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Muscle synergies analysis in semi-recumbent cycling: effect of different cadences and power outputs.

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Abstract

Semi-recumbent ergometer cycling is a common rehabilitation modality for individuals after spinal cord injury: cycling engages neurons located in the spinal cord, which have been identified as a therapeutic target to improve sensorimotor function after paralysis. In individuals with motor impairment, this is achieved through functional electrical stimulation (FES), which enables the activation of paralyzed muscles to produce movements. However, current stimulation strategies are generally not personalized and do not adapt to changes in cycling conditions, highlighting the need for more physiologically informed control approaches. In this context, muscle synergies, known as low-dimensional motor modules through which the nervous system coordinates muscle activity, have emerged as a promising framework for the development of adaptive FES controllers. Yet, while previous studies have shown that muscle synergies are largely preserved during upright cycling, it remains unclear whether the same modular organization is maintained during semi-recumbent cycling. A better understanding of the neuromuscular organization underlying semi-recumbent cycling may therefore support the development of more physiologically relevant FES control algorithms.

This study aims to investigate whether muscle synergies change across different cadences and power outputs during semi-recumbent cycling. Surface electromyography was recorded from 13 muscles of the dominant leg in healthy participants while cycling at three different cadences (40, 60, and 80 rpm), combined with three different power outputs (15, 30, and 45 W). Muscle synergies were extracted using non-negative matrix factorization, and the effects of cadence and power output on synergy weights and activation patterns were evaluated using repeated-measures statistical analyses.

Five muscle synergies were sufficient to consistently reconstruct muscle activation patterns across all participants and experimental conditions. Statistical analysis revealed no significant effect of power output and no cadence-power output interaction on the activation patterns. Meanwhile, cadence significantly affected only the fifth synergy activation pattern, with temporal differences occurring during the late phase of the pedaling cycle (311° to 332°). Since no statistical evidence for different power outputs, muscle synergies were analyzed across different cadences.

These findings suggest that the modular organization of muscle synergies is preserved during semi-recumbent cycling only across different power outputs. Indeed, cadence influences the temporal recruitment of specific synergies, particularly the fifth synergy, with activation occurring earlier in the pedaling cycle at higher cadences. In conclusion, while cycling across different power outputs shares a common modular organization, temporal adaptations related to different cadences need to be accounted for when designing an FES control strategy.

Chapter 1

Introduction

1.1 Background

Functional electrical stimulation (FES) during cycling is a common rehabilitation technique for people with spinal cord injury (SCI). Stimulation electrodes are placed over the main lower limb functional muscle groups (quadriceps, hamstrings, calves), and electrical pulse trains are delivered to evoke action potentials that propagate along the muscle fibers, leading to contraction and subsequent joint movement [1]. Individuals with SCI performing FES cycling are typically seated in a semi-recumbent position, facing a motorized rehabilitation ergometer, with their feet secured to the pedals.

FES cycling rehabilitation for SCI has been shown to have benefits, including functional neurological recovery [2], increased muscle cross-sectional area [3], muscle strength [4], and endurance [5], improved body composition [6], and bone health [7]. Whilst FES cycling provides extensive positive health benefits, current approaches to designing FES patterns commonly rely on trial-and-error methods, whereby clinicians manually determine stimulation parameters that produce cycling movements, or on generic stimulation patterns that are not tailored to the individual. Trial and error and generic approaches may not provide the optimal stimulus required for tissue adaptation [8], as they fail to account for inter-individual differences in anthropometry or crank configuration, both of which are known to influence optimal FES parameters [9]. Thus, FES cycling still faces challenges in defining stimulation strategies that are both efficient and individualized. [10, 11].

One approach to designing FES patterns is to mimic the neural solution adopted by the nervous system to generate movements, as the mimesis of biological control has the highest likelihood of successfully restoring motor function [11]. It is widely accepted that in order to generate voluntary movements, a certain set of muscles is simultaneously activated and coordinated [12]. Indeed, the CNS simplifies motor tasks in low-dimensional motor modules by linear combinations of muscle synergies. [13, 14].

Previous studies have investigated muscle synergies under different mechanical conditions, performing upright cycling [15, 16], where the results show that muscle synergies can be repeated under different cycling conditions. However, these findings have not been deeply explored in semi-recumbent cycling, which represents the typical configuration adopted in FES rehabilitation after SCI. Therefore, it remains unknown whether muscle synergies are preserved under these conditions. This study aims to quantify muscle activation patterns using muscle synergies during semi-recumbent cycling across different conditions (cadences and crank power outputs) while accounting

for participant anthropometry and crank configuration. This will enable the development of improved, physiologically plausible control algorithms for FES cycling, which will reduce clinician reliance on trial-and-error approaches and overcome the limitations of generic stimulation patterns that are not individualized.

1.2 Research Questions

This study addresses the following research questions:

- Is the structure of muscle synergies preserved across different cadences and power outputs in semi-recumbent cycling?
- What is the effect of cadence and power output on synergies across different individuals in semi-recumbent cycling?
- Are there any differences on muscle synergies across different cadences and power outputs in semi-recumbent cycling?

Chapter 2

Literature review

This chapter provides a background of human movements, the physiology of muscles, and how to record their electrical activity. It then explores how muscular-neural pathways can be disrupted, leading to motor impairments, focusing on spinal cord injury. Thus, it will introduce an overview of rehabilitation techniques, highlighting FES and describing different control methods and performing strategies. Finally, this chapter will explore how muscles are controlled by the central nervous system through what are called 'motor modules', or 'muscle synergies', linking them to neuromusculoskeletal modelling.

2.1 Human movement and muscle physiology

Human movement results from the interaction between the nervous system, musculoskeletal structures, and sensory feedback mechanisms. Voluntary human movement of the body is initiated within the brain's motor areas, followed by descending signals that travel through the spinal cord to the targeted muscles, resulting in action potentials that initiate muscle fiber contraction, force production, and finally the desired motion [17]. This process is continuously modulated by afferent sensory inflows that provide information about body position, force, and environmental conditions, allowing for adaptation and control of movement in dynamic contexts. To investigate how the nervous system controls muscles through this chain, it is first necessary to understand the basic building blocks of the neuromuscular system.

The electromyography (EMG) signal represents the electric potential generated by depolarization from the action potential propagating along the muscle fibers. Moreover, it provides us with information about the strategies that the central nervous system adopts to generate volitional movement. Therefore, a description of the human neuromuscular system is essential to understand how muscles generate bioelectrical signals [18].

The basic unit of the neuromuscular system is the motor unit (Fig. 2.1). It is formed by a motor neuron, including its dendrites and axons, and the muscle fibers innervated by the axon. The cell body of the motor neuron is located in the ventral horn of the spinal cord, and its axon exits through the ventral roots. Motor neurons and muscle fibers are connected together through the neuromuscular junction: a chemical synapse that allows the neuron to transmit a signal to the muscle fibers, leading to muscle contraction.

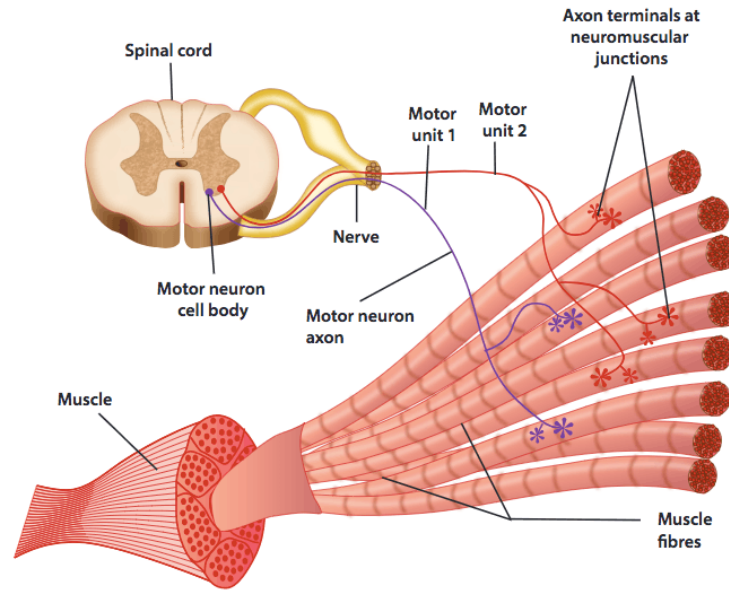


Fig 2.1. Axons of motor neurons extend from the spinal cord to the muscle. Each axon divides into several branches that terminate in neuromuscular junctions with muscle fibers distributed throughout the muscle.

Motor information travels along axons in the form of an action potential (AP). An AP is a rapid, temporary reversal of electric charge across a cell membrane, allowing neurons and muscle cells to send signals and communicate with each other. The neuronal AP travels through the axon, where the presence of myelin increases conduction velocity by enabling saltatory conduction (Fig. 2.2). The AP jumps from one node of Ranvier to the next rather than traveling continuously along the axon, thereby increasing conduction velocity [19]. Indeed, when the AP reaches the neuromuscular junction, acetylcholine is released from the synapse and binds to specific receptors on the muscle cell membrane. This binding opens ion channels, allowing sodium ions to flow inside the muscle cell, causing depolarization and consequently triggering a muscle AP. The muscle AP propagates along the muscle fiber, coupling electrical excitation to mechanical contraction, from the innervation zone to the tendons, where the potential dies out. To represent the propagation of the AP from the innervation zone toward the tendons, a triplet of charges model is commonly used (Fig. 2.3). Its main characteristic is that the total charge intensity is zero, because there are two negative charges and a positive one, which balance each other when they reach the tendons. The resulting electrical potential distribution propagates along the muscle fibers and can be detected by the electrodes placed on the skin surface.

