the peel-off approach did not compromise performance; in all analyses, the RoA for SCD consistently surpassed that of cBSS, which employs a deflation method.

Overall, the simulations indicated high performance of the proposed method in terms of MU yield and accuracy in decomposition. We further validated SCD experimentally using a two-source approach by comparing HD-iEMG and HD-sEMG recordings (Farina, Merletti, & Enoka, 2014; Mambrito & De Luca, 1984). Matching the decomposition results between these two modalities was crucial for validating the proposed algorithm. SCD decomposed significantly more MUs than cBSS from experimental signals, with a higher number of matched MUs extracted from the HD-iEMG recordings (41.6 ± 12.1 and 12.0 ± 5.3 vs 13.7 ± 3.1 and 2.1 ± 0.7 , $p < 0.001$). Interestingly, we observed that the distribution of exponents required in the contrast function had greater mean value for HD-sEMG signals compared to HD-iEMG signals (Figure 5.8, **d**). This finding suggests the need for a higher exponent when dealing with sources that are smaller or more similar to one another, a characteristic often seen with non-invasive signals.

Overall, the new approach significantly increased the number of detected MUs across all conditions without compromising accuracy, which actually improved. The yield varied widely depending on the conditions, with increases ranging from approximately 50% to over 300%, but yet an increase was achieved consistently in all cases, across a broad range of scenarios. This consistent increase implies we can now achieve better results in conditions that previously yielded borderline unit detections. Future work could explore how adjusting certain hyperparameters used in SCD might further enhance decomposition performance. For instance, lowering the silhouette threshold or adapting it dynamically during decomposition could potentially yield better results and recover sources that might otherwise be refined during manual editing. Also, future work could investigate whether all six swarm particles are essential for effectively capturing non-linearities or if computational time can be reduced by using fewer exponents. Nevertheless, since SCD performs six optimizations in

parallel and identifies more than twice as many MUs as cBSS, we believe the additional computational time is justified and does not pose a significant barrier to practical application.

The method is particularly useful in conditions where the number of successfully decoded units is typically small. For instance, in cases where the MUAPs are highly similar—such as when recording from deep muscles or from muscles covered by a large layer of subcutaneous fat—when discriminating between units becomes a major challenge. In these scenarios, standard decomposition techniques may fail to distinguish MUAPs, often leading to failure in the decomposition. By dynamically optimizing the separation of sources, SCD is a step forward to mitigate this problem, maximizing the contrast between similar MUAPs and ensuring a higher yield of distinct MUs even in such complex scenarios. An example of such conditions is the decoding of MUs from female individuals. The proposed method demonstrated superior MU yield, as highlighted earlier. This suggests that the technique is particularly effective in addressing physiological differences, enhancing the overall accuracy and applicability of MU detection across diverse populations. However, due to the limited number of experimental data presented, these findings should be validated through further analysis and additional research.

We presented representative results from female individuals for both the TA muscle, typically known for successful decomposition, and the forearm muscles, where decomposition tends to be less effective. The proposed method consistently improved the yield of decomposed MUs with respect to the SoA cBSS in these subjects for both muscle groups. However, the limited number of participants and experimental conditions precluded a statistical analysis, highlighting the need for additional data to evaluate the significance of these findings.

In most conditions, in female individuals, we could enhance the number of decomposed units by around 50%. While this does not match the numbers observed in males, it represents a substantial improvement in many cases. In some instances, adding 5 to 6 reliably extracted MUs, as shown in this study, can have a significant impact on the physiological interpretations.

Increasing the number of concurrently decoded MUs is critical for advancing our understanding of the neural control of movement. For example, a larger pool of concurrently sampled MUs provides more comprehensive information on the distribution of common synaptic inputs to MN populations

(Farina, Negro, & Dideriksen, 2014). This is particularly valuable for characterizing how MNs are grouped to generate force and for predicting the net muscle force generated by the population of active units (Caillet, Phillips, et al., 2023). Indeed, the association between estimated neural drive to muscle and muscle force output becomes stronger and more reliable as more MUs are included in the cumulative analysis.

The expansion in MU sample size not only enhances the precision of neuromuscular research but also opens up new possibilities for clinical and assistive applications, such as refined neural control strategies in prosthetics and advanced neural interfaces for rehabilitation (Barsakcioglu et al., 2020; Farina et al., 2017; Gogeaescoechea et al., 2020; Tanzarella et al., 2023). In these applications, adapting SCD for real-time, online decomposition is a key future direction.

However, SCD has been validated exclusively in scenarios where the muscle geometry remains static. Its current framework does not account for dynamic changes in muscle fiber length or significant shifts in firing patterns. Consequently, SCD's performance is likely to degrade in more complex conditions, such as during dynamic muscle contractions where fiber lengths or recruitment strategies change over time. This limitation highlights the need for further development to adapt the algorithm for non-stationary conditions, where changes in muscle architecture and firing behavior could substantially impact decomposition accuracy.

5.5 Conclusions

This study establishes SCD as the new benchmark for HD-sEMG decomposition. By dynamically adapting its contrast function and adopting a unique peel-off strategy, SCD consistently outperforms traditional methods, particularly in detecting small and deep MUs. These findings, validated through comprehensive simulations and experimental data, pave the way for SCD's use as a new tool for the study of the neural control of movement as well as for applications ranging from clinical diagnostics to advanced human-machine interfaces. Its ability to resolve finer differences between MUAPs marks a significant leap forward in capturing the full complexity of neuromuscular activity.

Building upon our optimized decomposition algorithms for both HD-iEMG and HD-sEMG signals,

the next chapter presents a robust proof-of-concept for an offline neural interface. This novel system leverages the decoded MU activity from HDEMG signals to demonstrate the feasibility of EMG-based neural interfaces for movement control.

These two worlds-the visible and the invisible-are in contact. Yet the difference between them is so great that the problem of the boundary that puts them in contact, that distinguishes them but also unites them, cannot fail to arise. How can this be understood?

Florenskij, Pavel

6

A Near-Perfect Neural Classifier

NOTE: Aside from minimal alterations to fit the format and flow of the thesis as a cohesive single document, the content of this chapter has been published in the following preprint article:

Grisson, A., Ibáñez Pereda, J., Muceli, S., Kundu A., Baracat, F., Indiveri, G., Donati, E., Farina, D. (2024). Intramuscular High-Density Micro-Electrode Arrays Enable High-Precision Decoding and Mapping of Spinal Motor Neurons to Reveal Hand Control. arXiv.

doi: <https://doi.org/10.48550/arXiv.2410.11016> (Under licence CC-BY)

Chapter 3 and Chapter 5 validated our proposed decomposition algorithm for HD-iEMG and HD-sEMG respectively, showing SoA performance in both realms. In this chapter, we propose a novel neural decoding system that enables unprecedented large-scale sampling of muscle activity. Using micro-electrode arrays with more than 100 channels embedded within the forearm muscles, we recorded high-density signals that captured multi-unit motor neuron activity. This extensive sampling was complemented by advanced methods for neural decomposition, analysis, and classification, allowing us to accurately detect and interpret the spiking activity of spinal motor neurons that innervate hand muscles. We evaluated this system in two healthy participants, each implanted with three HD-iEMG micro-electrode arrays (comprising 40 electrodes each) in the forearm. These arrays recorded muscle activity during both single- and multi-digit isometric contractions. For the first time under controlled conditions, we demonstrate that multi-digit tasks elicit unique patterns of motor neuron recruitment specific to each task, rather than employing combinations of recruitment patterns from single-digit tasks. This observation led us to hypothesize that hand tasks could be classified with high precision based on the decoded neural activity. We achieved perfect classification accuracy (100%) across 12 distinct single- and multi-digit tasks, and consistently high accuracy (96%) across all conditions and subjects, for up to 16 task classes. These results significantly outperformed conventional EMG classification methods. The exceptional performance of this system paves the way for developing advanced neural interfaces based on invasive high-density EMG technology. This innovation could greatly enhance human-computer interaction and lead to substantial improvements in assistive technologies, offering new possibilities for restoring motor function in clinical applications.

6.1 Introduction

Neural interface research aims to restore or establish connections between the nervous system and the external environment, for example in individuals with neurological impairments (Hatsopoulos & Donoghue, 2009; Lebedev & Nicolelis, 2006). These interfaces, based on stimulating (encoding) and/or recording (decoding) from neural pathways, are used to assist individuals with disabilities or enhance the capabilities of healthy individuals. For this purpose, the decoding of neural signals

provides commands to virtual systems, wearable robots or tele-operated devices.

Direct brain signal decoding is the only option for severe impairments; however, for many other applications, nerve or muscle interfacing may be preferable (Farina & Aszmann, 2014; Oskoei & Hu, 2007). The sEMG is particularly simple to record and is used in several interfacing applications (Shenoy et al., 2008; Wołczowski & Kurzyński, 2010; Zheng et al., 2022). Although recording signals from muscle tissue is not a direct neural interface, the EMG captures the electrical activity produced by the muscles, which reflects the neural activation sent from the spinal cord's output layers to the muscles (Farina et al., 2017). Therefore, sEMG signals can be decoded to reflect the activities of individual spinal MNs, which can then be mapped into estimated motor intent.

Despite the potential of surface EMG-based decoding of neural activity, current decoding and mapping systems are limited in accuracy and, particularly, vary significantly in performance across individuals and conditions. The primary cause of this variability is the dependence of surface EMG characteristics on the volume conductor separating the sources of signal (muscle fibers) and the recording electrodes. Associated to this limitation are the sensitivity of surface EMG features to electrode misplacement, typically due to donning and doffing of the wearable device (Mesin et al., 2009), and EMG signal cross-talk (Farina, Merletti, Indino, et al., 2002; Muceli et al., 2014). These and other limitations of wearable EMG sensors can be addressed with invasive electrodes. Recent advances in iEMG technology have provided micro-electrode arrays with tens of electrodes closely spaced with respect to each other (high-density EMG micro-electrode arrays) (Muceli et al., 2015, 2022).

Here, we present an innovative neural decoding system that extracts neural information from HD-iEMG micro-electrode array signals, mapping precise discharge timings of the decoded MNs into the executed tasks. This system was experimentally validated offline on two healthy individuals, using three concurrently implanted intramuscular electrodes in each subject (Figure 6.1). The validation involved classifying single- and multi-digit isometric tasks. The performance of the HD-iEMG mapping was then compared to the use of traditional pattern recognition techniques applied to the HD-sEMG and HD-iEMG signals. Therefore, we present four key advances in the field of neural decoding: 1) recordings of 120 intramuscular signals concurrently detected, paired with corresponding

HD-sEMG signals. This is so far the largest reported number of implanted electrodes in the forearm muscles; 2) new perspectives on the neural command structure underlying finger movements; 3) a highly accurate neural classifier (100% accuracy in single- and multi-digit tasks for 12 classes), using physiological and interpretable features; 4) a comprehensive comparison of mapping performance across invasive and non-invasive high-density recording modalities. These findings enhance our understanding of neuromuscular control of the hand and have significant implications for neuro-prosthetics and human-machine interfaces.

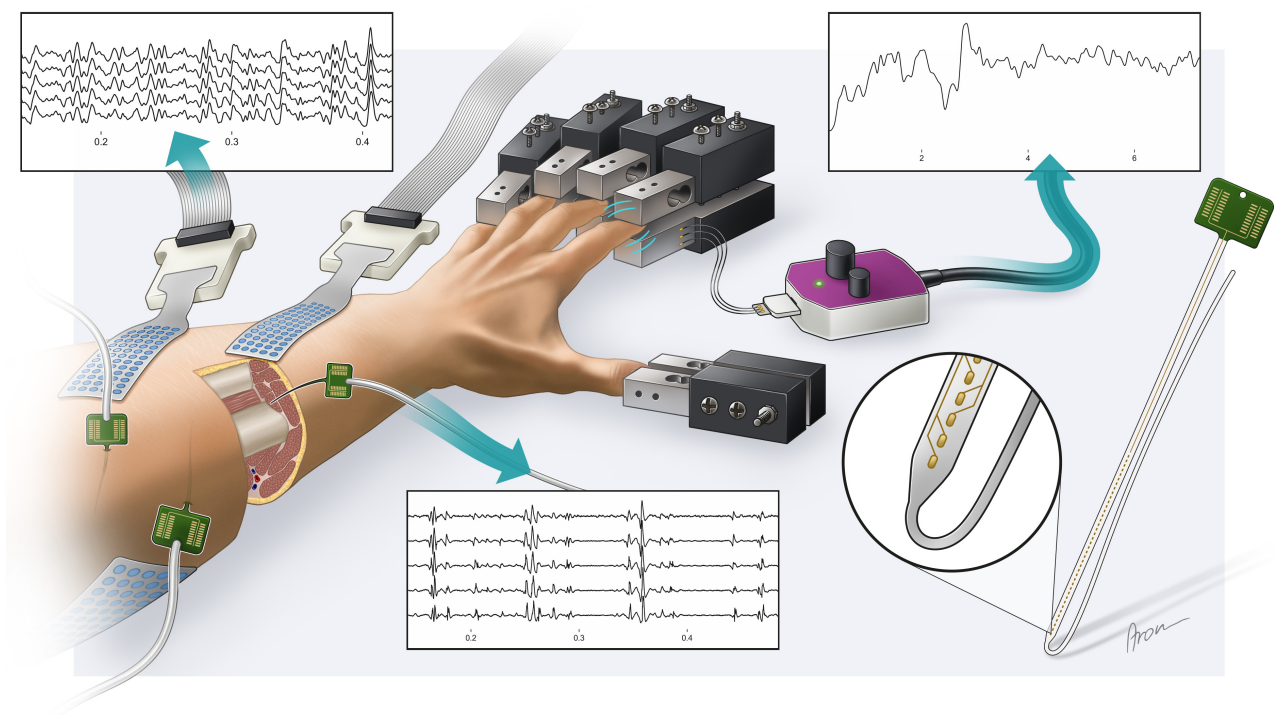


Figure 6.1: Experimental setup: Three micro-electrode arrays were implanted into the forearm muscles of two participants. Three high-density surface electrode grids were placed on the skin overlying the intramuscular recording sites. Two load cells per finger were used to measure flexion and extension forces. Representative data are shown for iEMG, sEMG, and force recordings. A schematic of the high-density intramuscular micro-electrode arrays, with a detailed zoom-in on the electrode tip, is shown on the bottom right.

6.2 Methods

6.2.1 Data Acquisition

6.2.1.1 Participants

Two healthy men, aged 39 and 30 years, were recruited for this study. Both participants had no history of neurological or musculoskeletal disorders that could affect MN functionality. The small subject sample was due to the relatively complex implant procedure, which included three micro-electrode arrays per subject, with ultrasound-guided insertion (see below). All experimental procedures adhered to the ethical guidelines set by Imperial College London. The studies were performed according to the Declaration of Helsinki, with an informed consent form signed by all participants before each experiment (ICREC Project ID 19IC5640 for the intramuscular recordings; JRCO Project ID 18IC4685 for the MRI acquisition).

6.2.1.2 Experiments

HD-iEMG signals were recorded from forearm muscles using multi-channel intramuscular electrodes designed for acute recordings (Muceli et al., 2015, 2022) (Figure 6.6 **a**). Additionally, three surface electrode grids (IED 4mm) with 64 recording sites each (Figure 6.6 **c**) were positioned over the targeted muscles to concurrently acquire HD-sEMG signals. This configuration enabled concurrent measurement of the electrical activity both within the muscle and at the skin surface. The EMG signals were acquired using a multi-channel amplifier (OT-Bioelettronica, Torino, Italy). All EMG data were sampled at 10,240 Hz, and digitised with 16-bit resolution. The HD-EMG signals were bandpass filtered (Butterworth, order 5) between 100-4400 Hz (HD-iEMG) and 20-500Hz (HD-sEMG). Recordings were made in bipolar configuration with a reference electrode placed on the wrist.

The participants performed force-tracking tasks following trapezoidal force trajectories at 15 % of their MVC (Figure 6.3 **b**). These tasks were executed individually for each finger (thumb, index,

middle, ring, and little finger) in flexion and extension. Additionally, forces were exerted by combinations of fingers (thumb and index; index and middle; middle and ring; index with middle and ring; thumb with index, middle, ring, and little finger) in flexion and extension, following the same trapezoidal profile. During all tasks, participants maintained isometric contractions focusing on the specified movements. The duration of the constant-force part of the contractions was 20 seconds. Two repetitions were recorded for each task.

A total of 20 types of contractions (classes) were performed. In the following, these classes were labelled as follows: thumb flexion (T-F), index flexion (I-F), middle flexion (M-F), ring flexion (R-F), little flexion (L-F), thumb and index flexion (TI-F), index and middle flexion (IM-F), middle and ring flexion (MR-F), index and middle and ring flexion (IMR-F), thumb and index and middle and ring and little flexion (TIMRL-F) thumb extension (T-E), index extension (I-E), middle extension (M-E), ring extension (R-E), little extension (L-E), thumb and index extension (TI-E), index and middle extension (IM-E), middle and ring extension (MR-E), index and middle and ring extension (IMR-E), thumb with index, middle, ring, and little extension (TIMRL-E).

We classified the different tasks with a series of analyses. First, we attempted the classification of 16 classes that included all individual fingers in flexion and extension, and three combinations of two fingers (T-F, I-F, M-F, R-F, L-F, IM-F, MR-F, IMR-F, T-E, I-E, M-E, R-E, L-E, IM-E, MR-E, IMR-E). Then, we focused on the tasks mostly associated to the targeted muscles, which resulted in 12 classes: I-F, M-F, R-F, IM-F, MR-F, IMR-F, I-E, M-E, R-E, IM-E, MR-E, IMR-E. Finally, as a proof of concept of a specific application of the mapping system - the control of a prosthetic hand - we selected tasks that are functionally important for amputees and challenging to decode with SoA methods (Jang et al., 2011; Kashiwakura et al., 2023). This group of tasks included power grip (TIMRL-F, TIMRL-E), pinch (TI-F, TI-E), and individual index/thumb finger activation (T-F, T-E, I-F, I-E), for a total of eight classes. All tasks were performed isometrically, with the corresponding fingers activated simultaneously. However, the power grip class was excluded for subject S1 due to the inability to correctly perform the coordinated movements of the thumb, index, middle, ring, and little fingers. Specifically, S1 exhibited oscillations in the force applied by the little finger, with fluctuations of amplitude of around 7 %MVC. These force variations led to inconsistent activation of the MUs, which were subsequently removed by the automatic cleaning process. As a result, the last analysis resulted

in six classes for S1 (T-F, I-F, TI-F, T-E, I-E, TI-E) and eight for S2 (T-F, I-F, TI-F, TIMRL-F, T-E, I-E, TI-E, TIMRL-E).

6.2.1.3 Instrumented Platform

A finger force measurement platform was designed to be adaptable to different hand sizes, adjustable in wrist angle, and equipped with high-resolution and cost-effective load cells to capture force signals. A 10-mm polycarbonate board was used for the platform body because of its light weight. Precision laser-cutting of load cell rails was executed using CAD software. The platform features ten 10-kg TAL220 load cells (two per finger, one for flexion, and one for extension), selected based on the maximum observed male exertion forces of 7-8 kg (Carabello et al., 2022; Cort & Potvin, 2011). The load cells are mounted on adjustable rails with rolling pins and connected to 3D-printed mounts to accommodate varying finger thicknesses. The EMG signals and the force data were acquired simultaneously by the Quattrocento amplifier. Force signals were amplified using Forza pre-amplifiers linked to the amplifier. A 3D-printed holder housed the 10 pre-amplifiers, optimising the setup for accurate force measurement across various hand sizes.

6.2.2 Classification

We examined the accuracy in classifying intended motor tasks into predefined categories by comparing two approaches: one that used decomposed MU activity (i.e., the timings of MN discharge events) and another that used global features derived from the interference signal (e.g., signal amplitude).

To classify tasks using MN behaviour, the HD-iEMG signals were decomposed into the contributing MUs. Several blind source separation approaches have been proposed in the literature (Buccino et al., 2018; M. Chen & Zhou, 2024; M. Chen et al., 2018; Jäckel et al., 2012; Leibig et al., 2016; Muceli et al., 2022). However, these methods often struggle to separate sources when their waveforms are similar, resulting in overlapping signals. Current contrast functions and non-linear metrics are not optimised to resolve these overlaps. We thus utilised a novel algorithm, SCD (Grison et al., 2024),

that addresses these limitations by using an adaptive contrast function that enhances spike sorting accuracy by adapting to the unique characteristics of spiking distributions. The filters associated with each source (MU) were aggregated into a comprehensive dictionary of filters for each task. Figure 6.5 panel **a** provides a schematic representation of this filter bank. In the diagram, each colour corresponds to a different class label, indicating the specific task being represented. The width of each bar reflects the number of filters associated with that task, illustrating how many MU filters contribute to the classification of each specific task. The zoomed-in section within the magenta box offers a closer look at a representative filter from the pooled filter bank. This filter visualises the amplitude of the MU across different channels, providing insights into how the neural activity is distributed spatially across the electrode array. Conversely, for global EMG classification, the root mean square of the EMG signals served as the feature for a C-Support Vector Machine classifier (Figure 6.5 **b**). To mimic a real-life mapping system, the calibration phase was conducted using the first recorded repetition, and the testing phase was performed using a repetition recorded later in the day.

6.2.2.1 Finger Task Classification Based on Motor Unit Behaviour

Motor unit spike trains were extracted through blind source separation of HD-iEMG recordings obtained during isometric finger tasks at 15 %MVC. The tasks included individual finger flexion and extension as well as multi-finger combinations performed isometrically. The decomposition process produced task-specific sets of MU filters, which were pooled to create a comprehensive bank of MU action potential filters $\mathbf{F}_i \in \mathbb{R}^{N \times M}$, where N represents the total number of extracted MU filters and M the number of EMG channels. Each MU filter $\mathbf{F}_i$ in this set was labelled according to the specific finger movement task it was derived from (e.g., thumb flexion corresponding to class 0).

These reference MU filters from the 15 %MVC contraction were then applied to the multi-channel iEMG signals $\mathbf{E}j \in \mathbb{R}^{M \times D_R}$, where D_R is the duration of the recording, recorded during a separate 15 % MVC trial involving all fingers performing both flexion and extension movements. This produced the corresponding discharge timings, represented as IPTs $\mathbf{I}ij \in \mathbb{R}^{N \times D_R}$ for each MU i and task j . Each IPT $\mathbf{I}_{ij}$ was clustered to separate the peaks (i.e., the source) from the noise components. Only

MUs with robust clustering quality ($SIL > 0.9$), physiological discharge periodicity ($CoV < 35\%$), and FRs within the physiological range for isometric contractions (mean discharge rate < 30 Hz) were retained for further processing. The discharge timings of these units were temporally segmented into 100-ms time bins with 50% overlap. This window was selected to be long enough for reliable classification while remaining short enough to minimize delay in real-time control applications. The windowing resulted in a binary matrix $\mathbf{B} \in \mathbb{R}^{N \times K}$, where K represents the number of bins. For each time bin k , the corresponding class label was assigned according to the filter $\mathbf{F}_i$ that produced the source, creating a final label vector $\mathbf{L} \in \mathbb{R}^K$. If no predictions were made for a given bin (i.e., no MUs were active), it was labeled as N/A. Finally, for each time bin, the class with the majority of votes was assigned to that bin. If there were no predictions overall for a bin, it was labeled as N/A and counted as a misclassification in the final accuracy calculation. The method, therefore, relies on unique MUs being consistently active throughout the entire task. Figure 6.5 **a** shows a schematic of the method.

6.2.2.2 Finger Classification Based on Global EMG

For myoelectric decoding of dexterous hand gestures, a linear SVM classifier with an L2 regularisation penalty factor of 0.1 was employed (Figure 6.5 **b**). The SVM classifier is a widely used method in EMG pattern recognition (Dhindsa et al., 2019; Oskoei & Hu, 2008; Purushothaman & Vikas, 2018). The HD-EMG signals were bandpass filtered (Butterworth, order 5) between 100-4400 Hz (HD-iEMG) and 20-500 Hz (HD-sEMG) to remove noise. Global normalisation factors based on the maximum absolute value across the pooled training set were derived and applied to the test set for consistent scaling. Outlier channels with baseline noise exceeding 3 standard deviations from the training mean were removed from all training and testing data. The pre-processed EMG was segmented into 100-ms intervals, with 50% overlap using a sliding window, preparing the bins similarly to how they were organised for the classification based on MUs. The r.m.s. values of the EMG were extracted as the input features. This was done in the same way for both surface and intramuscular high-density recordings. Moreover, to simulate a lower-density iEMG electrode configuration, we selected the first and last channel for each electrode, resulting in an IED of 2 cm (Figure 6.6 **b**). The difference between the two electrodes was computed for each array, resulting in 3 feature chan-

nels. This configuration was chosen to emulate structures like the implantable myoelectric sensors (IMES) electrodes, currently being tested to control multifunction hand prostheses (Merrill et al., 2011; Salminger et al., 2019; Weir et al., 2008). For the sEMG, we selected eight channels in the central column of the electrode grid, mimicking the configurations found in commercial low-density systems such as the MYOband (Figure 6.6 d).

6.3 Results

6.3.1 Targeted Muscle Insertions

The recording of multiple high-density intramuscular data arrays represents one of the key innovations of this study. HD-iEMG enables recordings from a significantly larger number of sites compared to traditional needle or wire electrode arrays (Farina & Gandevia, 2024). Furthermore, HD-iEMG signals exhibit greater spatial and temporal sparsity than HD-sEMG, allowing for more precise analysis of MN activity (Muceli et al., 2022). In this study, three HD-iEMG electrodes were inserted in the forearm muscles of two healthy participants, with one electrode placed in each of the most relevant extrinsic muscles for finger flexion and extension. Interfacing with MUs that control hand function poses inherent challenges due to the precise targeting required for intramuscular electrode placement in the primary muscles responsible for dexterous control. Optimal electrode placement sites were determined via a magnetic resonance imaging (MRI)- and ultrasound-based anatomical assessment of the forearm. MRI was employed to gain a detailed understanding of the forearm muscle anatomy and to guide the insertion point and angle of the electrodes. Specific forearm points were tracked using MRI markers across scans, allowing precise localization of marker positions and detailed insights into forearm structure (Figure 6.2 a). Each electrode array, comprising 40 platinum electrodes ($140\ \mu\text{m} \times 40\ \mu\text{m}$) linearly distributed over 2 cm with 0.5 mm IED spacing (Muceli et al., 2022), targeted muscles involved in finger movements. The insertion of each array was facilitated by a 25-gauge hypodermic needle, which was attached to the electrode with a guiding filament. Once the electrode was positioned, the filament was cut, allowing the needle to be removed while the electrode remained securely in the muscle.

The first micro-electrode array targeted the EPL and EDC muscles, activated for the extension of the thumb and the digits respectively. A distal location in the forearm was chosen to allow concurrent monitoring of the two muscles. The second array was inserted transversely to target the extensor digiti minimi (EDM) and the EDC, employing an unconventional technique that represents a novel approach to intramuscular electrode placement, thereby maximizing coverage of the muscles relevant to digit extension. The third array targeted the FDS muscle, chosen for its accessibility and essential role in flexion tasks.

MRI provides a comprehensive anatomical view of the forearm, but practical constraints limit its use during needle insertions. Once the electrode insertion points and general positioning were identified using MRI, precise muscle targeting during the actual insertion became essential to avoid veins, arteries, and nerves. This necessitated real-time imaging guidance to ensure accurate and safe insertion. A portable ultrasound device (Butterfly iQ+) was used to precisely visualise electrode paths to target muscles in real-time (Figure 6.2 **c** and **d**, for Subject 1 (S1) and Subject 2 (S2), respectively). Post-insertion, subjects performed maximal muscle contractions (MVC) as reference for submaximal tasks. This procedure also confirmed secure electrode placement and minimised the risk of displacement.

While electrode placement followed the above rigorous procedure, some errors in the exact target placements occurred. For example, the limited field of view of the ultrasound probe impeded the full tracking of the micro-electrode array targeting the EDM for both subjects (panels **c**, **d**, second column). The extension of the insertion to the boundary of the ECRB suggests that the electrode might not have fully targeted the EDM, potentially failing to record specific information related to the little finger tasks. Moreover, although the EPL was targeted in both subjects (panels **c**, **d**, first column), the number of channels within the muscle was relatively small (panels **c**, **d** first column). This potentially impacted the amount of information recorded from the EPL during these tasks. The micro-electrode arrays were acutely implanted in the target muscles with percutaneous wires.

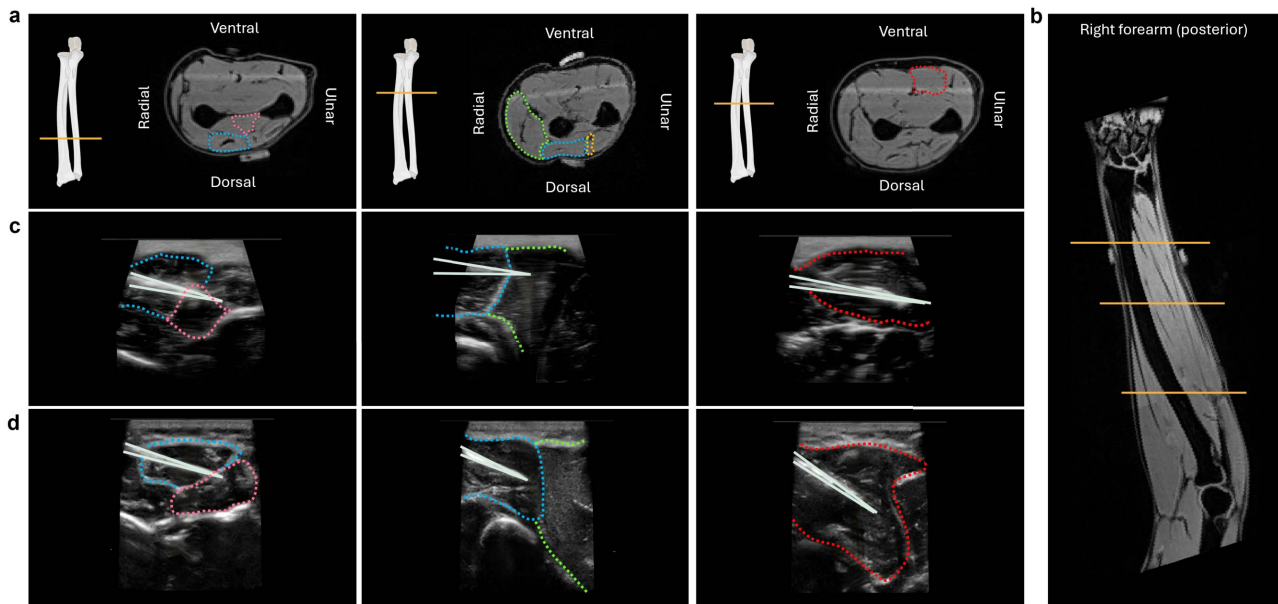


Figure 6.2: MRI and ultrasound images of the forearm with the boundaries of the relevant muscles highlighted in color (EPL: pink, EDC: blue, EDM: orange, ECRB: green, FDS: red). **a** MRI scans of the forearm of one subject with highlighted boundaries of the targeted muscles. The first MRI image includes a marker for precise positioning within the forearm’s anatomical structure. **b** Detailed MRI view of the forearm. **c, d** Ultrasound image during the micro-electrode array insertion for subject S1 (**c**) and subject S2 (**d**). The guiding needle is highlighted in white.