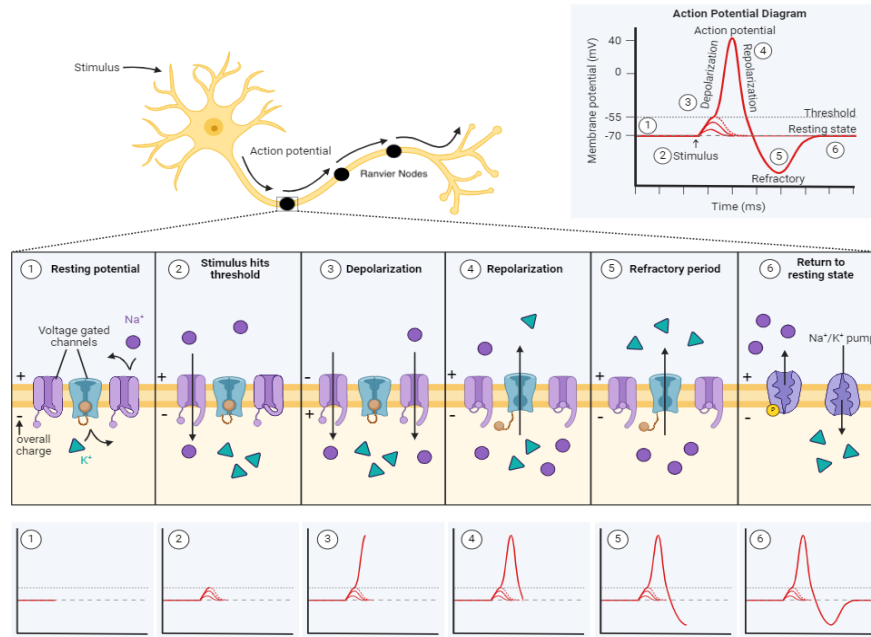


Fig 2.2. Saltatory conduction in a myelinated neuron and the main phases of an AP propagation with the corresponding ion movements across the membrane. A stimulus on the neuron triggers an AP, which travels by jumping between nodes of Ranvier. The top-right graph shows the membrane potential changing over time, divided into six phases reported in the table: 1) resting potential, 2) threshold, 3) depolarization, 4) repolarization, 5) refractory period, and 6) the return to the resting state.

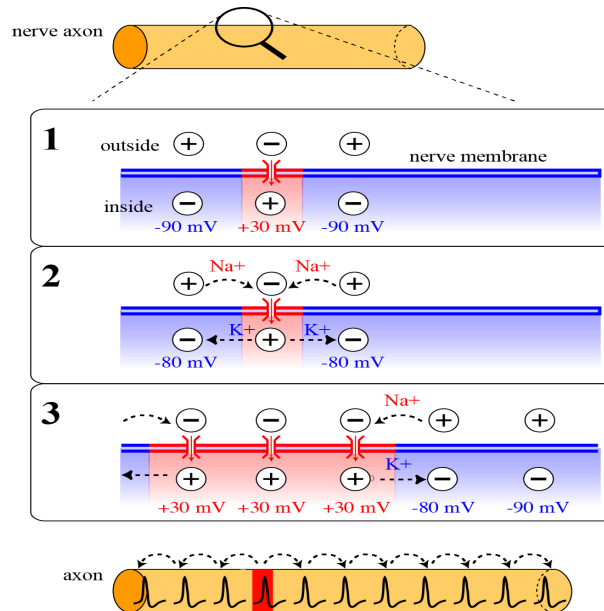
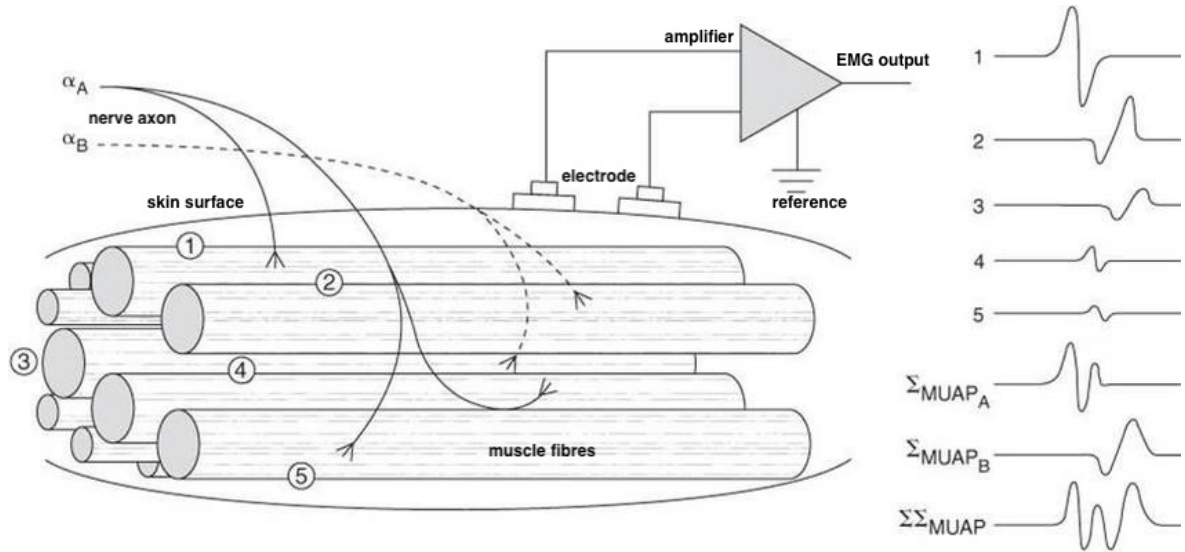


Fig 2.3. AP propagation using the triplets of charges model. Top: schematic of an axon. 1) Resting condition. 2) The AP reaches the nerve membrane: the process of depolarization begins. 3) AP propagates along the axon fiber. Bottom: schematic of action potential propagation.

2.1.1 Introduction to electromyography

EMG signal can be recorded through the use of electrodes, which are either inserted into the muscle (i.e., intramuscular recording) or placed on the skin above the muscle (i.e., surface recording). Surface EMG (sEMG) is the most used technique to detect EMG signals in research and clinical environments, due to its advantage of being a non-invasive method. Although multiple electrode configurations exist to measure sEMG, a common approach used in biomechanics is the bipolar configuration. It involves the application of two electrodes placed above the skin and oriented along the muscle fibers (Fig 2.4). Several factors must be taken into account for a good sEMG recording [20]. First is electrode placement: they should be spaced approximately 20 mm one for another. A higher inter-electrode distance provides a higher acquisition volume, containing more muscle fibers, but could cause crosstalk phenomena with nearby muscles. Second is the skin preparation. The skin-electrode interface is a source of noise. Hair and dead skin are two recognized problems in a good recording. Skin should be shaved, abraded, and cleaned prior to applying the electrodes. Third, the orientation of muscle fibers. Since different muscles have different pennation angles, electrodes should be placed along the orientation of muscle fibers to get the correct propagation of the AP signal. As described in **2.1** section, the AP propagates from the motoneuron to muscle fibers to tendon, causing depolarization and contraction: this potential is called motor unit action potential (MUAP). sEMG is the result of the superposition of every MUAP (i.e., their spatiotemporal summation)(Fig. 2.4).



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Fig 2.4. A schematic of MUAP spatiotemporal summation. Each number represents a different muscle fiber, and each letter represents a different MUAP. Each electrode records the resultant MUAP. An instrumented amplifier is used to compute the difference between two electrodes placed at nearby points, finding their relative electrical activity.

2.2 Spinal cord injury

Neuromuscular disorders are defined as a group of conditions that impair the motor neurons controlling voluntary muscles, causing progressive weakness, atrophy, twitching, and pain. Among these conditions, spinal cord injury (SCI) represents an example in which damage to the spinal pathways disrupts the transmission of neural signals between the brain and peripheral muscles [21]. Direct injury to the spinal cord itself or damage to the tissue and bones (vertebrae) that surround the spinal cord can cause an SCI. As a result, individuals with spinal cord injury often experience profound changes in muscle activation, coordination, and sensory feedback, making it a key focus in the study of neuromuscular dysfunction and rehabilitation.

The symptoms of SCI depend on the part of the spinal cord that is damaged and the extent of damage (Fig. 2.5). An injury to the upper part of the spinal cord can cause paralysis in most of the body and affect all limbs. This condition is called tetraplegia or quadriplegia. On the other hand, an injury that happens lower down the spinal cord may only affect a person's lower body and legs, and is called paraplegia. Paralysis can happen immediately upon injury (i.e., primary damage) or develop over time from bleeding and swelling in the spinal cord and cell death (i.e., secondary damage) [21]. An SCI can damage a few, many, or almost all of the nerve fibers that cross the site of injury. If the injury causes little or no nerve cell death, a person can make an almost complete recovery [22]. SCI injuries are divided into two categories depending on whether the injury is complete or incomplete. A complete injury is defined by the complete loss of sensory and motor function below the injury level. Whereas, it is called an incomplete injury if some sensation or motor function remains below the injury level. Together with the type of injury, his severity is accurately conveyed by the simple five-level (A–E) American Spinal Injury Association (ASIA) impairment scale [23]. The ASIA scale is shown in Table 2.1.

Grade	Description
A	Complete; no sensory or motor function preserved in the sacral segments S4–S5
B	Incomplete; sensory but not motor function preserved below the neurological level and extending through the sacral segment S4–S5
C	Incomplete; motor function preserved below the neurological level; most key muscles have a grade lower than 3
D	Incomplete; motor function preserved below the neurological level; most key muscles have a grade greater than 3
E	Normal motor and sensory function

Table 2.1: ASIA impairment scale

2.2.1 Functional Electrical Stimulation in rehabilitation

FES is a common rehabilitation approach that has shown promise in the improvement of motor function [25]. FES is defined as a rehabilitation technique capable of eliciting muscle contraction through externally applied current. These contractions are coordinated together to produce movement, such as walking or cycling. In FES applications, two main control strategies are commonly used: open-loop and closed-loop. In an open-loop system, the controller does not receive information about the internal and actual state of the system, but usually relies on an external manual switch,

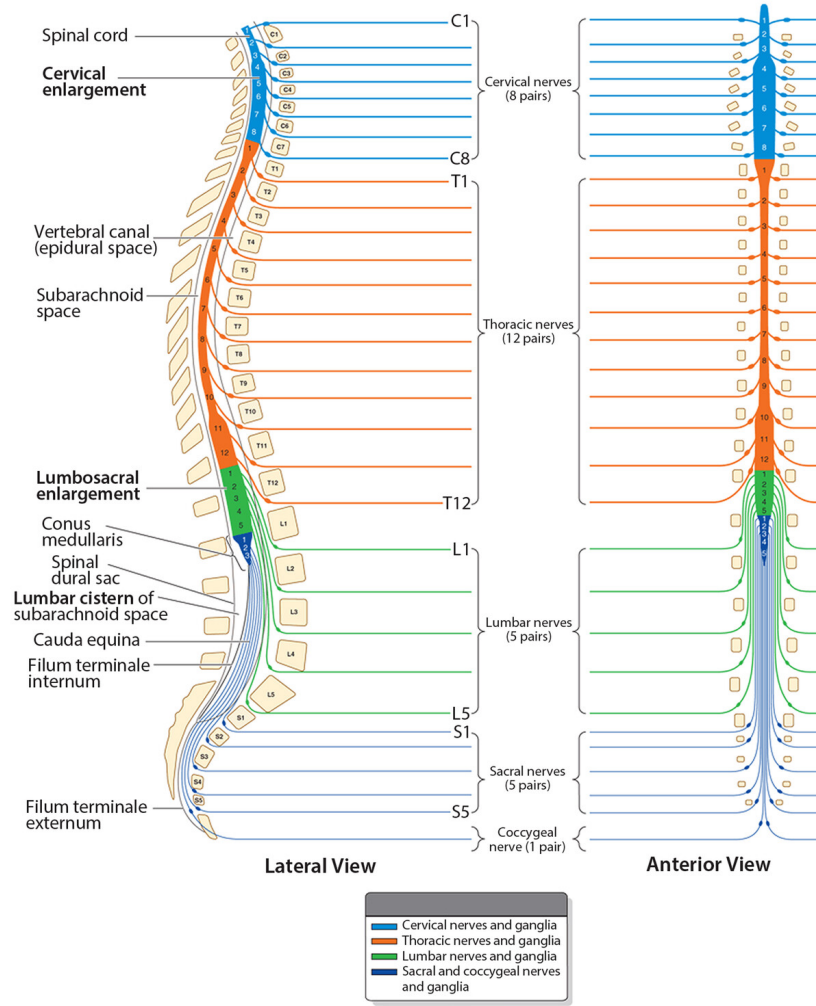


Fig 2.5. Anatomy of the spinal cord and adjacent spinal nerve roots [24]

or the pressure, acceleration, and other sensors to trigger the FES system to start or stop [26]. However, stimulation is delivered according to control strategies decided a priori, and the system parameters are typically obtained through repeated attempts, following the trial-and-error approach. On the other hand, a closed-loop system [27] uses real-time feedback from sensors, such as joint position sensors, cycling speed or angle, or muscle EMG, to automatically adjust the intensity, timing, and pattern of the electrical stimulation applied to the muscles. A closed-loop approach is more accurate than an open-loop, as it takes into account the patient's characteristics and movement feedback, but more expensive and requires more maintenance.