6.3.2 Data Characteristics

Concurrent recordings of HD-iEMG and HD-sEMG were obtained during flexion and extension tasks for each finger of the hand individually and for four combinations of finger tasks. By accurately decoding the electrical activity into the spiking activities of spinal MNs, we uncovered distinct neural signatures associated with each task, providing deeper insights into the neural mechanisms underlying fine motor control. Leveraging this new understanding, we then performed classification analyses across multiple conditions, further validating the robustness of these task-specific neural patterns. First, we classified 16 classes of gestures, which included all individual finger movements and three two-finger combinations, in both flexion and extension. Next, we analysed the 12 classes that corresponded to tasks primarily controlled by the implanted muscles, ensuring that these tasks involved movements whose neural activity was captured by the intramuscular micro-electrode arrays. Finally, as a proof of concept for prosthetic hand control (one representative application of this mapping system), we selected eight functionally relevant tasks, which are challenging to decode with current methods (see Section 6.2.1.2 in Methods for more details).

Figure 6.3 **a** shows a representative example of the data recorded from one of the micro-electrode arrays within the muscle. The multi-channel data recorded from all three micro-electrode arrays was subsequently decomposed to identify the spiking times of individual MUs (Figure 6.3 **b**). On average, 27.1 ± 12.9 (S1) and 8.0 ± 6.1 (S2) MUs were identified from the HD-iEMG per task (mean and std across two repetitions, the three electrodes, and the 16 tasks). The average discharge rate of the decomposed MUs across the two participants was 11.0 ± 3.9 spikes/s. The average number of MUs from the HD-sEMG was 5.1 ± 4.50 (S1) and 1.79 ± 1.86 (S2). On average across the two participants, the number of MUs decomposed from the HD-sEMG was significantly lower than the one from the HD-iEMG ($p < 0.0001$, Mann–Whitney U test). The average firing rate of MUs decoded from the HD-sEMG was 8.86 ± 3.03 spikes/s, also significantly lower compared to those from the HD-iEMG ($p < 0.0001$, Mann–Whitney U test). Figure 6.3 **c** presents the average amplitude maps of MU activation during different finger extension tasks. These maps were generated by first computing the spike-triggered average for each MU action potential, per electrode, and per task, followed by normalisation and rectification. The spike-triggered averages were then averaged across all active MUs for each electrode and task to highlight the areas of the electrode array that were most active for each task. As shown in Figure 6.3 **c**, the electrodes exhibited task-specific activity, indicating localised activation within each array. This observation reflects the high spatial selectivity of each HD-iEMG channel. While some improvements in insertion procedures may be needed in future studies, the results indicated that the recorded information was of high quality for the subsequent analyses.

6.3.3 Neural Control of Digit Tasks

This study marks the first instance in which we have been able to sample a relatively large number of MNs across multiple single- and multi-digit tasks under standardized conditions. By analyzing the activities of these MNs, we uncovered distinct neural patterns associated with different tasks, enhancing our understanding of the neural mechanisms underlying fine motor control.

Figure 6.4 shows the distribution of active MUs for individual finger movements and three two-finger combinations, with flexion tasks in panel **a** and extension tasks in panel **b**. Tasks are arranged along

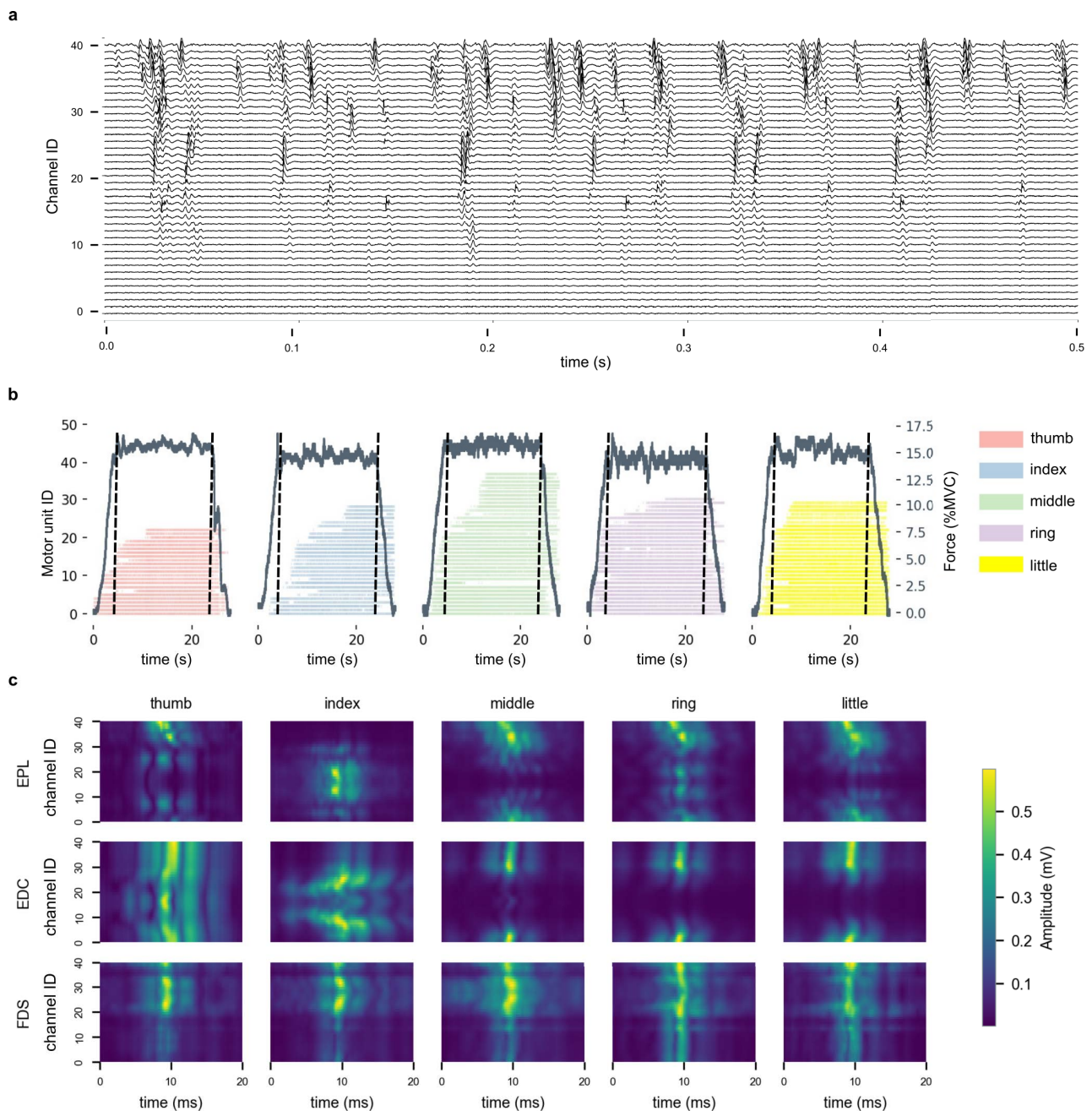


Figure 6.3: **a** A 0.5-second segment of HD-iEMG data captured during the extension of the little finger from the EPL electrode. **b** Raster plots showing the extension movements of individual fingers for the second repetition for subject S1. The sections within the dashed lines indicate the parts of the signal considered for this study. **c** Average amplitude map of MU activities during the extension task of each finger and for each electrode (S1). The x-axis represents time in ms, and the y-axis represents the 40 channels recorded per electrode.

the axes, with bold squares highlighting MUs engaged during the specific task. The data reveal that the highest number of active MUs were consistently task-specific. Motor units active during other tasks are shown using distinct colors and patterns—solid colors for flexion and diagonal hatching for extension—facilitating comparisons of MU engagement across different movements. While some

MUs were engaged during other tasks, their contribution was minor compared to the dominant task-specific activity, emphasizing the precision and task-specific recruitment of MUs. Notably, MUs involved in single-digit tasks were rarely recruited for multi-digit tasks involving the same finger. On average, MUs from single-digit tasks contributed for only $2.3 \pm 3.7\%$ of the MUs recruited across all multi-digit tasks and movement directions, underscoring the specialized recruitment of MUs for multi-digit movements. Furthermore, MUs that were active across all tasks, but not specifically tied to the task in focus, contributed for $44.6 \pm 11\%$, though task-specific MU activity consistently accounted for the majority of the recruitment. These findings highlight the nuanced and highly selective recruitment patterns of MUs during complex hand movements.

Figure 6.4 c provides a representative example of MU action potential waveforms for index flexion (blue), middle flexion (green), and the combination of index and middle finger flexion (pink). The distinct waveform shapes between the combination and individual finger movements visually confirm, for this representative example, that mostly unique MUs were activated for each task. This suggests that specific MUs are recruited in different patterns depending on the complexity and nature of the finger movements. The ability to distinctly identify these patterns is crucial for accurate decoding and mapping systems. The distinct activation of MUs for each task suggests that this decoding system is well-suited for highly accurate gesture classification. This consistency supports the use of MU activity to reliably differentiate gestures, as explored in the subsequent classification analysis.

6.3.4 Decoding Hand Function

The following sections present the results of three offline analyses: 1) classification of all 16 classes of recorded tasks; 2) classification of a subset of 12 classes associated to tasks primarily requiring the muscles that were targeted by the multi-electrode arrays (see Section 6.3.1), and 3) classification of six (S1) and eight (S2) classes chosen as important for prosthetic control (Kashiwakura et al., 2023) (considered as a potential application of the proposed decoding system). In all analyses, results are reported when using three feature spaces: MU discharge timings as decoded from HD-iEMG, root mean square (r.m.s.) values for each channel of the HD-iEMG, and r.m.s. values for each channel of the HD-sEMG. The different classification pipelines are depicted in Figure 6.5. Additionally, in the

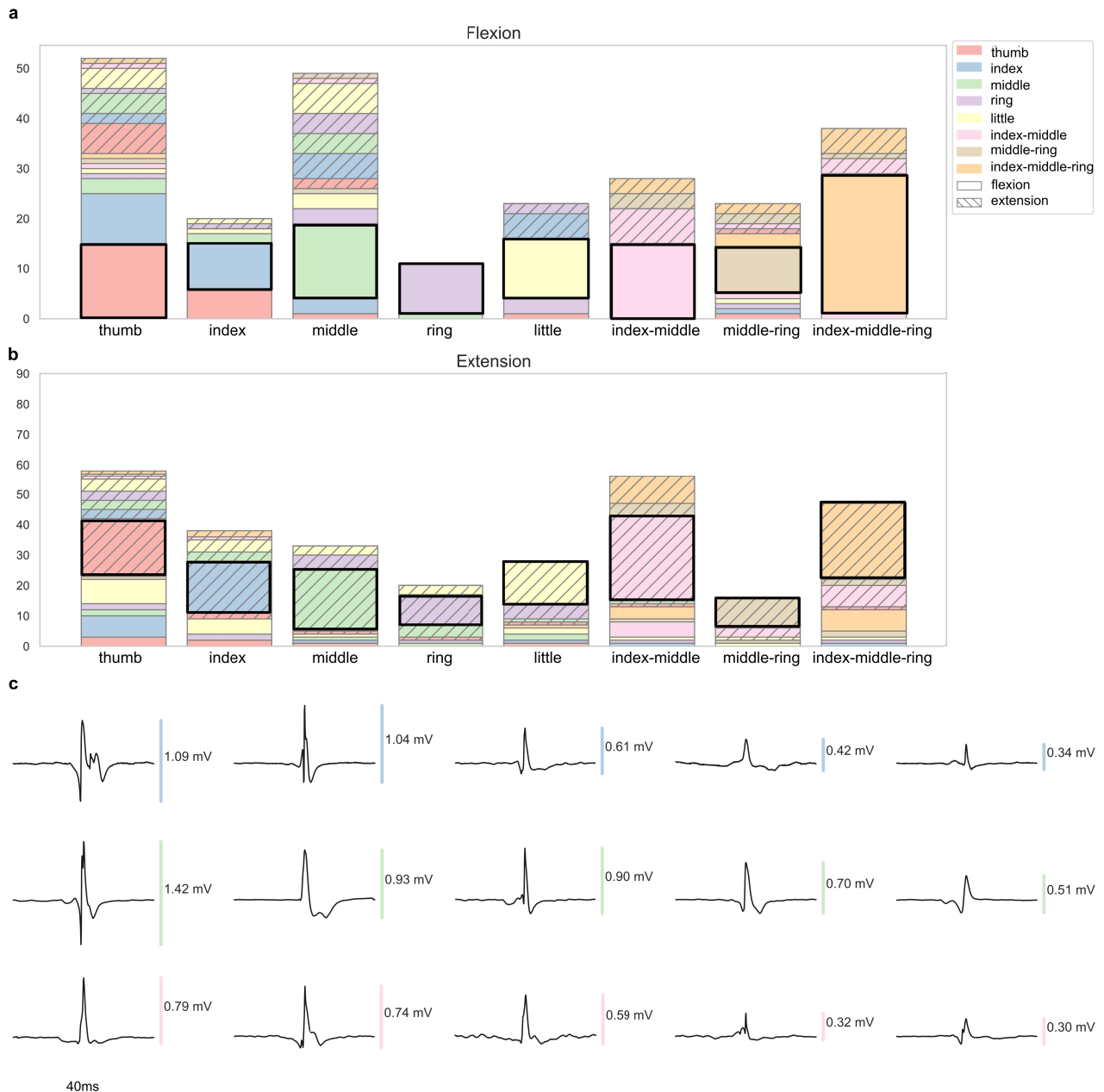


Figure 6.4: Motor unit counts for S1 during flexion (**a**) and extension (**b**) tasks classified from the 16 classes. Each bar represents the number of MUs that were active during the specific task, labelled according to the filter that produced the accepted source. The hatch pattern distinguishes the extension task from the flexion task, providing a clear visual differentiation between the two movements. This highlights the distribution and activity levels of MUs across different tasks. **c** Representative MU action potentials decomposed from the FDS electrode for S1 during various tasks: index finger flexion (blue), middle finger flexion (green), and the combination of index and middle finger flexion (pink). The majority of the MUs in the combination task originate from unique MUs specific to the combined movement, rather than being a combination of the MUs active during the individual index and middle finger flexion tasks. This indicates distinct MU recruitment patterns for the combined task compared to the individual tasks.

first two analyses we report results from lower-density iEMG and sEMG signals (in Figure 6.6 **b** and **d** respectively). This analysis was performed to investigate whether high-density configurations were essential for achieving high prediction accuracy. Throughout the rest of the study, the classification results based on MNs activity decoded from HD-sEMG signals were omitted due to insufficient MU decoded in the recordings. This limited yield likely stemmed from suboptimal electrode grid coverage and placement, resulting in most tasks having no detectable MUs activity.

6.3.4.1 Full Classification (16 classes)

In the analysis of motor tasks spanning all 16 flexion and extension categories, the MN discharge timings decoded from HD-iEMG provided a perfect classification accuracy for subject S1 of 100% and an accuracy of 96.1% for subject S2 on average across the tasks. The mis-classification for subject S2 was exclusively related to the little finger, and it was due to the fact that the electrode placement did not adequately target the EDM, the muscle primarily responsible for the activation of the little finger. The accuracy using the decomposition from the HD-iEMG recordings was higher than that achieved using the r.m.s. values of the HD-iEMG (90.1% and 94.3%) or the r.m.s. values of the HD-sEMG (96.6% for S1, 76.5% for S2). Figure 6.7 presents the confusion matrices per subject and classification type.

6.3.4.1.1 Lower-density Electrode Configurations When using low-density EMG configurations, the accuracy decreased substantially compared to the high-density systems, with the bipolar intramuscular achieving 47.8% for S1 and 69.0% for S2, and the sEMG achieving 92.5% for S1 and 64.3% for S2. These results indicate that the reduction in accuracy when shifting from a high-density to a lower-density configuration for pattern recognition is particularly large for intramuscular recordings and more modest for surface signals (Bian et al., 2017; Toledo-Pérez et al., 2019). The confusion matrices for the bipolar and low-density sEMG signals are shown in Figure 6.8.

6.3.4.2 Targeted Muscle Classification (12 classes)

Figure 6.9 shows the confusion matrices for the classes fully captured by the electrodes, as confirmed by ultrasound imaging (Figure 6.2). The classifier using MUs decomposed from HD-iEMG signals

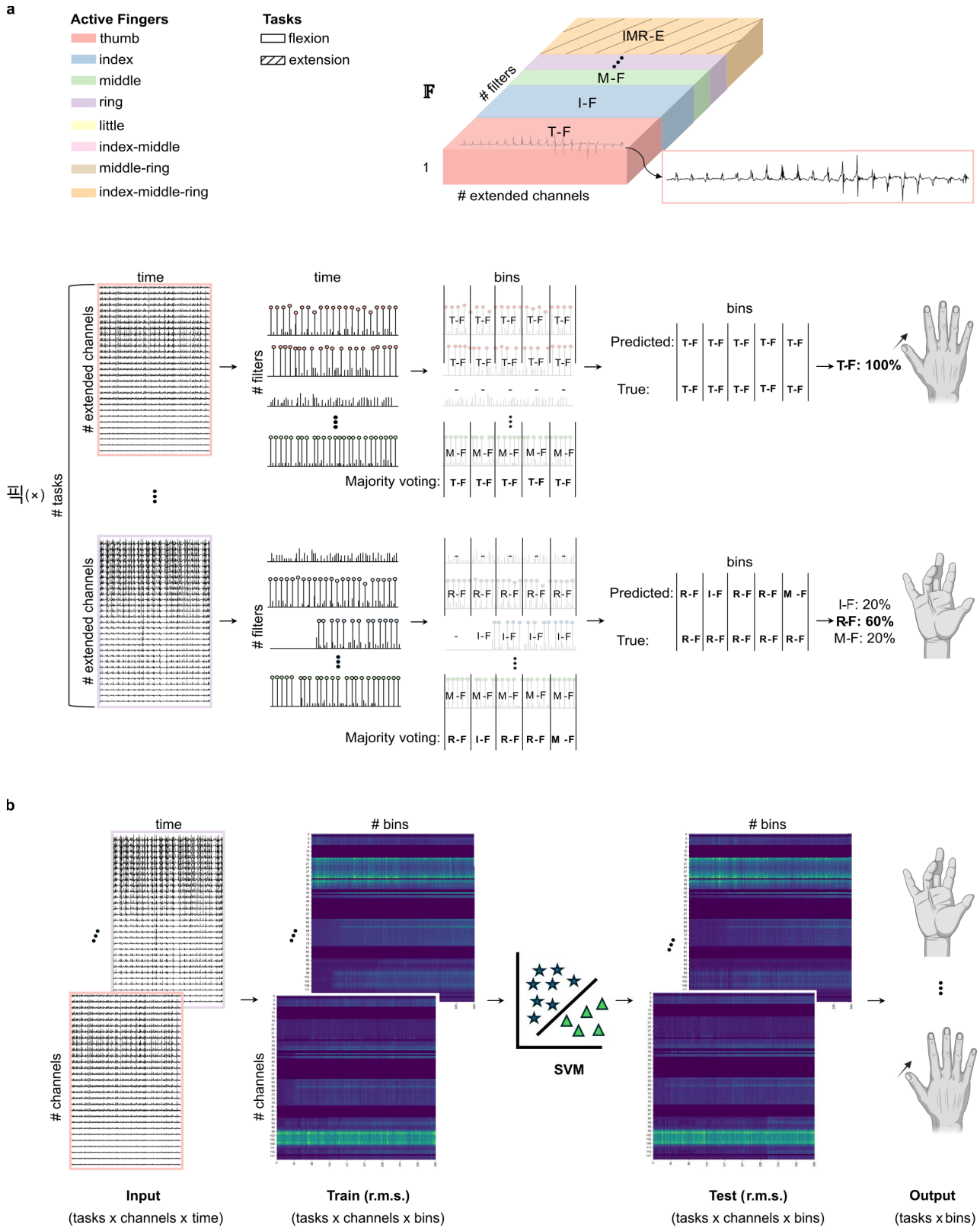


Figure 6.5: Analysis pipelines. **a** Classification using the MU discharge times as features. The EMG signals from each task (finger flexion and extension) were decomposed into individual MU activities. The MU action potential filters were then pooled into a filter bank, denoted as $\mathbf{F}$. These filters were applied to the EMG signals of each task, generating a set of IPTs. Each IPT was clustered into source and noise components. If the clustering quality was robust, the source was accepted and segmented into sliding windows. Each time bin was assigned a class label based on the filter that produced the corresponding source. Majority voting was applied across the set of IPTs for each segment. The final classification label was determined by the ratio of correctly labelled segments to the total number of labelled segments. **b** Classification from global EMG features. The r.m.s. values of the EMG signals were extracted and segmented into sliding windows. These formed the input to a linear C-Support Vector Machine (SVM) classifier. The model was fit on the training data and then applied to the test data for classification. The resulting output provided the final classification labels.

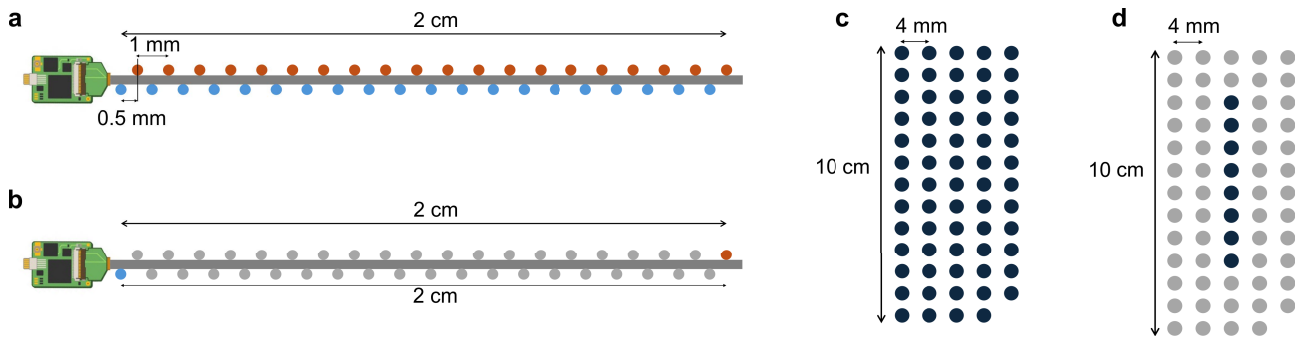


Figure 6.6: The four electrode configurations considered in this study. **a** HD-iEMG electrode, 40 channels with 0.5 mm IED. **b** Lower-density iEMG electrode, two channels with 2 cm IED. **c** HD-sEMG electrode, 64 channels with 4 mm IED. **d** Lower-density sEMG electrode, eight channels with 4mm IED.

reached 100% accuracy for both S1 and S2, which shows a perfect classification when targeting all muscle structures responsible for the tasks. The classifier using r.m.s. values from HD-iEMG achieved an accuracy of 99.3% for S1 and 99.7% for S2, while the classifier using r.m.s. values from HD-sEMG reached an accuracy of 95.3% for S1 and 71.4% for S2.

These results underscore the superior performance of intramuscular configurations over surface configurations for the classification based on neural features. When the electrodes accurately targeted the relevant muscles, the classification accuracy was 100% in the two subjects.

6.3.4.2.1 Lower-density Electrode Configurations On average across the two subjects, the bipolar iEMG achieved an accuracy of 55.4% for S1 and 78.8% for S2, while the sEMG achieved an accuracy of 89.1% for S1 and 57.2% for S2. These results show a notable reduction in the accuracy compared to the high-density configuration systems, highlighting the importance of a high-density setup in achieving high classification accuracy. The confusion matrices for the 12-class classification from the lower-density configurations are shown in Figure 6.10.

6.3.4.3 Mapping Specific Functional Classes (6 and 8 classes)

Figure 6.11 presents the classification results when focusing on the classes most relevant from a control perspective. Results are reported for six (S1) and eight (S2) classes (refer to Methods for details). The accuracy achieved from the MU activities decoded from HD-iEMG was 99.4% for S1 and 100%

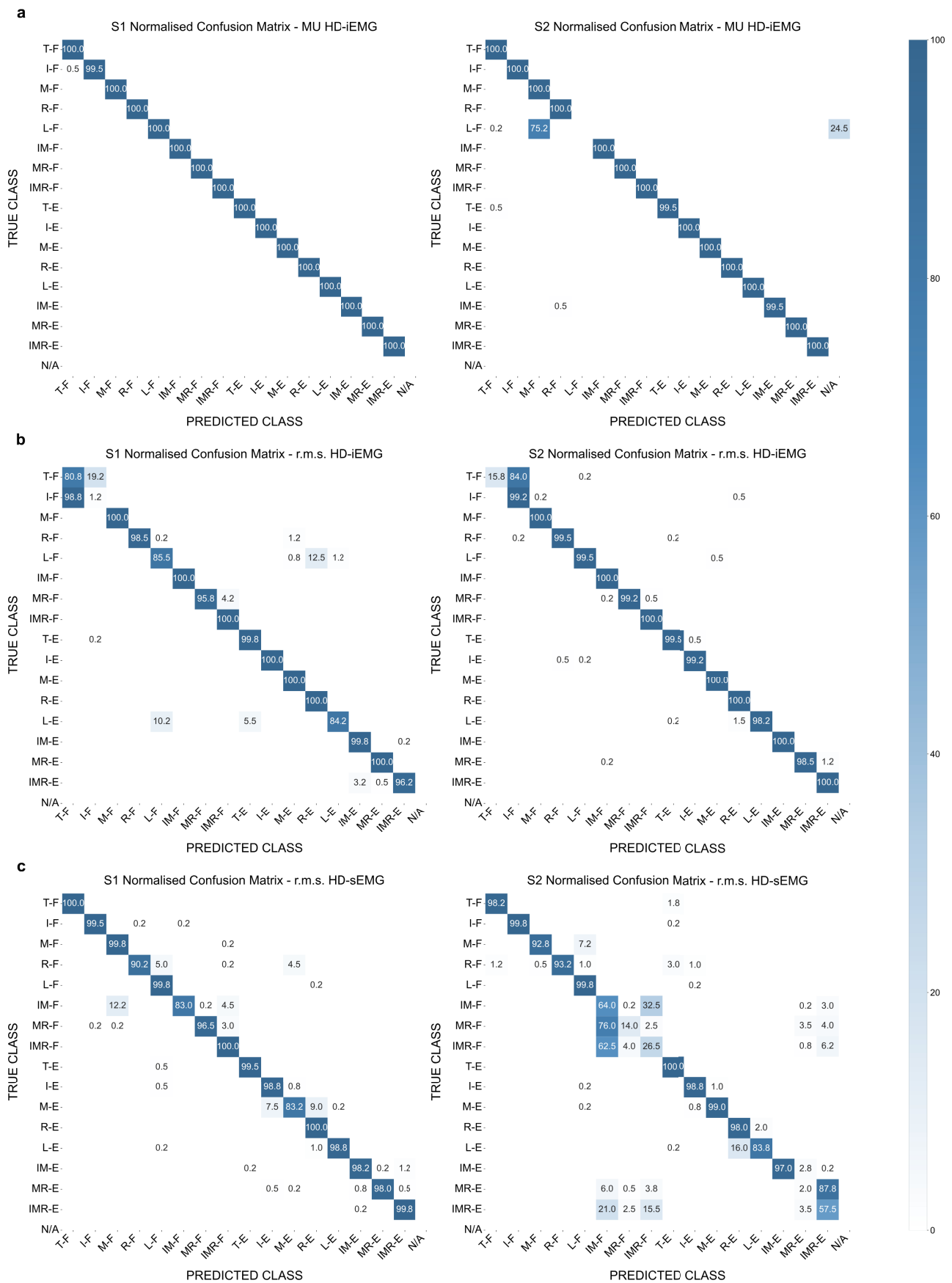


Figure 6.7: Normalised confusion matrices illustrating the percentage of predictions for each of the 16 flexion and extension gestures, comparing true labels (y-axis) with predicted labels (x-axis). The matrices show predictions for S1 on the left and S2 on the right, across different data types: **a** HD-iEMG MUs. **b** r.m.s. values of HD-iEMG. **c** r.m.s. values of HD-sEMG.