Due to the great difficulty individuals with SCI experience in standing, cycling is often combined with FES technology as a rehabilitation strategy. The FES cycling system presents mainly three components: the controller, the stimulator, and the angle encoder [28]. The controller is responsible for receiving feedback information from the encoder about the state of the system and communicating with the stimulator to provide stimulation. The stimulator uses a short electrical pulse to generate muscle contraction. Indeed, the intensity of the stimulation is determined by three parameters: pulse amplitude, pulse duration, and pulse frequency. Stimulation amplitude ranges between 2-120 mA for surface stimulation, whereas pulse frequencies generally range from 20-50 Hz [1]. Both parameters are inversely related, so that an increase in pulse

duration may require a pulse with lower amplitude or different stimulation frequency to generate a response. By changing those three parameters, muscles can be stimulated in different ways [29]. The pulses are provided to actuate joints by stimulating one or more muscles that exert torques on the joint. The movement is the resultant coupling between the controller, the stimulator, and the encoder. Besides, the resulting joint angle can be regulated by stimulating flexor and extensor muscles, which actuate the joint in opposite directions [30].

2.3 FES control methods and strategies

To achieve precise control of the FES cycling, a wide variety of control methods have been applied to the FES cycling system:

- **Bang-Bang (BB) stimulation strategy:**
BB control strategy is a heuristic procedure that involves full activation or deactivation of large muscle groups at specific time intervals [31]. The algorithm switches the stimulation either completely on or completely off according to pre-defined thresholds or phases of the movement. Although robust and simple, this method does not fully represent the reality of the movement. It leads to inefficient power production, and muscles are likely to fatigue [32].
- **Proportional Integral Derivative (PID) method:**
PID control strategy adjusts electrical stimulation parameters (amplitude or pulse width) to manage muscle contraction and movement. The algorithm continuously computes the error between the desired output and the actual response, and then modifies the stimulation according to proportional, integral, and derivative terms. It improves precision and stability by reducing the error between a target movement, such as knee motion [33] or crank position and cadence [34], and the actual muscle response.
- **Fuzzy Logic (FL) method:**
FL control strategy is primarily used because it can deal with uncertainty and nonlinearity [35]. Given the nonlinear nature of the musculoskeletal system, its application in FES helps to better control the intensity and frequency of the stimulus for more stable and natural motion. The algorithm translates input variables, such as tracking error or cadence, into rules that determine how the stimulation parameters should be adjusted [36].
- **Neural Network (NN) method:**
NN control strategy is used thanks to its structure: inputs, weights, and activation functions can be used to analyze and predict the outcome of the system by implementing training and learning rules [37] [38]. As FL method, this approach is particularly useful as NN can model the nonlinear nature of the musculoskeletal system, although its performance strongly depends on the quality and amount of training data available.

However, all these methods still rely on the binary BB stimulation patterns, continuous feedback, or training data available, which limits their physiological fidelity.

2.4 Muscle synergies

Many researches since Bernstein [39] have aimed to understand how the CNS system organizes movements. How this coordination is accomplished by the CNS is a long standing question in the study of motor control. One widely accepted hypothesis is that this complexity is simplified by a modular organization of the neural system controlling movement. In this framework, the CNS solves the redundancy problem by relying on generating movement as a combination of a small number of invariant muscle patterns, commonly referred to as motor modules or muscle synergies [12]. Although the hypothesis is debated, an increasing number of studies in humans and animals using simulation and behavior experiments have shown compelling results in the last few decades. [12, 14, 13]. The concept of muscle synergy has been formalized with a mathematical model based on factorization algorithms that decompose the EMG signal into the product of two components. There are several factorization methods used to extract muscle synergies [40]: Principal Component Analysis (PCA), Independent Component Analysis (ICA), Factor Analysis (FA) and Non-Negative Matrix Factorization (NMF). Together with methods, three different synergy models have been proposed (Fig. 2.6):

- Time-invariant model (i.e., synchronous model) [40]:

$$u(t) = \sum_{i=1}^k a_i(t) \times w_i$$

where $u(t)$ is the muscular activation pattern, k is the number of synergies, w is a synchronous scalar value (i.e., weight coefficient matrix), which represents the relative weight of each muscle within the synergy, a is the time-dependent coefficient (i.e., activation coefficient matrix), which represents the relative contribution of each synergy for each muscular pattern.

- Time-varying model (i.e., asynchronous model) [41]:

$$u(t) = \sum_{i=1}^k a_i \times w_i(t - \tau_i)$$

where $u(t)$ is the muscular activation pattern, k is the number of synergies, w is the time-varying function, in which the scalar value a is applied, and the time shifted value τ

- Space-by-time model [42, 43]:

$$u(t) = \sum_{i=1}^k \sum_{j=1}^h \omega_i(t) \times a_{i,j} \times w_j$$

where $u(t)$ is the muscular activation pattern, ω_i and w_j are the temporal and spatial modules respectively, and $a_{i,j}$ is a scalar activation coefficient.

In this study [45] is reported that NMF (Fig 2.7) was the most appropriate extraction method in walking and running conditions by comparing four extraction methods (i.e., NMF, PCA, ICA, and FA). Each muscle synergy contributes to the mechanical output needed to generate task-specific biomechanical functions.

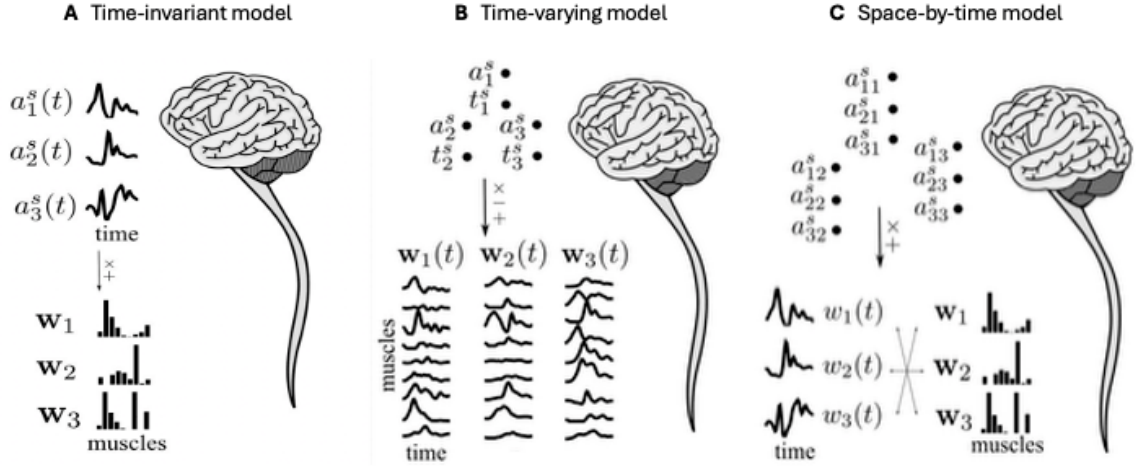


Fig 2.6. Existing models for muscle modularity used to extract and represent muscle synergies [44].

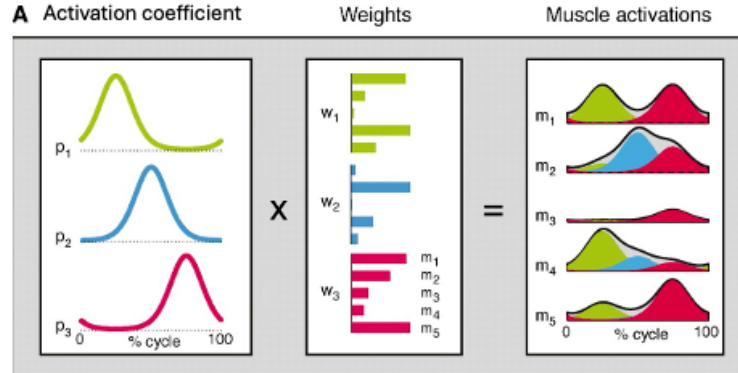


Fig 2.7. Schematic of muscle synergies extracted from NMF: simulated example of muscle activation patterns as a combination of activation patterns and weight coefficients. [46].

2.5 Neuromusculoskeletal modelling

Following the concept of muscle synergies, neuromusculoskeletal (NMSK) modelling provides a quantitative framework to investigate how coordinated neural control strategies are translated into mechanical movement. While muscle synergies describe the organization of muscle activations into low-dimensional control modules, NMSK models extend this perspective by linking neural commands to the biomechanical behavior of muscles, tendons, joints, and sensory pathways [47]. NMSK models are used to study not only how muscles are coordinated, but also how such coordination generates movement and force. NMS models offer a more complete understanding of human movement than approaches that focus solely on musculoskeletal structure or kinematics. Neuro-musculoskeletal modelling has become an important tool in both research and clinical applications. It is widely used to investigate motor impairments [48], evaluate reha-

bilitation strategies [49], and optimize interventions such as FES [50]. In populations with neurological disorders, including spinal cord injury, these models offer valuable insight into altered neuromuscular mechanisms and can support the development of personalized therapeutic solutions [8].