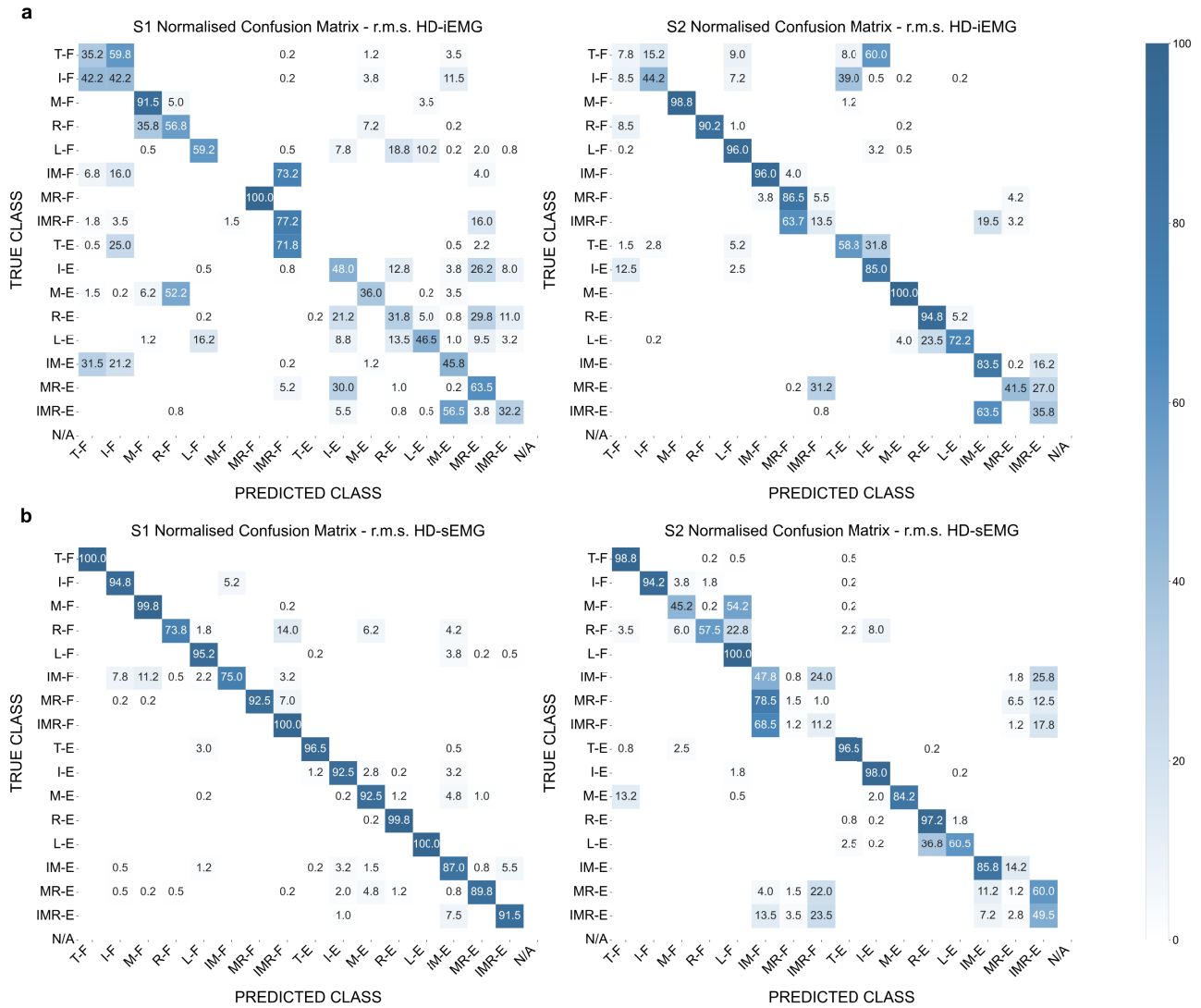


Figure 6.8: Normalised confusion matrices illustrating the percentage of predictions for each of the 12 flexion and extension gestures, comparing true labels (y-axis) with predicted labels (x-axis). The matrices show predictions for S1 on the left and S2 on the right, across different data types: **a** r.m.s. values of simulated bipolar, **b** r.m.s. values of 8-channel sEMG.

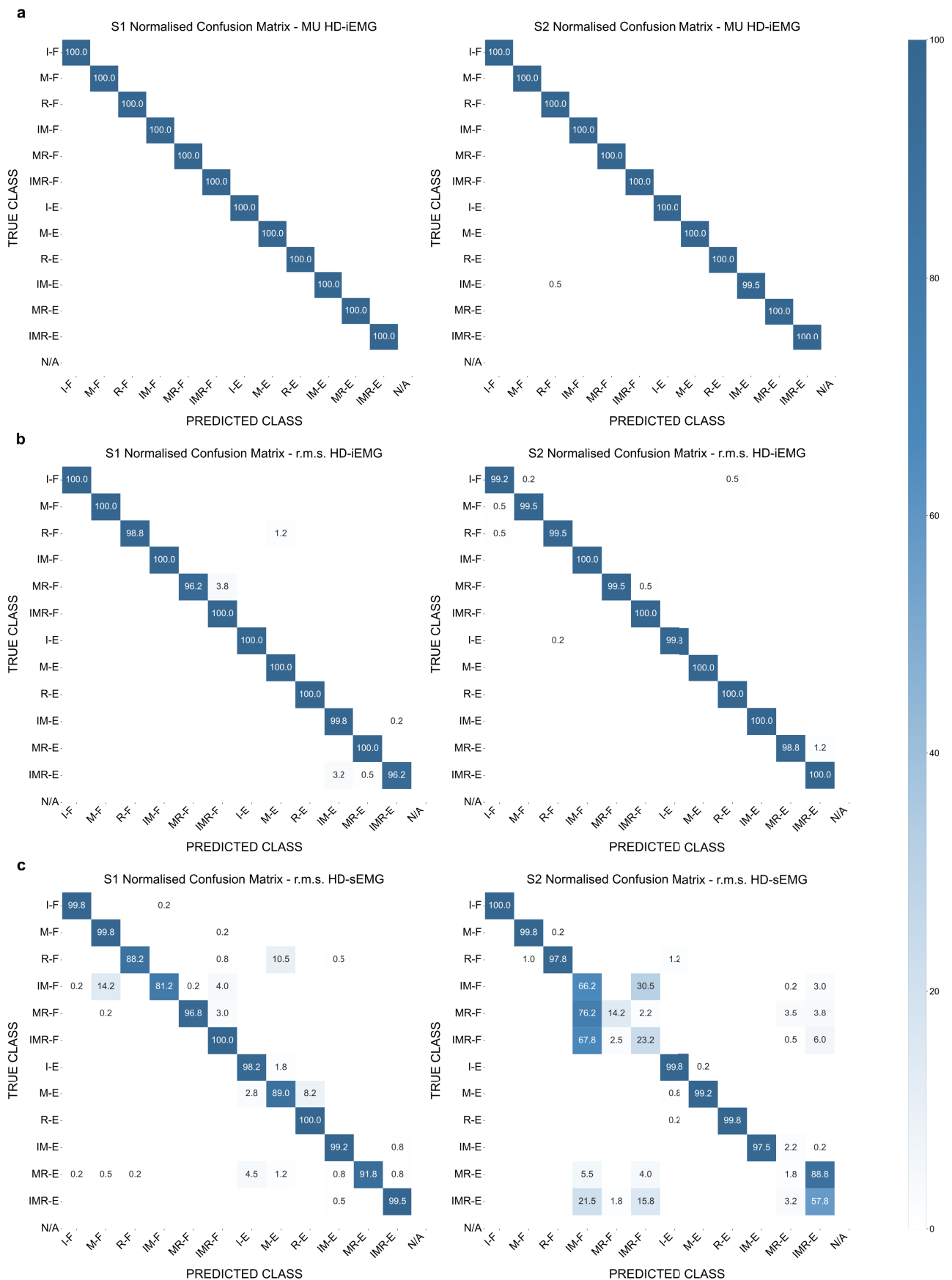


Figure 6.9: Normalised confusion matrices illustrating the percentage of predictions for each of the 12 flexion and extension gestures, comparing true labels (y-axis) with predicted labels (x-axis). The matrices show predictions for Subject S1 on the left and S2 on the right, across different data types: **a** HD-iEMG MUs. **b** r.m.s. values of HD-iEMG. **c** r.m.s. values of HD-sEMG.

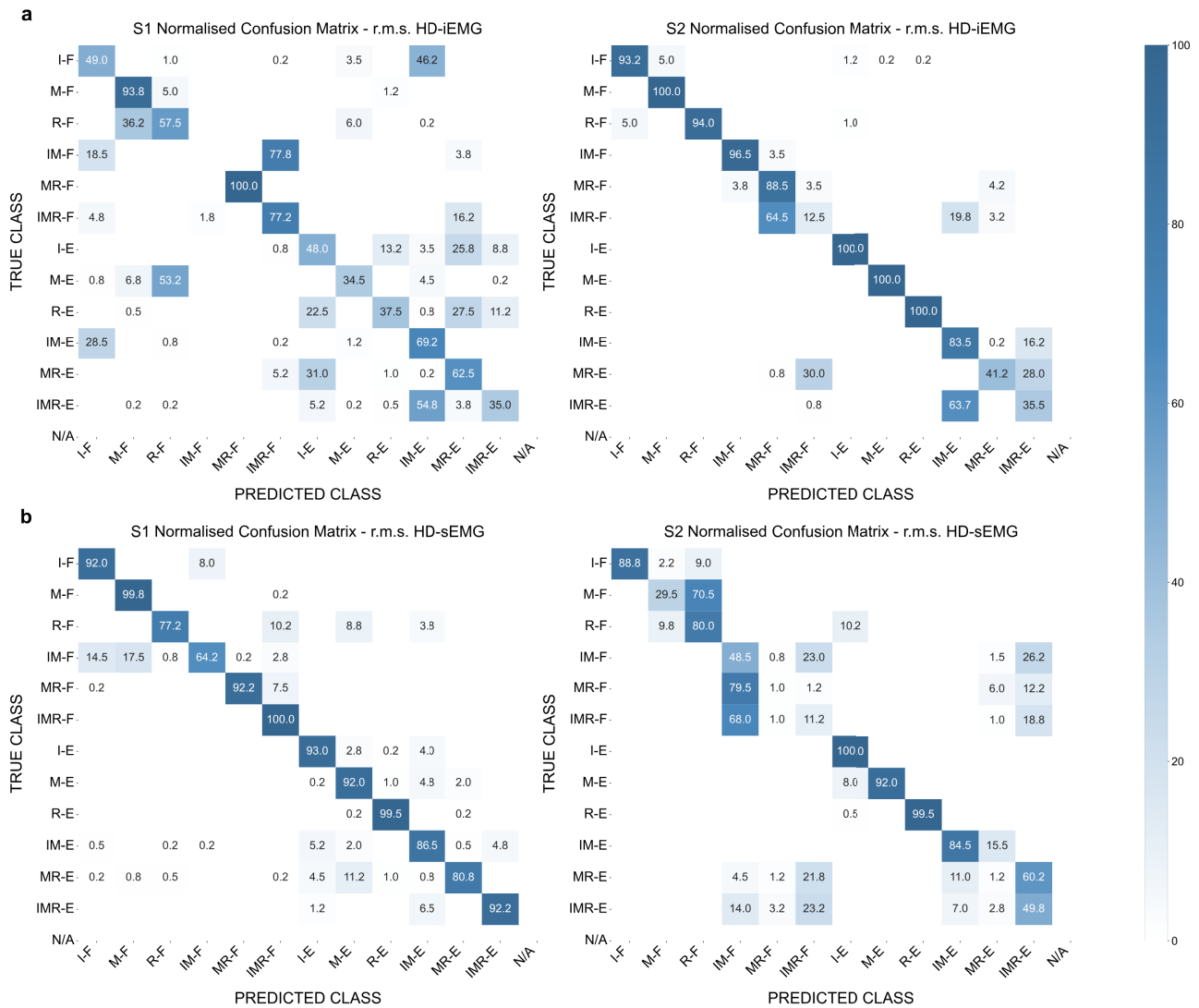


Figure 6.10: Normalised confusion matrices illustrating the percentage of predictions for each of the 12 flexion and extension gestures, comparing true labels (y-axis) with predicted labels (x-axis). The matrices show predictions for S1 on the left and Subject 2 (S2) on the right, across different data types: **a** time domain and r.m.s. values of simulated bipolar, **b** time domain and r.m.s. values of 8-channel sEMG.

for S2, while the accuracy from the r.m.s. values of the HD-iEMG was 76.4% for S1 and 89.0% for S2. The accuracy from the r.m.s. values of the HD-sEMG was 87.0% for S1 and 74.4% for S2. The effectiveness of using high-density intramuscular EMG recordings combined with MU decomposition for neural prosthesis control is demonstrated also in this analysis by the very high accuracy rates ($>99\%$). This near-perfect accuracy in classifying gestures typically used for prosthetic hand control indicates the potential of the proposed interface in external device control.

6.4 Discussion

Decoding motor intent is critical for accurate control in neural interfacing systems. At the peripheral level, decoding performance depends heavily on the type and quality of recorded muscle signals. We developed an advanced intramuscular neural system capable of decoding the precise discharge timings of spinal MNs by deconvolving HD-iEMG recordings. These recordings capture detailed neural signals from MN pools, offering rich insight into underlying motor control processes. The proposed system allowed us to observe MN recruitment in single- and multi-digit tasks, revealing that a majority of MUs recruited during each task are task-specific, even when the same digits are used. These insights into MN behavior suggest a strong potential for precise discrimination between hand tasks, which our system successfully validated with high accuracy.

Our recording and decoding system allowed us to explore the neural control during digit tasks. Notably, we observed that the MUs recruited during multi-digit tasks were almost entirely distinct from those recruited during the tasks of the corresponding single digits. This finding aligns with previous research by Oßwald et al. (Oßwald et al., 2023), which also suggested that MU recruitment during multi-digit tasks is well separated from that of single-digit tasks; however, this previous study was limited by the unconstrained and dynamic nature of the tasks investigated. Our results strongly indicate that hand control in multi-digit tasks is characterized by maximal separation of degrees of freedom, likely due to the demands of dexterous control. This was evidenced by the count of MUs active per task (Figure 6.4). Additionally, we provided representative examples of the distinct action potential waveforms associated with different tasks, further supporting the highly

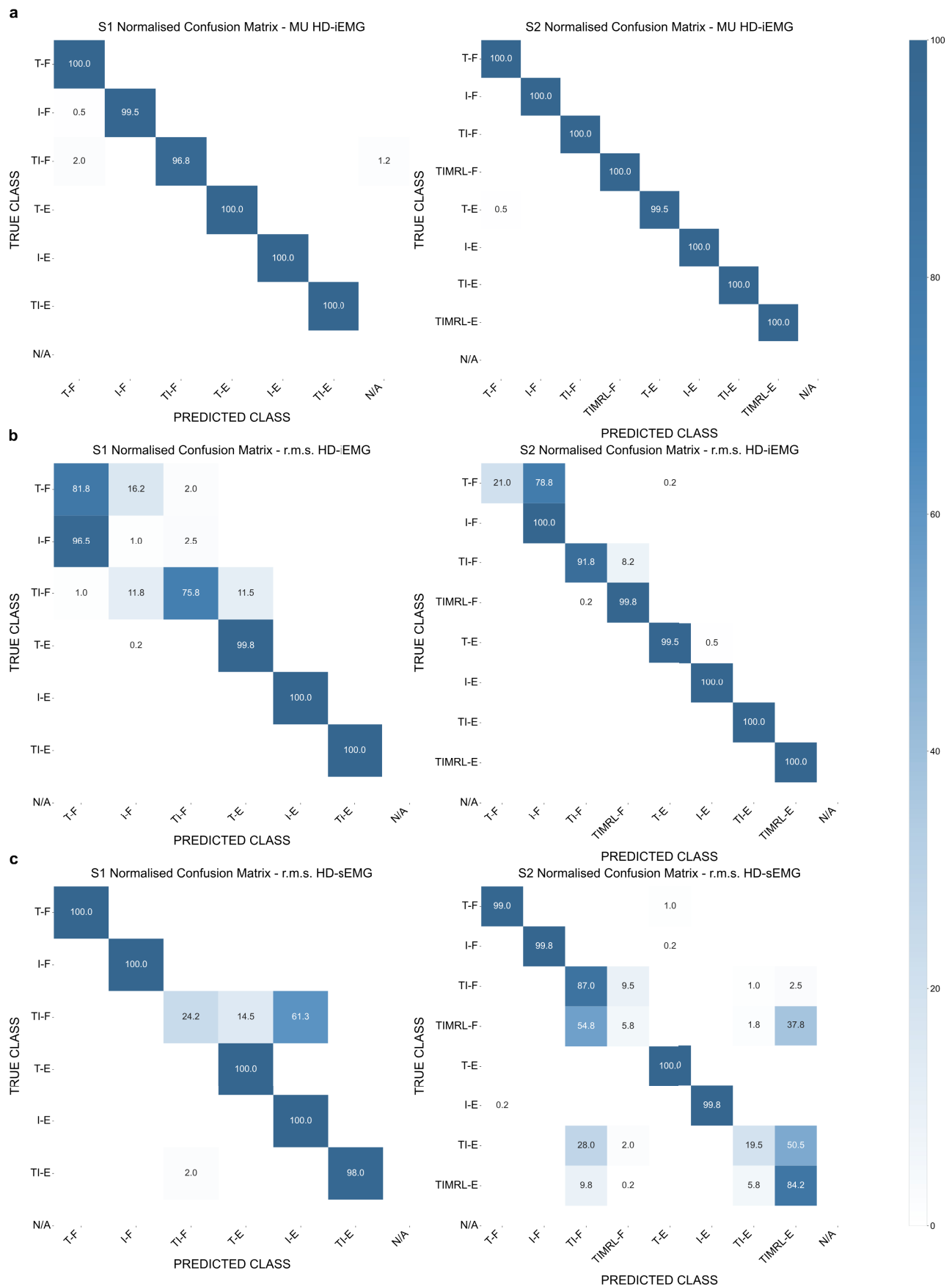


Figure 6.11: Normalised confusion matrix illustrating the percentage of predictions for each of the six (S1) and eight (S2) flexion and extension gestures, comparing true labels (y-axis) with predicted labels (x-axis). **a** MU HD-iEMG. **b** r.m.s. values of HD-iEMG **c** r.m.s. values of HD-sEMG.

specific recruitment of MUs during single- vs multi-digit tasks.

Building on this understanding of the neural mechanisms underlying fine motor control, we hypothesized that a system leveraging the decoded activities of MNs could achieve near-perfect accuracy in gesture classification. This is because the detection of task-specific MUs is a perfect indicator of a task. The accuracy of the proposed mapping system was validated through a series of experiments. A novel signal classification method was applied to the decoded series of discharge timings, achieving near-perfect discrimination for six, eight and 12 classes (average >99%), and up to 16 classes (average >96%). While traditional HD-iEMG and HD-sEMG features demonstrated solid performance (over 71%), the MNs identified through HD-iEMG consistently yielded superior accuracy, exceeding global features by 0.3% to 28.6%, depending on the conditions. The substantial improvement in classification accuracy is highly relevant for applications in neuroprosthetics, as this increase in accuracy can significantly enhance the effectiveness and responsiveness of assistive devices, leading to better user experiences and outcomes. Moreover, in all tasks, the classification accuracy from HD-iEMG MUs for the combined finger movements approached 100% (Figures 6.7 a, 6.9 a), further validating the task-specific recruitment of MUs during multi-digit tasks.

Decoding motor intent can be approached through global EMG features or MU discharge timings, each with distinct advantages and challenges. Global EMG features represent the spatiotemporal convolution of many MN discharges but are variable across individuals, particularly when recorded at the skin surface. This variability, alongside issues like amplitude cancellation and cross-talk, complicates precise control in complex tasks (Farina et al., 2004; Mesin, 2020). Due to these challenges, global HD-sEMG achieved classification accuracies ranging from 71% to 97%, highlighting variability in performance across tasks.

Although relatively high classification accuracies were achieved in some tasks, the reliance on features that are not directly linked to the underlying physiological mechanisms diminishes interpretability and presents challenges for clinical applications and motor control research. Understanding the specific contributions of muscle groups and neural activity is essential for validating model predictions and enhancing practical utility. In contrast to global EMG features, MN discharge timings provide a direct and physiologically relevant representation of neural activity, closely re-

flecting the spinal-level coding mechanisms of movement. By decomposing the EMG signals into their constituent MU activities, we demonstrated that MN spike trains can serve as critical sources of information for identifying complex tasks. This approach addresses the variability present in global EMG by separating neural information from muscle fiber action potentials, ensuring that the shapes of action potentials do not influence classification outcomes. Additionally, this separation mitigates the effects of EMG amplitude cancellation and enhances spatial and temporal sparseness, thereby reducing issues related to cross-talk (Farina, Merletti, & Enoka, 2014).

A key novelty of this work is the recording of high-density data intramuscularly, enabling detailed and accurate decoding of neural activity. The importance of intramuscular measurements is underscored by the large number of MUs that could be decomposed per task. High-density surface EMG has also been used for MU decoding (Farina et al., 2017) but it presents evident limitations with respect to implanted systems. One of these limitation is that it typically allows for the decoding of fewer MUs. This limitation is compounded by the challenge of accurately determining which muscles are activated, primarily due to a lack of spatial selectivity in the signals captured. This lack of resolution can lead to overlapping activity from adjacent muscles, making it difficult to pinpoint specific muscle contributions during movement (Farina et al., 2004; Mesin, 2020).

Precise targeting of the appropriate muscles and decoding of direct neural information resulted in nearly perfect decoding accuracy from the MU activity derived from HD-iEMG. Ensuring accurate muscle targeting remains critical for effectively extracting useful information from these recordings. As we reported in Section 6.3.4.2, when there was high confidence in the targeted muscles, the accuracy of classification from decoded MU activities was 100%.

Lower-density configurations were extracted from the high-density iEMG and sEMG data to evaluate whether multi-channel configurations were necessary for achieving high classification accuracies. The results showed that classification accuracy from global EMG features dropped with fewer channels, more significantly so for the iEMG. Regarding the 16 class classification, the bipolar iEMG configuration dropped by 40% for S1 and by 25% for S2 for 16 classes, while sEMG dropped by 4% for S1 and just over 10% for S2. A similar trend was observed for the 12 classes. This highlights the important of high-density configurations to accurately classify gestures from the EMG. It is important

to note that classification based on MNs would not be feasible with lower-density configurations, as high-density setups are essential for the decomposition methods utilised to extract MN activity.

Various approaches have been explored in previous research for classifying hand gestures from global EMG features. For eight classes, time domain and deep learning methods have shown variable level of success, with accuracies ranging from 81.54% (Fajardo et al., 2021) to 95% and about 91% (Simão et al., 2019). A convolutional auto-encoder combined with a CNN achieved 99% accuracy for 10 finger gestures (Jia et al., 2020), but this approach was tested on a smaller set of 10 classes compared to the 16 and 12 classes used in our study.

There have also been previous studies that presented results on classification of MU activities, extracted from surface EMG, associated to hand tasks. For example, neural drives to muscle regions were estimated by grouping MNs based on the spatial location of their corresponding muscle units, achieving over 97% accuracy across 11 classes in patients with targeted nerve reinnervation (Farina et al., 2017), while similar methods using MU action potential waveform activations reached around 95% accuracy for 11 classes (C. Chen, Yu, et al., 2020). A related technique employing MU filters achieved 94.6% accuracy for 12 gestures (C. Chen et al., 2023). Additionally, neuromorphic approaches have shown promise, with approximately 95% accuracy for 10 classes (Tanzarella et al., 2023).

Overall, our method outperforms all previous approaches, achieving accuracies exceeding 99% for six, eight, and 12-class classification tasks and greater than 96% for 16 classes. Notably, the precision obtained from MU activity significantly surpasses prior results, highlighting the robustness and reliability of our system for decoding neural information. The use of MUs not only enhances performance but also provides a theoretical framework for achieving 100% accuracy. As long as MU discharges are accurately detected, and each task involves at least one unique MU, it becomes theoretically possible to reach perfect classification. This implies that, with precise muscle targeting and continued advancements in decoding algorithms, flawless performance is achievable — a potential we have already demonstrated in some cases in this study (e.g., 100% accuracy for both S1 and S2 in the 12-class classification).

Under the experimental conditions of our study, the proposed approach based on decoding MNs

demonstrated superior accuracy compared to traditional pattern recognition methods. However, the small sample size, driven by the need for intramuscular insertions, limits the generalization of these results. Further validation with a larger population is needed.

Transitioning to online implementation for real-world applications presents significant challenges. Real-time HD-iEMG decomposition is computationally intensive, requiring high sampling frequencies to capture neural signals accurately (Rossato et al., 2023). While advances in microprocessor speeds have made real-time neural data processing more attainable, the challenge remains significant due to the intensive computational demands, especially since even offline data recordings are often hindered by the high sampling rates required (this problem is clearly exacerbated when increasing the number of channels, as in this study).

A promising future direction is the integration of spiking neural networks directly onto low power mixed-signal neuromorphic chips (Chicca et al., 2014; Mead, 2023). As decomposition methods continue to advance and become more capable of adapting to signal nonstationarities (Mendez Guerra et al., 2024), implementing real-time decomposition methods with neuromorphic electronic circuits will significantly accelerate the development of practical neural interfaces. The combination of adaptive signal processing and the computational efficiency of neuromorphic architectures represents a crucial step toward deploying neural prosthetics and other neurotechnologies that rely on real-time neural decoding (Donati & Indiveri, 2023). This integration would not only enhance the system's ability to handle dynamic, real-world scenarios but also reduce the need for constant retraining.

Despite the clear advantages of intramuscular systems over non-invasive ones, practical challenges hinder their widespread adoption. Precise muscle targeting is essential, requiring careful planning and execution by trained personnel. Mis-targeting can lead to mis-classifications, while increasing the number of electrodes could improve targeting accuracy. Our study focused on well-controlled isometric contractions, where signal decomposition is highly reliable. The system remains untested in dynamic, naturalistic conditions where movement alters action potential shapes.

6.5 Conclusions

In conclusion, we have described new findings on the motor control of multi-digit tasks. Additionally, we have proven the possibility of decoding the behaviour of the pools of MNs that physiologically innervate some of the muscles responsible for finger movements. This decoding was demonstrated in two healthy individuals with three implanted electrodes each and was shown to provide information that can be used for highly intuitive and accurate control commands. The accuracy of control derived from MUs extracted from HD-iEMG was near-perfect for both subjects and was successfully tested across a range of conditions, encompassing six to 16 distinct fine finger gestures.

*If one idea is more modern than
another it is a sign that neither
are immortal.*

Gadda, Carlo Emilio

7

Conclusion

This chapter provides a summary of the research and key contributions of this thesis. It concludes with recommendations for future work to advance the applicability of the novel MN-based classification in real-time applications. Suggestions include a large-scale data collection with HD-iEMG electrodes to validate findings in diverse scenarios. Additionally, potential implementation challenges are examined, along with proposed strategies to address them.

7.1 Summary of Thesis Achievements

The aim of this PhD was to develop an EMG-based interface that integrates advanced decoding algorithms with multi-channel intramuscular micro-electrode EMG arrays to enable precise neural

signal extraction and interpretation.

The physiological background of neuromuscular anatomy and AP transmission was provided in Chapter 2, together with an in-depth review of common methods for EMG decomposition. Limitations and gaps of previous decomposition methods were identified. Moreover, key distinctions in the signal characteristics between sEMG and iEMG were provided, underscoring how the volume conductor introduces fundamental constraints on signal quality in the sEMG and the ability to reliably detect individual MU activity.

In Chapter 3, a novel BSS decomposition algorithm, optimized for HD-iEMG signals was proposed. The key innovation of SCD lies in its adaptive contrast function, which dynamically adjusts the separation process to better capture the unique spatiotemporal characteristics of each individual MUAP. The algorithm was validated on experimental HD-iEMG with ground truth annotations provided by manual decomposition on EMGLAB. The results demonstrated that SCD was able to detect 57.7% more MUs than current SoA techniques. Moreover, the accuracy of the MUs identified by both SCD and the compared method increased by 1.2%, highlighting the efficacy of the adaptive contrast function in also improving decomposition performance.

Given the ability to automatically and reliably decompose HD-iEMG data, in Chapter 4 different configurations of the HD-iEMG electrodes were explored. The results revealed that the density of detection points was the most critical factor for maximizing the number of identified MUs with very high accuracy. Comparisons between intramuscular and surface recordings also confirmed that HD-iEMG consistently captured larger and more stable numbers of MUs across subjects and contraction levels.

Aware of the inherent limitations of HD-sEMG signals, in Chapter 5 SCD was adapted to try and increase the yield of decomposed MUs from non-invasive signals. SCD's ability to address key challenges faced by existing methods was demonstrated, particularly in identifying low-amplitude MUAPs and effectively handling complex decomposition scenarios, like high-interference signals. SCD was extensively validated using simulated and experimental HD-sEMG recordings and compared it with current SoA decomposition methods under varying conditions, including different excitation levels, noise intensities, force profiles, sexes, and muscle groups. The proposed method

consistently outperformed existing techniques in both the quantity of decoded MUs and the precision of their firing time identification. For instance, under certain experimental conditions, SCD detected more than three times as many MUs compared to previous methods, while also significantly improving accuracy. These advancements represent a major step forward in non-invasive EMG technology for studying MU activity in complex scenarios.

Finally, in Chapter 6, a novel neural decoding system was presented. This system enabled large-scale sampling of muscle activity using micro-electrode arrays with over 100 channels embedded in forearm muscles. These arrays captured HD-iEMG signals that were decoded into patterns of activation of spinal MNs. In two healthy participants, intramuscular activity during single- and multi-digit contractions was recorded, revealing distinct MN recruitment patterns specific to each task. Based on these patterns, perfect classification accuracy (100%) for 12 single- and multi-digit tasks and over 96% accuracy for up to 16 tasks was achieved, significantly outperforming SoA EMG classification methods. This HD-iEMG system and decoding/classification method represents a major advancement in neural interfacing, with the potential to improve human-computer interaction and the control of assistive technologies, particularly for replacing or restoring impaired motor function.

7.2 Discussion

This thesis introduces a novel decomposition algorithm designed to effectively separate sources from HD-iEMG and HD-sEMG signals, leveraging this to decompose a unique dataset collected at the forearm for gesture classification. This approach demonstrated highly accurate MN control based on signals from HD-iEMG recordings, contributing significant advancements to neuromuscular decoding. The comprehensive nature of this work spans algorithm development, experimental data collection, and practical applications in neural interfaces, establishing new methodologies for investigating MU behavior while acknowledging important limitations that guide future research directions.