Typically, NMSK frameworks include anatomical representations of bones, joints, muscles, and tendons, together with mathematical descriptions of muscle contraction dynamics, motor control strategies, and afferent pathways [51](Fig. 2.8). NMSK models can estimate internal variables that are difficult or impossible to measure directly, such as muscle forces, fiber lengths, joint contact loads, and reflex responses. [52]. However, the resulting estimations of NMSK models are dependent upon the neural drive utilized.

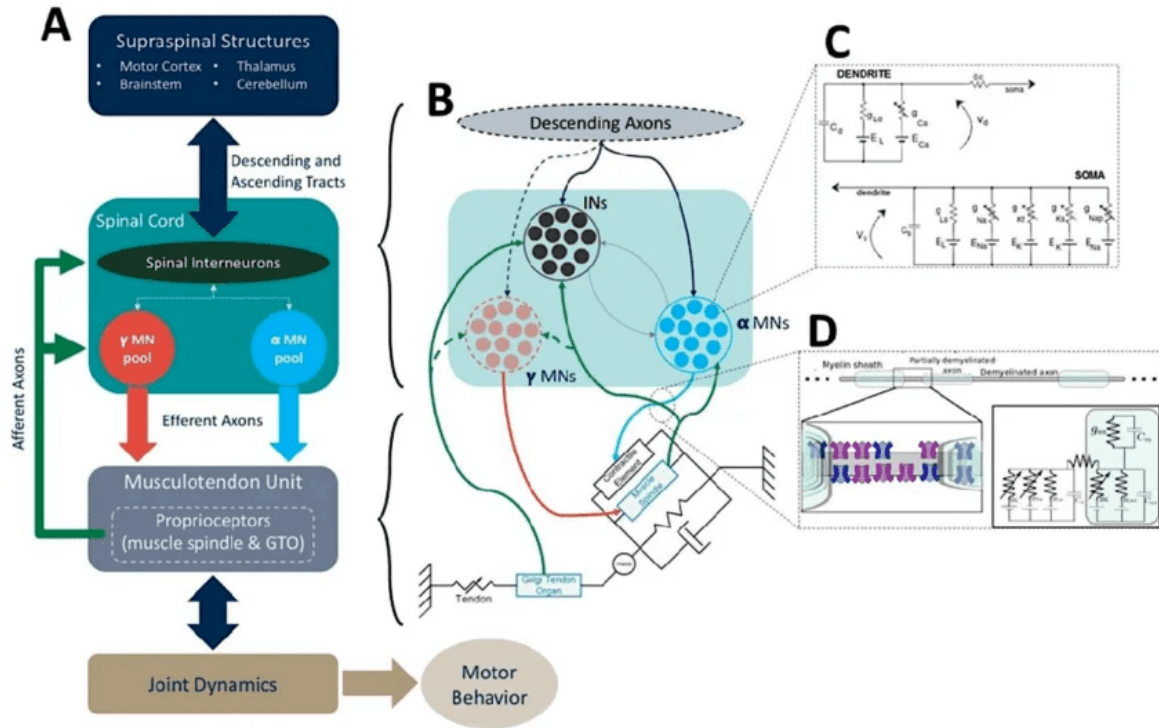


Fig 2.8. Hierarchical view of the neuromusculoskeletal system and its major structures. **A** Overview of the neuromusculoskeletal system and the interaction between its different components. **B** Detailed view of the spinal cord (top) and Hill-type muscle model with muscle receptors (bottom). **C** Common α -MN model equivalent circuit, divided into its soma and its dendrite. **D** A schematic of a Ranvier node (left) and an axonal segment (right) [51].

Traditionally, neural drive is calculated using a computational algorithm, such as static optimization or computed muscle control, that determines how muscles should activate to produce movement: these algorithms can assume minimization of a priori criteria (e.g., sum of muscle activation squared) [53] subject to dynamic constraint [54]. However, individual-specific motor control strategies vary across individuals even when external joint biomechanics are the same [55].

Recently, EMG-driven neuromusculoskeletal (NMSK) models have emerged as a promising alternative to traditional neural algorithms and are known to provide physiologically plausible estimates of internal states, such as joint contact forces [56, 57, 58]. The Calibrated EMG Informed Neuromusculoskeletal Modelling (CEINMS) toolbox [59] uses EMG signal along with joint kinetics and external loads to estimate internal

variables (muscle forces, fiber length, joint contact loads, etc). CEINMS has the capacity not only to use experimental EMG but also to adjust experimental EMG and synthesize unknown EMG to compute joint kinetics that closely match experimental kinetics.

2.5.1 Neuromusculoskeletal modelling with synergies approach

Muscle synergies and neuromusculoskeletal modelling can be integrated to provide a more physiologically meaningful representation of motor control. Following this framework, muscle synergies describe the low-dimensional organization of muscle excitations, while NMSK models translate these excitations into biomechanical outputs such as muscle forces, joint moments, and joint contact forces. Previous studies have shown that synergy-informed NMSK approaches can estimate joint moments and contact forces using a reduced number of EMG recordings, with performance comparable to more complete EMG-assisted models [48]. This suggests that muscle synergies can be used as a descriptive tool for motor coordination and as inputs to personalized computational models capable of estimating internal biomechanical variables. Muscle synergy-based stimulation has already been proposed as a way to design more physiological and personalized FES strategies, by stimulating groups of muscles according to coordination patterns associated with specific biomechanical functions [60]. Moreover, EMG-informed NMSK models have been applied during electrical stimulation to estimate muscle forces and joint moments, supporting their potential use for monitoring and adapting FES rehabilitation [50].

Therefore, combining muscle synergy analysis with personalized NMSK modelling may provide a useful framework to improve FES control. Synergies can reduce the dimensionality of the stimulation problem by identifying coordinated muscle groups, while NMSK model outputs can quantify the mechanical consequences of these neural commands. In this context, the present work investigates muscle synergies during semi-recumbent cycling as a first step toward understanding how coordinated activation patterns may be used to inform more personalized and physiologically grounded FES strategies for individuals with SCI.

Chapter 3

Material and methods

3.1 Participants

Eleven healthy individuals (age 26.64 ± 1.50 y, body mass 74.56 ± 15.28 kg, height 1.76 ± 0.14 m) were recruited to participate in this study. None of the participants had prior experience with semi-recumbent cycling. Prior to participation, each participant gave their informed written consent. This study was approved by the Griffith University Human Research Ethics Committee (2021/171).

3.2 Data acquisition

Motion, pedal reaction forces, and electromyograms (EMG) were acquired synchronously during the semi-recumbent ergometer (Tigo, Thera-Trainer, Hochdorf, Germany) cycling. To record marker trajectories, a 12-camera Vicon motion capture system (Vicon Motion System Ltd., Oxford Metrics, Oxford, UK) was used (200 Hz). To track the movement of the trunk and lower limb (pelvis, thigh, shank, ankle, and foot segments), a total of 41 markers (25 single markers and 4 clusters of 4 markers) were placed on the subject's body. Surface EMG signals (2000 Hz) were recorded from 13 muscles of the dominant leg (Myon Aktos, Schwarzenberg, Switzerland): biceps femoris, gluteus maximus, gluteus medius, gracilis, lateral gastrocnemius, medial gastrocnemius, rectus femoris, semimembranosus, soleus, tensor fasciae latae, tibialis anterior, vastus lateralis, vastus medialis. The skin was shaved, abraded, and cleaned with isopropyl alcohol before placement of the bipolar EMG gel electrodes (Ag/AgCl Duo-Trode, Myotronics Inc, 19 mm center-to-center inter-electrode distance) according to the SENIAM guidelines [20]. In addition, crank kinematics, bilateral radial, and tangential crank reaction forces were measured synchronously. [56]

In order to recreate the recommended FES setup for individuals with SCI [30], the ankles were fixed to the crank pedals using orthotic braces (OAPL, Clayton, Victoria). Participants were asked to cycle at different cadences (40, 60, and 80 revolutions per minute [rpm]) and power outputs (15, 30, and 45 Watts [W]) [61]. A custom real-time biofeedback interface was developed in MATLAB (MathWorks, MA, USA), and together with a metronome, the interface provided real-time auditory and visual feedback to help participants maintain the desired cadence and power output. Meanwhile, to achieve the desired power output, resistive torque from the ergometer was adjusted accordingly. Fifteen Crank rotations were recorded after at least 30 s of cycling at the prescribed cadence and power output.

3.3 Data processing

Data were analyzed using a custom-made Python script (Python Software Foundation, Wilmington, DE, USA). EMG signals were band-pass filtered (second-order zero-lag Butterworth digital filter, bandwidth 20 to 400 Hz) to attenuate DC offset, motion artifacts, and high-frequency noise [20]. Filter magnitude and phase are reported in Fig. 3.1. Filtered signals were full-wave rectified and low-pass filtered (second-order zero-lag Butterworth digital filter, cut-off frequency 6 Hz) to obtain muscle activation patterns (envelope). Applying a filter can introduce edge artifacts; therefore, all values below zero were clipped to zero to ensure a non-negative matrix. Since the crank angle is a periodic circular signal, it was filtered using a different approach. The raw signal was first converted from Volts [V] to degrees [°] and then mapped into its sine and cosine components. These components were independently low-pass filtered (second-order zero-lag Butterworth digital filter, cut-off frequency 6 Hz). Filter magnitude and phase are reported in Fig. 3.2. Finally, the filtered crank angle was reconstructed using the arctangent function (`atan2`). The same approach was repeated, converting the raw signal from Volts [V] to degrees [°] after filtering, yet not effective. The result of the filtered crank angle is reported in Fig. 3.3.

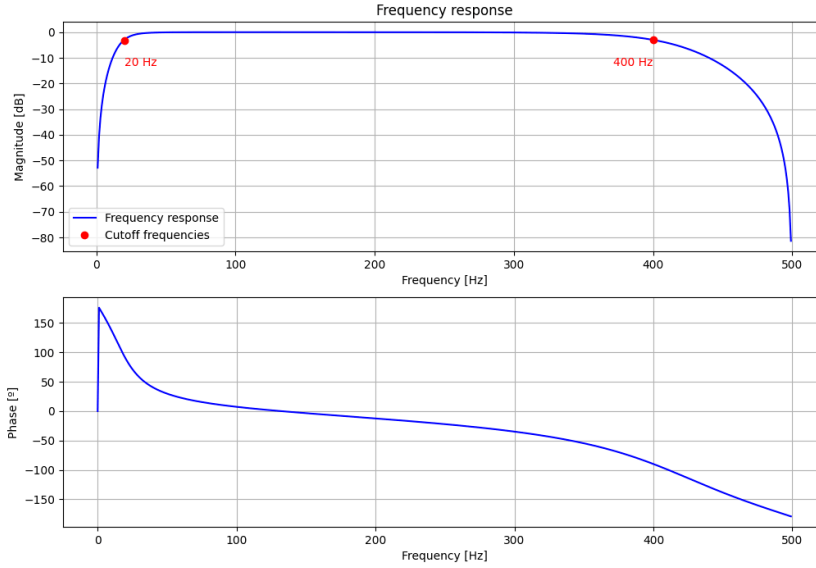


Fig 3.1. Band Pass filter frequency response. The top figure shows the magnitude of the filter, while the bottom shows the phase. The two red dots correspond to the two cutoff frequencies, 20 Hz and 400 Hz, respectively.

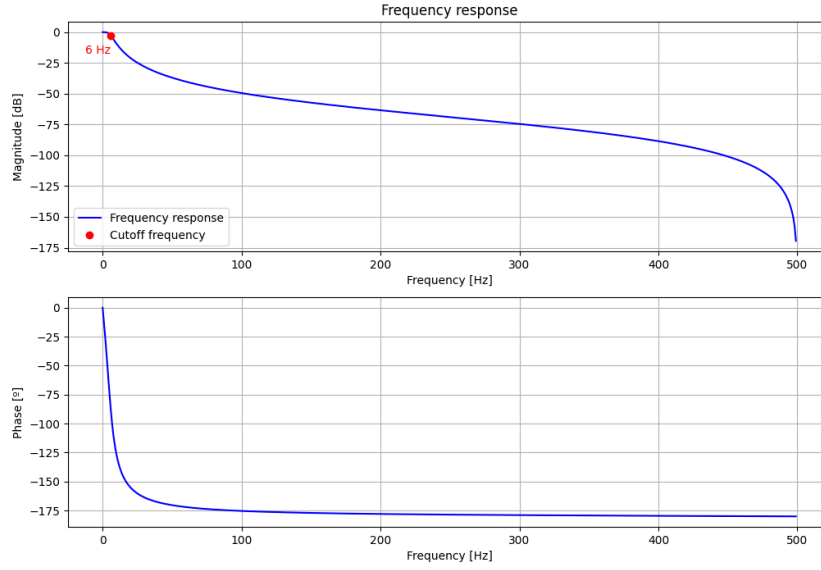


Fig 3.2. Low Pass filter frequency response. The top figure shows the magnitude of the filter, while the bottom shows the phase. The red dot corresponds to the cutoff frequency at 6 Hz.

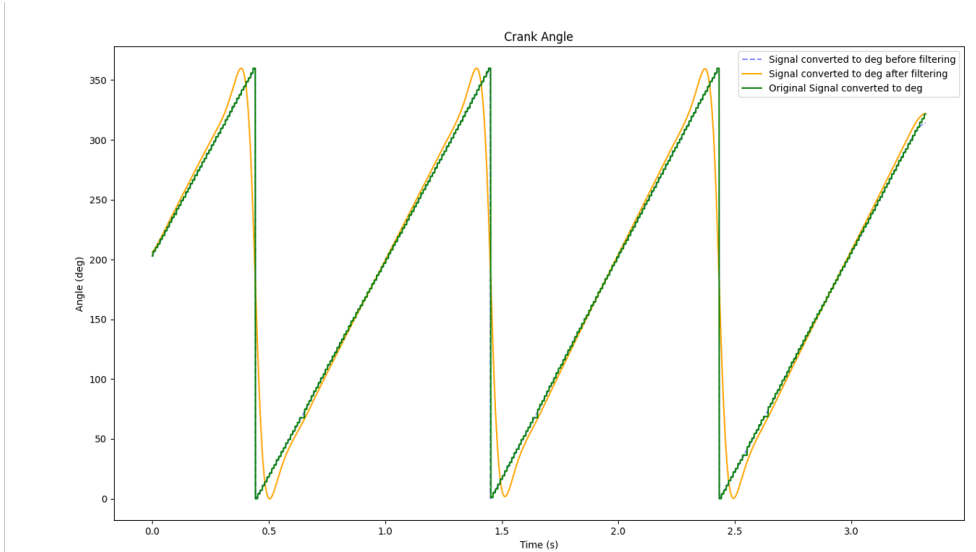


Fig 3.3. Crank angle and its filtering: two different filtering approaches were tested. The green line represents the raw signal. The yellow line shows the signal converted to degrees after filtering, while the blue line (not visible because it overlaps with the green) represents the conversion to degrees before filtering.

3.4 Neuromusculoskeletal modelling

After a visual inspection of the EMGs envelopes, Calibrated EMG-Informed NMSK Modelling Toolbox (CEINMS) [59] was used to add and improve signal quality, and muscle synergies were subsequently extracted from the CEINMS adjusted EMG signals. This decision was made after examining the semi-recumbent cycling EMG data,

which showed that many EMGs were discarded due to poor quality. This was likely caused by the semi-recumbent position, where EMGs placed on muscles around the pelvis were pinched, or calf muscles inside the orthotic braces were knocked. Muscles reconstructed from CEINMS used for extracting muscle synergies were: adductor (ADD), biceps femoris long head (BFLH), gluteus maximus (Gmax), gluteus medius (Gmed), gracilis (GRA), lateral gastrocnemius (GL), medial gastrocnemius (GM), rectus femoris (RF), semitendinosus (SMT), tensor fasciae latae (TFL), tibialis anterior (TA), vastus lateralis (VLAT), vastus medialis (VMED). We decided to exclude the soleus (SOL), since the muscle crosses the ankle joint, and the ankle was fixed with the pedal using the orthotic braces.

Peaks were extracted from the crank rotation signal, and they were used to segment the EMG signal. Each cycling cycle was then time-normalized to 360 points [62]. 12 cycling cycles were granted for each combination of cadence and power output (Fig. 3.4). Thus, for each repetition, all EMG segments were stacked into a matrix $V = m \times (t \times n)$, where m is the number of muscles, n is the number of cycling cycles, and t is the number of time points. V is the matrix that contains the muscular activation patterns.

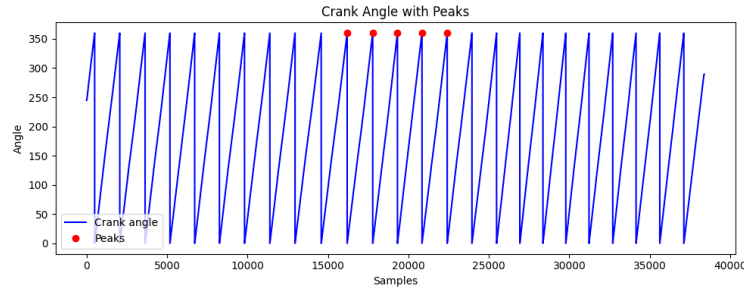


Fig 3.4. An example segmentation of crank angle: in this combination, cadence-power output was available for 3 different trials, so in order to grant 12 cycles, 4 different segments were taken from each trial.

3.5 Muscle synergies extraction

To extract muscle synergy weight vectors and synergy activation primitives, non-negative Matrix Factorization (NMF) method [63][45] was used. The NMF approximates V in the form $V = W \times H + e = \hat{V} + e$, where W is a $m \times n$ matrix (m is the number of muscles and n is the number of synergies) that specifies the relative weight of each muscle within each synergy, H is a $n \times t$ matrix (t is the number of cycles $\times$ time points) that specifies the relative contribution of each synergy for each muscular pattern. At each iteration, the algorithm updates matrices W and H in order to minimize the Frobenius norm between V and $\hat{V}$. The number of synergies being an input of the NMF algorithm, the algorithm was run with two to seven synergies. For each run, the Variability Accounted For (VAF) was computed to assess the goodness of the reconstructed signal.

$$VAF_{\text{total}} = 1 - \frac{\sum_{i=1}^m \sum_{j=1}^t (V(i, j) - \hat{V}(i, j))^2}{\sum_{i=1}^m \sum_{j=1}^t (V(i, j))^2}$$

A minimum value of 90% for VAF_{total} was used to consider the quality of reconstruction acceptable [64][65].

Each activation coefficient was matrix then normalized to its maximum value (range 0 to 1), and the corresponding weight matrix was scaled consequently, in order to preserve the product $V = W \times H$. In this way, all information related to amplitude was transferred to the weight coefficient matrix. To account for variability in both temporal and spatial components, the first set of synergies within subjects was reordered according to the peak timing of H . This pair was then removed from the sets. All subsequent synergies were reordered by maximizing cosine similarity to this reference set [16].

3.6 Statistical analysis

After reordering and before proceeding with further analysis, a two-way repeated-measures ANOVA was performed separately for each synergy activation profile to evaluate the main effects of cadence, power output, and their interaction [66]. When no significant effect of one variable was observed, data were averaged across that feature while varying the other. Thus, a one-way ANOVA was performed for each muscle within the same synergy.

Chapter 4

Results

4.1 Structure of muscle synergies

Fig 4.1 shows the mean computed first across conditions within a subject, and then across all subjects. Overall, the VAF increases monotonically with the number of synergies, indicating an improvement in the quality of EMG reconstruction. Here, it can be found that the mean VAF value was 84.67% as the synergy number was two, and the VAF value reached 91.86% as the number increased to three. A further increase to four synergies, with 95.40% VAF led to a smaller gain (+3, 54%), suggesting a reduction in marginal improvement. Finally, for five and six synergies, the VAF approached saturation, reaching 97.28 and 98.32, respectively. In table 4.1 are reported all the values of VAF from two to six extracted synergies.

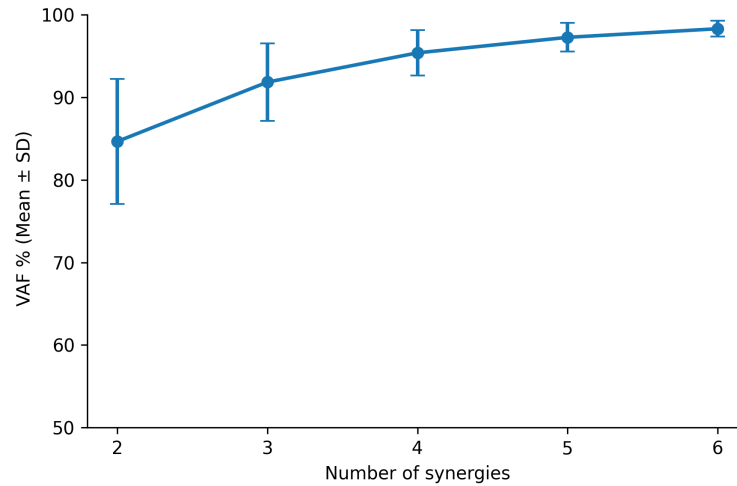


Fig 4.1. VAF value across all conditions and across subjects from two to six synergies. The plot shows the mean and standard deviation across all twelve cycling cycles and across subjects.