The first key strength is the collection of two unique datasets that provide unprecedented insight into neuromuscular activity patterns. The first dataset, recorded from the forearm over three con-

secutive days, and the second, from the TA muscle over two consecutive days, both feature multiple HD-iEMG insertions per participant, systematically paired with HD-sEMG recordings. The controlled, longitudinal nature of these recordings mirrors the precision typically associated with small-sample animal studies, offering detailed temporal patterns of MU behavior. The data collection process demanded extensive preparation and expertise development, including specialized training in invasive recording techniques, strict adherence to medical safety protocols, proficiency in ultrasound imaging for identifying critical anatomical structures, and the development of a hardware recording platform capable of simultaneous acquisition from multiple load cells and multi-electrode arrays. This comprehensive approach yielded datasets of exceptional quality that not only underpin the analyses presented in this thesis but also serve as valuable resources for multiple ongoing studies and international collaborations, enabling detailed investigation of MU properties and control strategies. However, several important limitations must be acknowledged. The primary limitation stems from the restricted sample size—two participants per dataset—which significantly constrains the generalizability of the findings across different populations, age groups, and skill levels. This limited sample size makes it challenging to account for individual variations in muscle architecture, MU distribution, and control strategies that might affect the recorded signals. Additionally, only a limited number of repetitions per task were recorded to capture a wider range of tasks, which restricts the amount of data available for each individual task. Moreover, while the consecutive-day recordings provide valuable insights into short-term stability, they do not capture potential longer-term variations in MU behavior or electrode performance. The focus on healthy participants also leaves questions about the applicability of these findings to clinical populations or individuals with different neuromuscular conditions. Furthermore, although the multiple insertion points provide comprehensive spatial sampling, the inherent variability in electrode placement across sessions and participants introduces potential inconsistencies in spatial sampling that could affect the interpretation of MU analyses. While the depth and quality of the collected data partially mitigate these limitations, future validation would benefit from a larger and more diverse subject pool, extended recording periods, and inclusion of clinical populations to strengthen the conclusions drawn and better understand the broader applicability of these techniques.

The second major contribution is the development of a fully automatic, high-yield, and high-accuracy

decomposition algorithm capable of processing both HD-iEMG and HD-sEMG signals. This advancement represents a significant leap forward in MU analysis methodology, particularly in terms of processing efficiency and accuracy. Traditional manual methods for HD-iEMG decomposition demand high time investments from expert operators—a ten-second recording of a low-force contraction (10 %MVC) from the TA muscle previously required approximately 128 hours of manual processing. In contrast, SCD accomplishes the same task in just 32 minutes while maintaining decomposition quality. While automatic methods for HD-iEMG signals were previously introduced by Negro et al. (2016), their capability was limited to identifying only a small subset of MUs present in the signal. The SCD algorithm distinguishes itself through its SoA performance on both HD-iEMG and HD-sEMG signals, automatically adapting to the underlying distribution of the data without requiring user intervention. The successful validation of SCD for non-invasive signals represents a significant advancement for the field, enabling the detection of larger MU pools through non-invasive means and thereby enhancing our ability to make robust inferences about motor control strategies.

However, several important limitations and technical challenges must be carefully considered. The fundamental limitation lies in SCD's core assumption of temporal stability in the muscle geometry model—any significant changes in muscle architecture during contraction or movement can violate this assumption and compromise SCD's performance. The application to intracortical data, while promising in concept, currently lacks rigorous validation against contemporary spike sorting techniques. This gap necessitates a systematic analysis pipeline, beginning with detailed simulations and progressing to experimental validation, to definitively assess whether BSS principles are suitable for intracortical applications. Technical challenges become particularly apparent when dealing with intracortical recordings, which typically employ high sampling frequencies (20-30 kHz) (Magland et al., 2020), making the model inversion computationally intensive. Signal drift in these recordings introduces non-stationarity that fundamentally challenges the algorithm's underlying assumptions, suggesting the need for incorporating adaptive mechanisms within the SCD framework (see Section 7.3).

The current implementation faces practical limitations that affect its immediate clinical applicability. SCD has been validated only in offline analyses, with real-time implementation remaining a crucial

next step. While established approaches exist for transitioning to real-time operation—typically involving offline decomposition to generate filters that can be reapplied in online settings (Barsakcioglu et al., 2020)—this capability has not yet been integrated into SCD. Parameter optimization also presents ongoing challenges; although most parameters are automatically determined based on sampling frequency, certain critical parameters require manual tuning for each dataset. Most notably, the extension factor ($L + R$) significantly influences decomposition yield, with optimal values differing from the $1000/M$ ratio proposed by Negro et al. (2016). While our findings suggest better performance with higher values, the lack of an automatic optimization method for this parameter introduces potential variability in algorithm performance across different applications and reduces its accessibility to non-expert users. Future development should prioritize implementing automated parameter optimization strategies to enhance the algorithm’s robustness and user-friendliness across diverse experimental conditions.

The development of SCD enabled valuable insights into future intramuscular electrode technologies through systematic investigation of recording configurations. By analyzing various electrode arrangements, quantitative relationships between recording density and decomposition outcomes were established, providing practical guidelines for expected MU yield and decomposition accuracy as a function of recording point density. These findings offer crucial guidance for future electrode design and implementation strategies. However, several limitations constrain the broader applicability of these results. The fundamental challenge lies in the absence of ground truth validation, forcing reliance on the algorithm’s internal consistency metrics rather than absolute accuracy measures. This limitation introduces uncertainty in the precise relationship between electrode configuration and decomposition performance. Additionally, the confined focus on the TA muscle, while pragmatically chosen for its accessibility and suitability for multiple electrode insertions, raises questions about the generalizability of these findings to muscles with different architectural properties, fiber arrangements, and activation patterns. A comprehensive validation across diverse muscle groups would be essential to establish broadly applicable design principles for future intramuscular recording technologies.

A significant achievement of this work was the successful demonstration of a proof-of-concept neural decoding and mapping system for motor control. The results showed that with sufficient spa-

tial sampling density from HD-iEMG signals in anatomically relevant muscles, near-perfect gesture classification accuracy is achievable using decomposed MU activity. However, several critical limitations must be addressed before practical implementation becomes feasible. Beyond the previously discussed sample size and data availability constraints, the current system faces significant challenges in transitioning to real-world applications. The implementation requires real-time decomposition capabilities coupled with an extremely efficient classification framework that are currently unavailable. The present approach, which involves tracking all decomposed MUs across multiple tasks and analyzing their activation patterns offline, while effective for proof-of-concept demonstration, is computationally prohibitive for real-time applications. Real-world implementation would require fundamental redesign of the processing pipeline, potentially incorporating optimized decomposition strategies, and selective MU tracking. Furthermore, the system would need to account for variations in electrode placement, muscle fatigue, and changing environmental conditions that could affect signal quality and classification accuracy. Future work must focus on developing these real-time processing capabilities while maintaining classification accuracy, possibly through the implementation of adaptive algorithms and efficient feature selection methods. This development path represents a crucial step toward creating a practical, reliable neural interface system suitable for clinical applications.

7.3 Future Work

A key next step for the SCD algorithm is a systematic validation against SoA spike sorting techniques for intracortical data. Recently, Pachitariu et al (2024) released KiloSort4, a highly optimized spike sorter that has demonstrated superior performance on a variety of intracortical datasets. While iEMG and intracortical data differ in their underlying signal characteristics, the fundamental model is conceptually similar. This suggests the potential for adapting BSS methods like SCD to intracortical applications, just as spike sorting algorithms can be applied to iEMG data with appropriate modifications (B. Chung et al., 2023). Validating SCD's efficacy on intracortical recordings, in comparison to the latest spike sorting tools, will be crucial for assessing its broader applicability beyond surface and intramuscular EMG, reducing the needs of manual operation that cause inherent vari-

abilities due to human error (F. Wood et al., 2004).

Further research will also focus on optimizing SCD's parameters, especially the extension factor, including methods to automatically select this factor based on data distribution characteristics. Moreover, to enhance SCD's applicability in real-world settings, a future step involves integrating it with adaptive decomposition techniques that adjust parameters by minimizing divergence from an initial calibration phase (Mendez Guerra et al., 2024). This will allow SCD to adapt seamlessly to both isometric and dynamic contractions in real time, broadening its utility and facilitating its translation into practical applications. Initial validations will be conducted on offline datasets, followed by tests in online environments.

Parallel efforts will also explore optimal HD-iEMG electrode configurations for various human muscles. Recent advancements in intramuscular micro-electrode array technologies have inspired other research groups to investigate configuration parameters suited for different animals and muscle groups (B. Chung et al., 2023). Extending this work to the key muscle groups in humans will provide valuable guidance for the design and deployment of future intramuscular recording systems.

An important future consideration is the potential impact of electrode shifts over time. Addressing this challenge may involve periodic re-insertion, alternative methods for stabilizing the electrode on muscle fibers, or developing algorithms that can automatically recalibrate to account for slight changes in recording locations. Each solution must be evaluated with specific use cases in mind. For instance, in clinical settings, an electrode with a more secure attachment to muscle fibers might be feasible, whereas in other applications, this may be less ideal. The optimal solution would involve robust algorithms capable of adapting to minor positional changes, which will be a priority for future testing and validation efforts.

Ultimately, the long-term vision is to realize a wireless, implantable intramuscular electrode system with embedded decomposition and control algorithms, enabling seamless and highly interpretable neuromuscular interfaces.

7.4 Final Remarks

This project focused on the development, validation, and application of a novel HD-iEMG interface. This goal was accomplished through: 1) the collection of a dataset, which required designing a complex experimental protocol and acquiring high-quality intramuscular data, 2) the development of a novel decomposition algorithm, 3) a systematic analysis of electrode design, offering practical insights for future technological advancements, and 4) the development of a novel decoding and mapping system, extensively tested across diverse conditions, achieving near-perfect classification accuracy.



Appendices

A.1 Data Collection - Forearm

Figure A.1 illustrates the experimental setup used for data collection from the forearm. This figure highlights two main components: the hardware developed for the experimental protocol and the electrode setup. The platform includes two load cells per finger, one for measuring flexion forces and the other for extension forces. The load cell positions can be adjusted using a railing system, and the width between the load cells can be modified by adjusting screws on the extension load cell. Additionally, the wrist angle is adjustable to accommodate different experimental conditions.

Each load cell connects to a force amplifier, which in turn interfaces with the main amplifier (Quat-

trocento) to ensure synchronization between force measurements and EMG signals. Alternatively, three Phidgets amplifiers, positioned adjacent to the load cells, facilitate data streaming to a laptop for use with custom-built data acquisition software.

The electrode setup includes one of the three HD-iEMG electrodes and two of the three HD-sEMG grids, which are clearly visible in the figure.

The full experimental protocol spanned three days, during which the electrodes remained implanted in the muscles.

A.1.1 Isometric Protocol

The first and third day of recording focused on isometric contractions. The isometric protocol consisted of a range of finger movements under various force levels and contraction patterns, divided into three categories:

1. Individual Finger Movements (Flexion and Extension)

- (a) Performed at 5 and 15 %MVC using a trapezoidal pattern.
- (b) Conducted at 10 and 20 %MVC using a pseudorandom pattern.
- (c) Executed at 60 %MVC in a triangular pattern.

2. Select Individual Fingers (Thumb, Index, Middle - Flexion and Extension)

- (a) Conducted at 20, 30, and 50 %MVC using a trapezoidal pattern.
- (b) The 50 %MVC trials were repeated on Day 3 and included wrist angles of 0° , $+40^\circ$, and -40°
- (c) Performed at 30 %MVC with a pseudorandom pattern.
- (d) Conducted at 40 %MVC using a ballistic pattern.
- (e) Executed at 50 %MVC with a chirp pattern in a sinusoidal wave.

3. Combination of Fingers (Flexion and Extension)

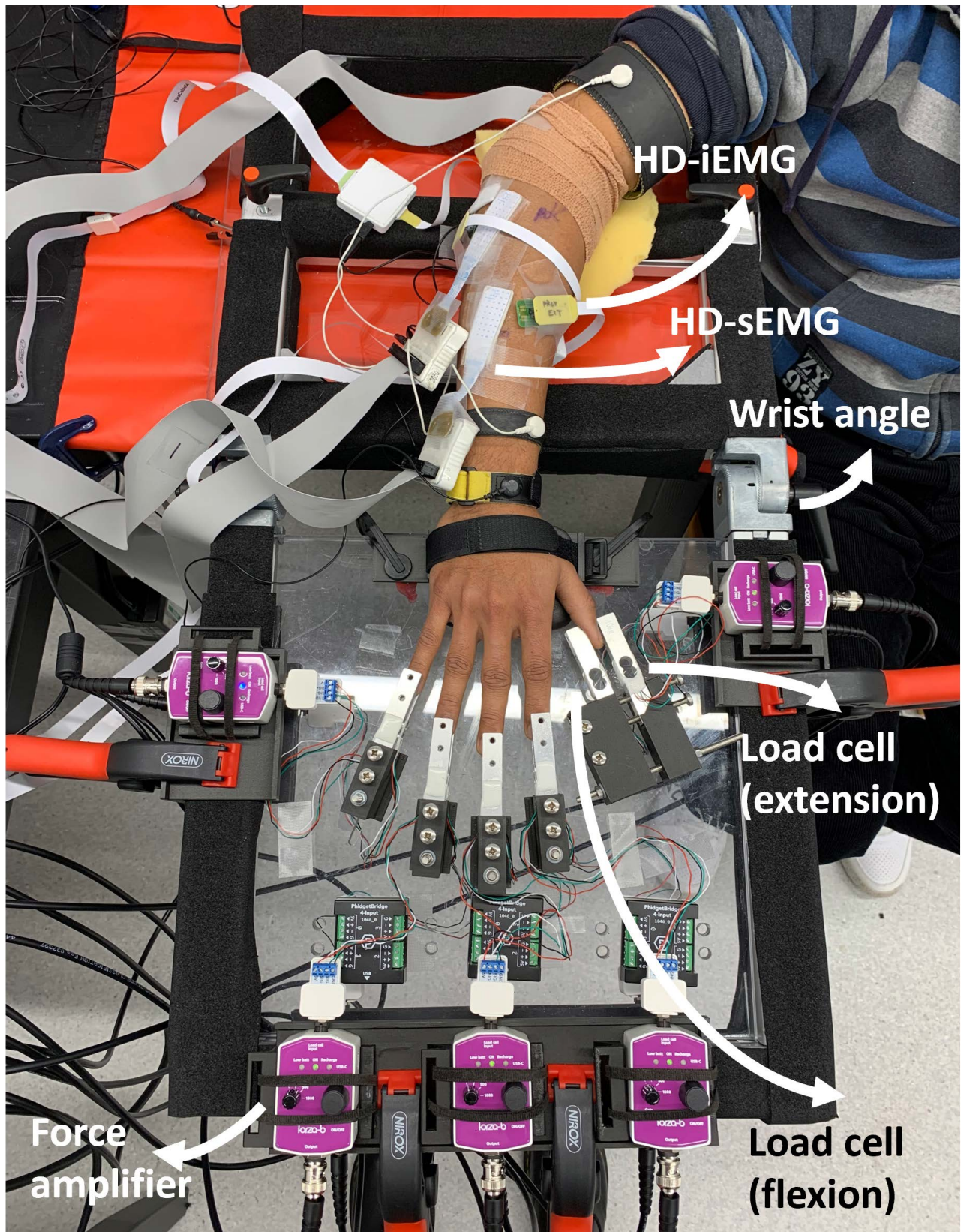


Figure A.1: Experimental setup for the isometric protocol.

- (a) Two, three, or five fingers contracting together using a trapezoidal pattern.
- (b) Trials varied in ramp times and target forces, with some tests maintaining consistent ramp times and force levels.