Table 4.1: Mean values of VAF (Mean $\pm$ SD)

Number of synergies	Mean $\pm$ SD
2	84.67 $\pm$ 7.56
3	91.86 $\pm$ 4.71
4	95.40 $\pm$ 2.75
5	97.28 $\pm$ 1.74
6	98.32 $\pm$ 0.96

Although EMG activity in most conditions was well accounted for by three synergies, to further exhibit similar trends for all the subjects within conditions, every single value of VAF was examined. Table 4.2 reports VAF values representative of four different subjects, presenting a value of $VAF_{\text{total}} \geq 90\%$ for five synergies. Hence, we decided to keep five as a consistent number for all subjects in our analysis. VAF critical values for the four subjects are reported in Table 4.2.

Table 4.2: VAF (%) values for four different representative subjects. Legend: n = number of synergies, (-) means no repetition is available for the trial.

RPM	Watt	Subject 5		Subject 6		Subject 8		Subject 10	
		n = 4	n = 5	n = 4	n = 5	n = 4	n = 5	n = 4	n = 5
40	15	95.2	98.5	93.4	94.8	97.5	98.1	95.9	97.3
	30	92.1	97.9	87.5	92.8	94.5	94.8	91.8	93.5
	45	92.5	97.9	87.5	92.2	94.6	95.7	91.1	94.4
60	15	87.9	95.9	94.1	97.2	94.9	95.0	94.2	96.7
	30	94.9	97.5	90.4	93.5	93.3	93.8	90.9	96.2
	45	95.9	98.8	90.8	94.1	90.4	92.7	91.0	97.4
80	15	98.7	98.7	–	–	93.3	96.0	94.2	96.6
	30	98.2	98.8	96.1	98.0	89.7	92.3	89.5	94.2
	45	96.4	97.1	95.1	97.6	94.6	96.4	94.6	95.4

Table 4.2 shows VAF critical values: Subject 5 40rpm30W, Subject 6 40rpm45W, Subject 8 60rpm15W and Subject 10 80rpm30W, which exceed the 90% threshold, even if the variability accounted for value is higher than 87% from 4 to 5 synergies. Thus, five synergies were fixed as a constant for all subsequent analyses.

4.2 Group-level effects of cadence, power output, and their interaction.

An spm1d two-way ANOVA repeated-measures was performed on each activation coefficient matrix to analyze the effect of cadence, power output, and their interaction within subjects. The table 4.3 shows the test results, reporting critical F-values and maximum F-values for each activation coefficient vector. Meanwhile, the following five figures, from 4.2 to 4.6, show the result of the test for every H activation coefficient. The dashed line represents the F-value corresponding to the threshold $\alpha = 0.05$, and the gray shaded area indicates regions where $p < 0.05$. In figure 4.6, main effects analysis showed that cadence did have a statistically significant effect on the activation coefficient H5. There were notable differences at angles from 311° to 332° . In contrast, the main effects analysis showed that power output did not have a statistically significant effect on any other activation coefficient. Finally, the test revealed that there was not a statistically significant interaction between the effects of cadence and power output for any activation coefficient vector. Following these results, data were averaged across power output while varying the cadence.

Table 4.3: Critical F-values and maximum F-values for each activation coefficient vector. F^* represents the critical threshold at $\alpha = 0.05$, while Max F represents the maximum F-value reached across the time vector.

	H1		H2		H3		H4		H5	
	F^*	Max F	F^*	Max F	F^*	Max F	F^*	Max F	F^*	Max F
Cadence	10.57	4.39	11.19	2.75	10.57	2.45	11.06	6.55	11.15	18.2
Power	10.57	1.66	11.19	8.16	10.57	3.07	11.06	5.02	11.15	4.5
Interaction	5.73	3.19	5.96	5.0	5.72	2.85	5.91	3.0	5.95	2.1

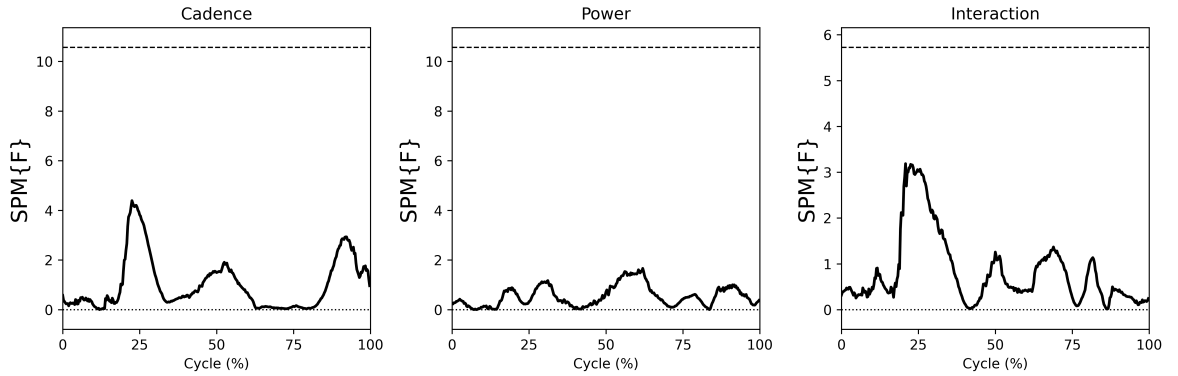


Fig 4.2. Result plots from spm1d repeated-measures ANOVA for $H1$ activation matrix. The dashed line represents the F-value corresponding to the threshold $\alpha = 0.05$.

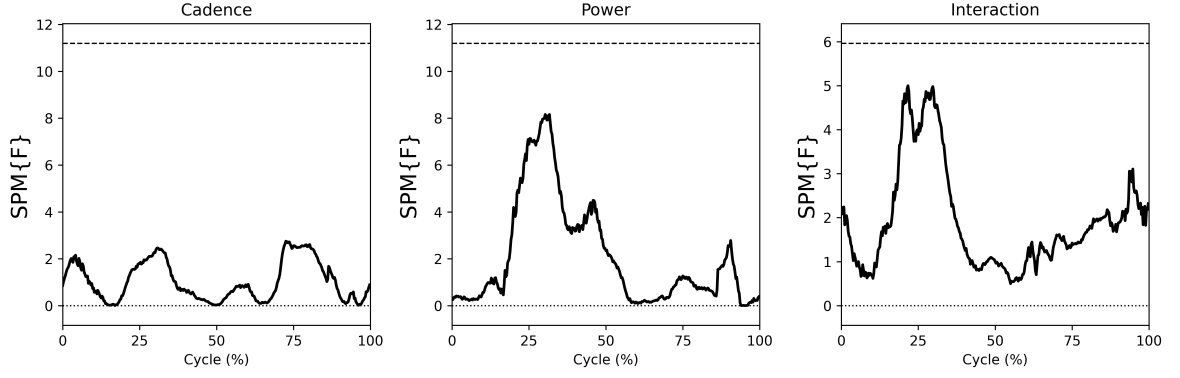


Fig 4.3. Result plots from spm1d repeated-measures ANOVA for $H2$ activation matrix. The dashed line represents the F-value corresponding to the threshold $\alpha = 0.05$.

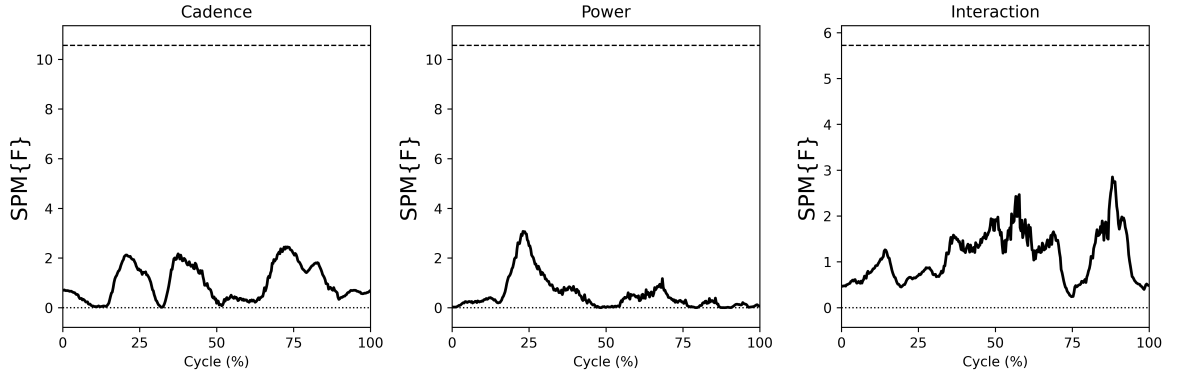


Fig 4.4. Result plots from spm1d repeated-measures ANOVA for $H3$ activation matrix. The dashed line represents the F-value corresponding to the threshold $\alpha = 0.05$.

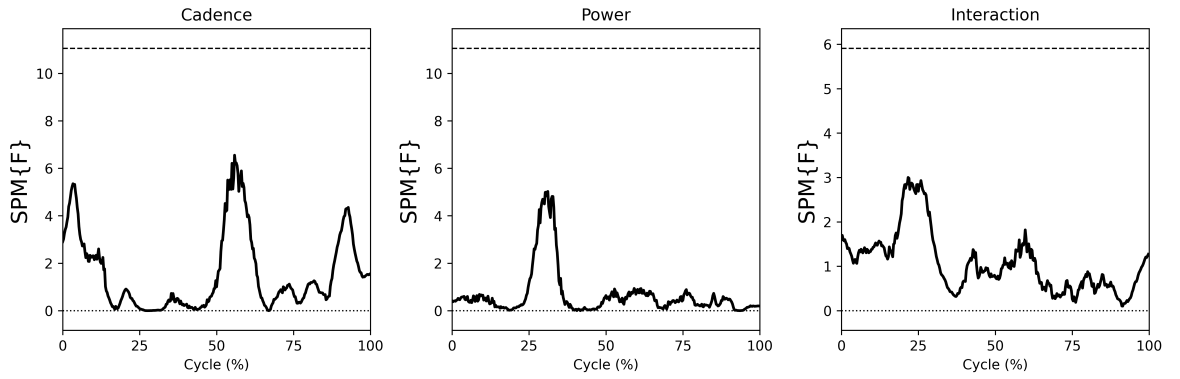


Fig 4.5. Result plots from spm1d repeated-measures ANOVA for $H4$ activation matrix. The dashed line represents the F-value corresponding to the threshold $\alpha = 0.05$.

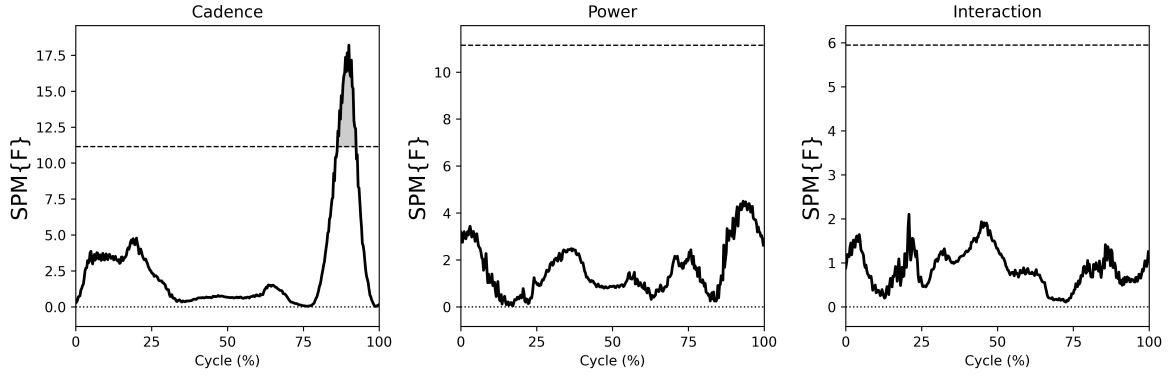


Fig 4.6. Result plots from `spm1d` repeated-measures ANOVA for $H5$ activation matrix. The dashed line represents the F-value corresponding to the threshold $\alpha = 0.05$, and the gray shaded area indicates regions where $p < 0.05$.

4.3 Effect of cadence at fixed power output

Figures 4.7, 4.8, 4.9, respectively obtained by averaging data at fixed power outputs of 15, 30, and 45 W, show muscle synergy weights and activation profile vectors.

Fig. 4.7 shows muscle synergies extracted at 15 W. In Synergy 1, the largest contributors are the gluteus group, VLAT, and VMED, with high activation of TFL and the calf group, although with large variability. Synergy 3 consistently shows high GRA contribution across cadence, a pattern also observed in Fig. 4.8 and in Fig. 4.9. In Synergy 4, weights are distributed between multiple muscle groups, with a decreasing trend for the quadriceps groups (VLAT, VMED, RF), going from 40 to 60 to 80 rpm. Muscles with significant differences were VLAT, VMED, and TFL for Synergy 1. GMAX, GMED, and MGAS for Synergy 2. SMT for Synergy 3, and RF and TA for Synergy 5. In the H activation matrix, Synergies 1, 3, and 4 show cadence-dependent shifts, with activation peaks occurring earlier as cadence increases. In Synergy 1, the peak of the 80 rpm curve is anticipated with respect to the 40 and 60 rpm curves. This trend is also present in Synergy 3 and 4: the peak is anticipated starting from 40, going to 60, and then to 80 rpm. In particular, the main activation peaks occurred approximately around 60–85% of the cycle at 40 rpm, around 60–80% at 60 rpm, and around 40–50% at 80 rpm.

Fig. 4.8 shows muscle synergies extracted at 30 W. The overall structure of the weight coefficient is comparable to that observed in 15 W, although cadence-related differences were more evident in specific synergies. The muscle GRA is shown to have a high contribution in Synergy 3. Synergy 5 shows marked differences across cadence in both weight coefficients and activation profiles. The 40 and 60 rpm activation profiles have the main peak toward the end of the cycle, while the 80 rpm activation profile is shifted toward an earlier phase. Muscles with significant differences are VLAT and TA, for Synergy 1, GMAX and MGAS for Synergy 2. VLAT, RF, SMT, and BFLH for Synergy 5.

Fig. 4.9 shows muscle synergies extracted at 45 W. Although sharing the same structure as those observed in 15 W and 30 W, Fig. 4.7 and Fig. 4.8, the H activation matrix is marked differently across cadences. In Synergy 1, only 60 and 80 rpm share the same profile with a peak in the center, whereas 40 rpm curves show two main peaks located near the beginning and at the end of the cycling cycle. A similar phenomenon

also happens in synergy five, where the peaks for 40 and 60 rpm are located at the end of the cycle, and the 80 rpm peak is at the beginning of the cycle. Muscles with significant differences are GRA, GMAX, VLAT, VMED, RF, and MGAS for Synergy 1. GMAX and MGAS for Synergy 2. RF for Synergy 3, VLAT, RF, SMT, and BFLH for Synergy 5.

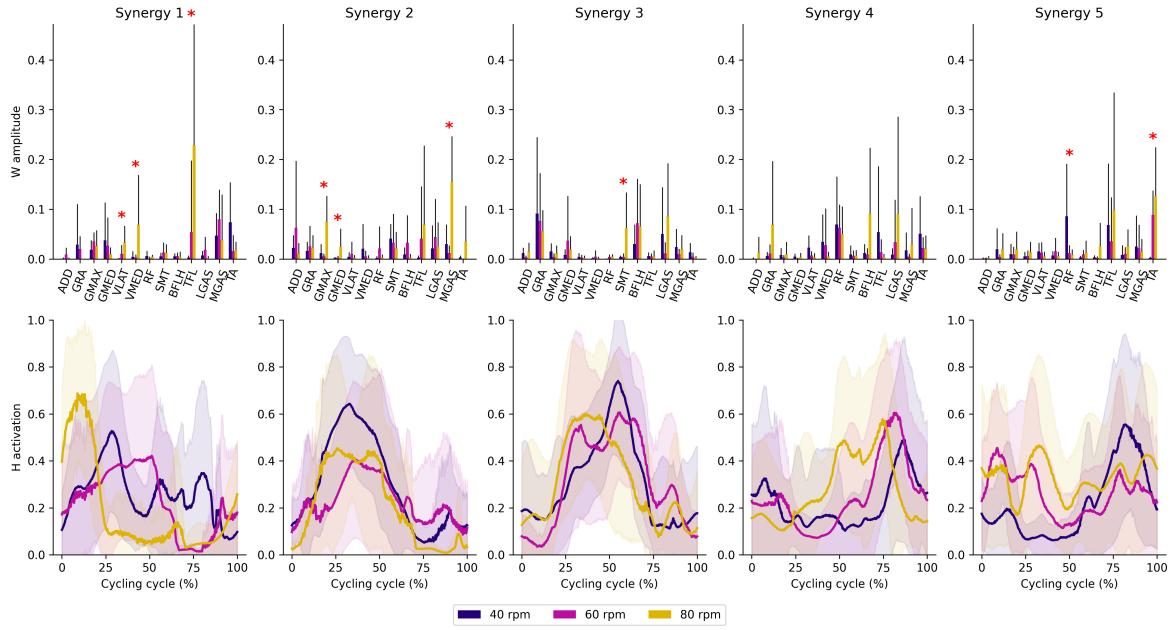


Fig 4.7. Muscle synergies extracted from the same power output condition (15 watts) but at different cadences. Top line: weight coefficient matrix. The values are reported as mean $\pm$ standard deviation, and * indicates a significant difference. Bottom line: activation coefficient matrix. The thicker line is the mean, while the shaded area represents the standard deviation. Legend: blue 40 rpm, magenta 60 rpm, yellow 80 rpm.

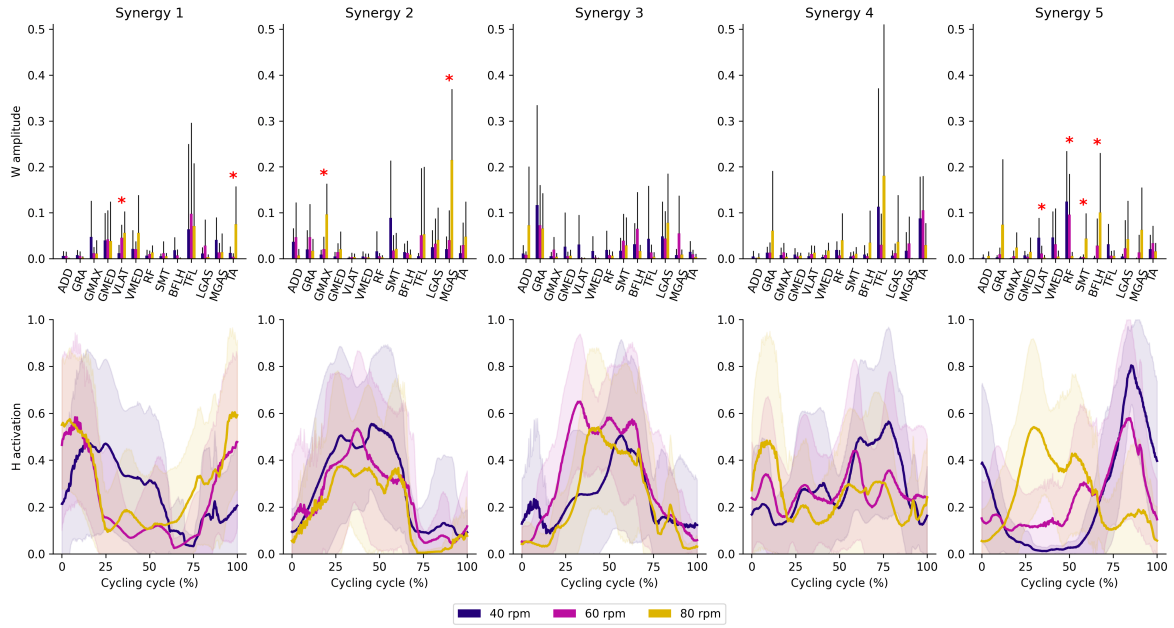


Fig 4.8. Muscle synergies extracted from the same power output condition (30 watts) but at different cadences. Top line: weight coefficient matrix. The values are reported as mean $\pm$ standard deviation, and * indicates a significant difference. Bottom line: activation coefficient matrix. The thicker line is the mean, while the shaded area represents the standard deviation. Legend: blue 40 rpm, magenta 60 rpm, yellow 80 rpm.