An example of the EMG signals recorded during the different tasks is shown in Figure A.2.

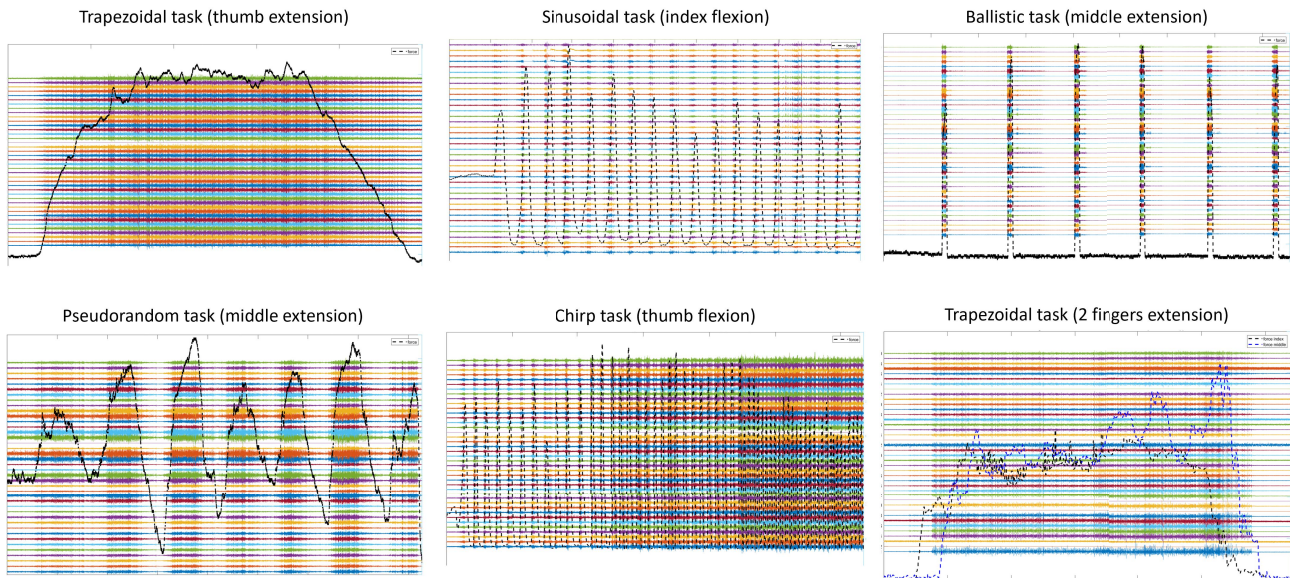


Figure A.2: HD-iEMG example data recorded from the forearm.

A.1.2 Dynamic Protocol

The second day of recordings focused on naturalistic movements. Subjects performed 29 distinct hand gestures, each repeated five times at two different movement speeds. Additionally, they executed the 26 letters of the sign language alphabet, also repeated five times. To capture detailed motion data, 19 markers were placed on the forearm and hand of each subject, with signals recorded concurrently using motion capture technology. The experimental setup for these recordings is depicted in Figure A.3.

A.2 Data Collection - TA

The second major dataset was recorded from the TA of two subjects over a 2-day experimental protocol. Three HD-iEMG electrodes were implanted in the TA of one subject, and four in the TA of

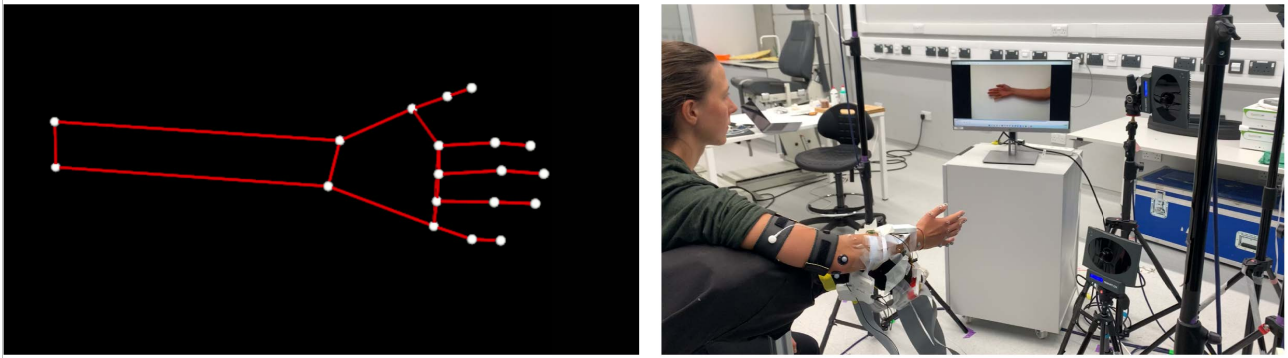


Figure A.3: Experimental setup for the dynamic protocol.

the other. The subjects performed isometric contractions at various force levels. Figure A.4 illustrates the experimental setup used for the recordings from the second subject.

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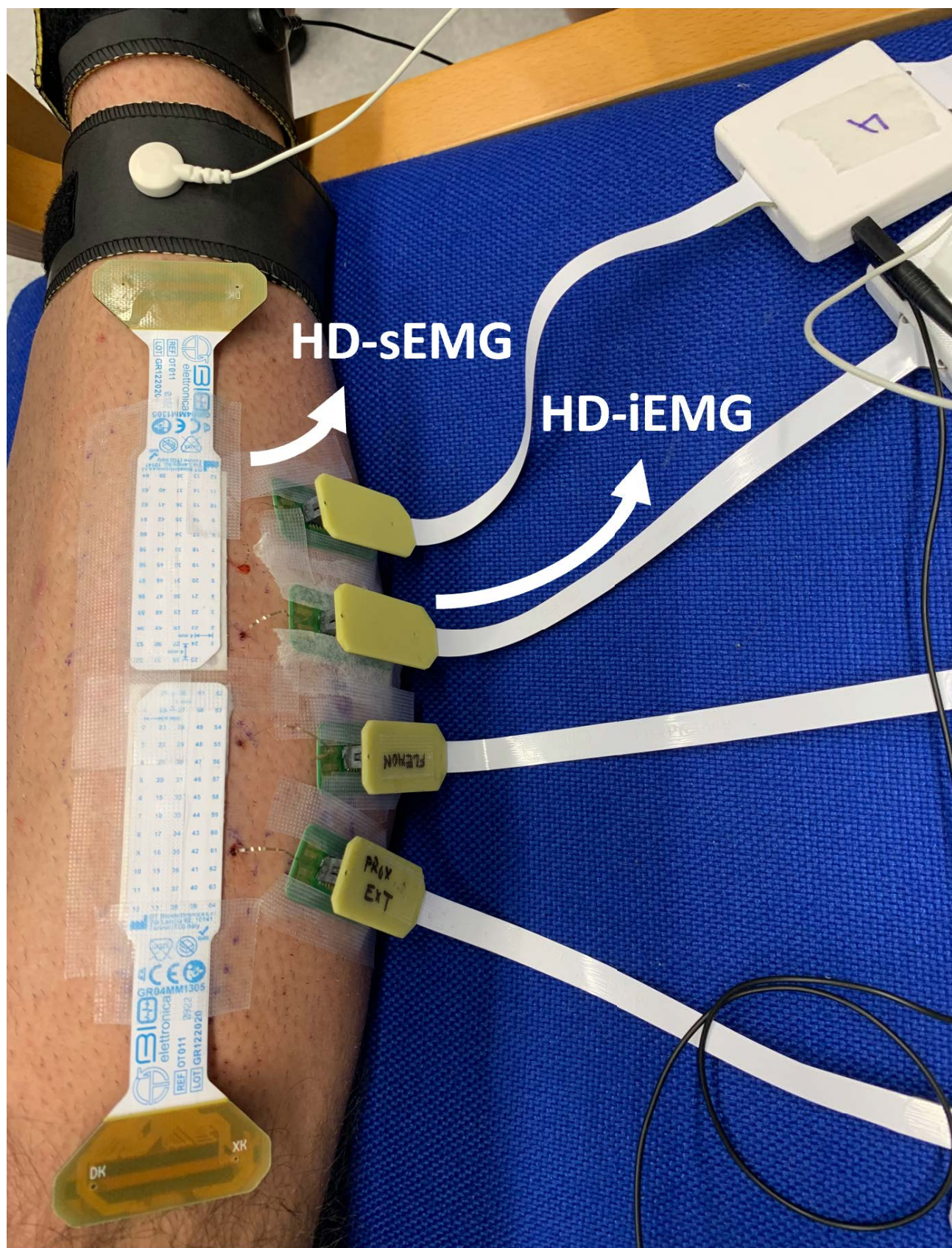


Figure A.4: Experimental setup for the TA experiments.



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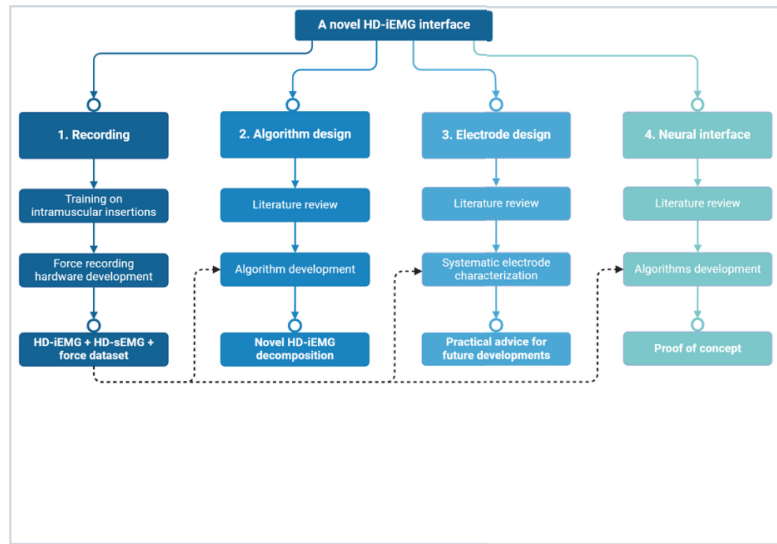
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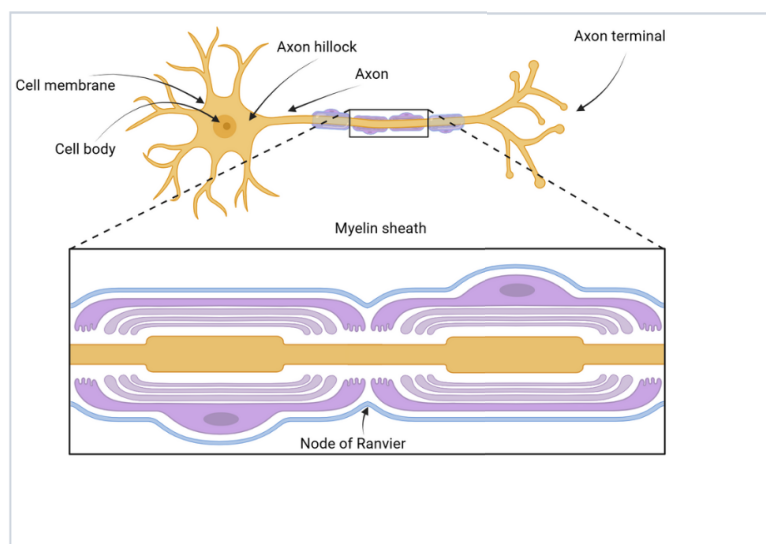
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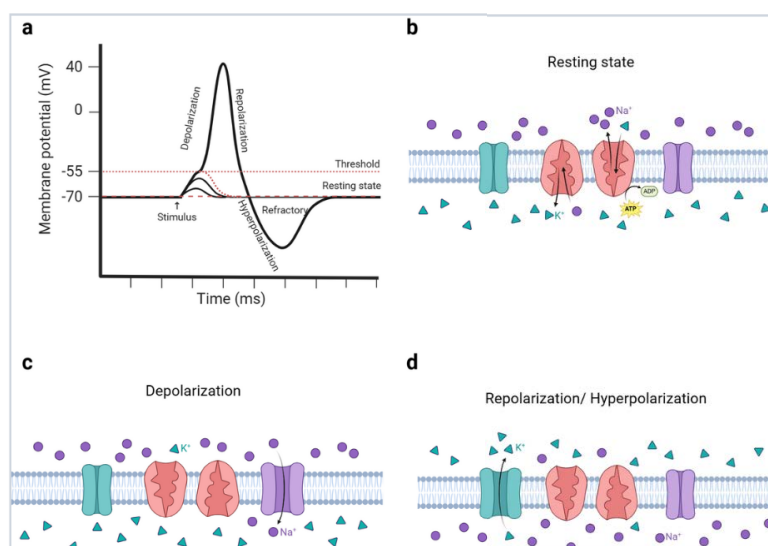
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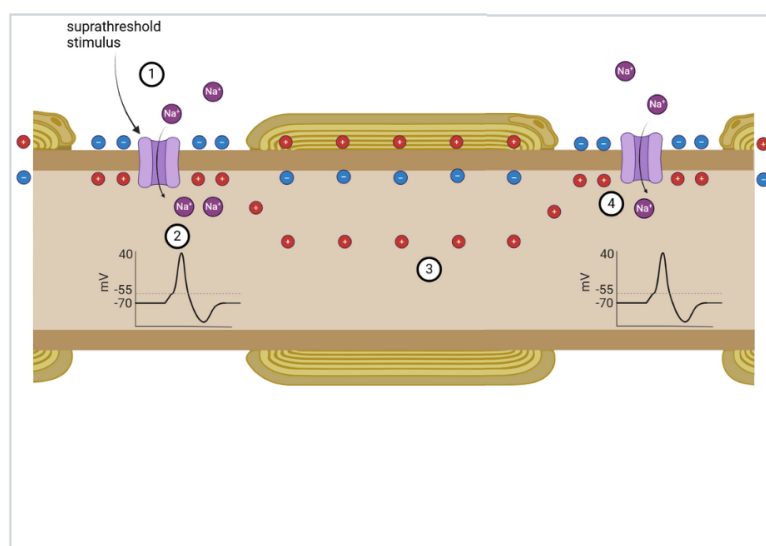
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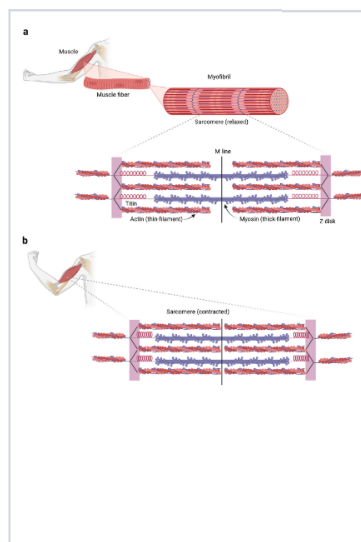
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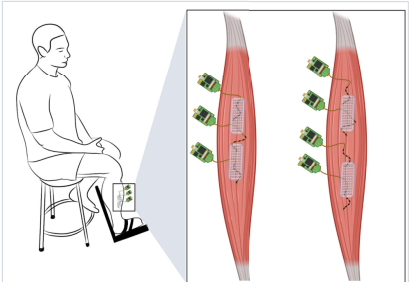
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