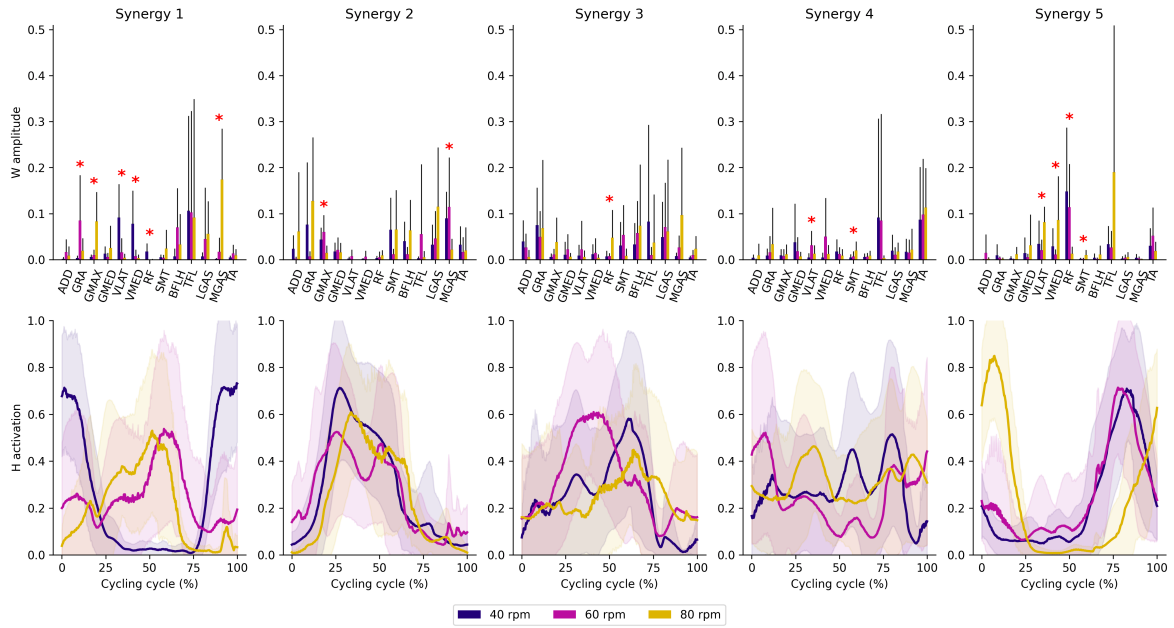


Fig 4.9. Muscle synergies extracted from the same power output condition (45 watts) but at different cadences. Top line: weight coefficient matrix. The values are reported as mean $\pm$ standard deviation, and * indicates a significant difference. Bottom line: activation coefficient matrix. The thicker line is the mean, while the shaded area represents the standard deviation. Legend: blue 40 rpm, magenta 60 rpm, yellow 80 rpm.

Chapter 5

Discussion

This study investigated whether muscle synergies change in semi-recumbent cycling across cadence and power output. Overall, the results suggest that the general structure of muscle synergies is preserved across individuals. The main findings, as regards muscle synergies matrices of weight coefficient and activation pattern, are that cadence has a statistically significant effect on the fifth activation coefficient, and several activation profiles showed a shift toward earlier phases of the pedaling cycle as cadence increases.

5.1 Organization of muscle synergies

The optimal number of muscle synergies required to reconstruct lower-limb muscle activity is not homogeneous across the literature, and this variability appears to be related to the motor task being analyzed. In several studies, three to five synergies have been reported as sufficient to obtain an adequate reconstruction of lower-limb EMG activity: some studies considered three synergies sufficient [65, 66], whereas others reported four [64, 67] or five synergies [68]. In upright cycling, Ambrosini et al. reported a number of synergies ranging from two to five in healthy participants [62]. These differences suggest that the dimensionality of muscle coordination is not fixed, but depends on methodological and biomechanical factors, including the experimental protocol, the specific task constraints, the muscles included in the analysis, the factorization algorithm used, and the reconstruction threshold adopted [69].

A closer examination of the mechanical constraints imposed by the experimental setup may help explain the observed synergy organization. In the present protocol, the ankle was fixed, reducing the available degrees of freedom compared with unconstrained cycling, walking, or running. This constraint limits the contribution of distal joint motion and may alter the relative role of ankle-related muscles during the pedaling cycle. At the same time, the task may remain mechanically redundant, since different combinations of muscle activations can generate similar external outputs. Under these conditions, the extracted synergies may reflect both the neural organization of muscle coordination and the mechanical constraints imposed by the experimental setup.

Another relevant aspect involves the muscles included in this study. As the EMG-assisted output of CEINMS includes both adjusted and synthesized EMGs, muscles that had poor quality signals were generated by CEINMS to create a complete muscle activation output. Following this, across all participants, thirteen muscles were used. Other experimental studies have been known to use nine muscles in cycling [62], eight muscles in cycling [70], which differs from our study that utilizes most of the lower

limb muscles. The benefit of using CEINMS to generate these activation signals is that poor-quality signals can be addressed by adjusting them to match external motion (inverse dynamics).

Muscle synergies can also be extracted starting from a dataset containing muscles recorded bilaterally, such as in the work done by Esmaeili and Maleki [16], whereas the present study extracts muscle synergies from unilaterally recorded muscles. This difference can change the interpretation of muscle synergies, depending on the muscles included initially. When muscles from both legs are analyzed, the extracted synergies may reflect not only the activation timing of individual muscle groups, but also inter-limb coordination and the contribution of both limbs across different phases of the pedaling cycle. In contrast, synergies extracted from a single leg provide a more local representation of unilateral muscle coordination. Therefore, differences in synergy structure, muscle weighting patterns, and phase-related interpretation between studies may partly depend on whether the initial EMG dataset includes one or both lower limbs.

5.1.1 Algorithm used and intrasubject variability

Previous studies have investigated whether muscle synergies during upright cycling remain consistent across different mechanical conditions [16]. They reported a high degree of similarity among time-varying muscle synergies across mechanical conditions and across participants, suggesting that cycling may rely on a relatively robust modular control strategy despite changes in external task demands. In their work, Esmaeili and Maleki used a different algorithm for extracting time-varying muscle synergies, whereas the present work used a time-invariant synergy model. Time-varying synergies include both spatial and temporal information within each module, while time-invariant synergies separate fixed muscle weightings from their corresponding temporal activation coefficients. Therefore, similarity across conditions may reflect different aspects of motor coordination depending on the selected algorithm.

Muscle synergy extraction aims to decompose complex, high-dimensional EMG data into a reduced set of spatial muscle weightings and temporal activation coefficients. However, when the biomechanical task is constrained and redundant, the factorization algorithm may have more difficulty identifying clearly separated modules. Figure 5.1 provides an example of the variability in the EMG signals used as input for the synergy extraction. In this condition, some muscles, such as ADD, LGAS, and MGAS, show clear and repeated activation bursts with relatively large amplitudes. In contrast, RF, SMT, BFLH, and TFL show lower and more intermittent activity. This difference in signal amplitude and temporal consistency can influence the structure of the extracted synergies. Since NMF reconstructs the original EMG matrix as the product of non-negative muscle weights and temporal activation coefficients, the algorithm tends to highlight the patterns that contribute most to the global reconstruction of the dataset. Therefore, muscles with larger amplitude or higher variability may be represented more strongly within each synergy, while muscles with lower activation intensity may have a smaller contribution to the extracted synergy. In the present dataset, this may explain why some muscles were more clearly represented in some synergies, whereas the quadriceps and the hamstring groups was not always fully captured as a distinct module.

These findings are relevant to the present study because they support the idea that

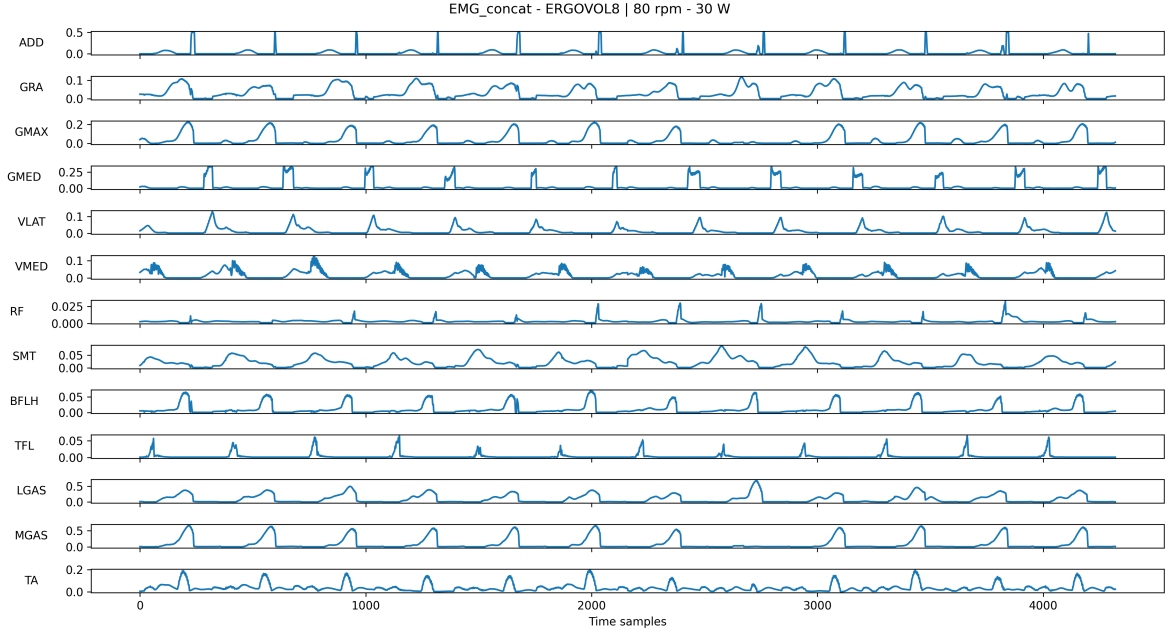


Fig 5.1. Example of concatenated EMG envelopes for one significant participant during cycling at 80 rpm and 30 W. Muscles such as MGAS and TA show repeated and high-amplitude activation, whereas the quadriceps group presents lower and more intermittent activation. This amplitude and temporal variability may influence the contribution of each muscle to the synergies extracted using NMF.

the central nervous system may preserve a common organization of muscle coordination during cycling, while modulating the intensity or timing of muscle activation according to the mechanical condition. In this framework, changes in cadence or workload do not necessarily require a completely different coordination strategy, but may instead be achieved by adapting or changing the recruitment of similar underlying motor modules. However, some methodological differences should be considered when comparing their results with those of the present study.

5.2 Effect of cadence at fixed power output

The comparison of muscle synergies at fixed power output suggests that cadence mainly affected the temporal recruitment of the synergies, rather than producing a complete reorganization of their spatial structure. Across 15, 30, and 45 W, several synergies preserved a comparable distribution of muscle weights, although significant differences were observed in specific muscles and conditions (Fig. 4.7, 4.8, 4.9). This indicates that the underlying muscle coordination modules were only partially modified by cadence, while their activation timing was more clearly adapted to the mechanical requirements of the task.

At a fixed power output, increasing cadence reduces the time available to complete each pedaling cycle and modifies the torque requirement at the crank. Since mechanical power is determined by the product of torque and angular velocity, a higher cadence can produce the same external power with a lower torque per cycle. This may explain why, in several synergies, the temporal activation profiles were shifted toward earlier phases of the cycle at higher cadences. The earlier recruitment observed at 80 rpm,

particularly in Synergies 1, 3, 4, and 5, depending on the power condition, may reflect the need to anticipate muscle activation in order to generate force within a shorter mechanical time window.

The changes observed in the activation profiles (H) were more evident than the changes in the W coefficients. This suggests that cadence modulation was achieved primarily through temporal adjustments of pre-existing coordination patterns, rather than through the recruitment of entirely different muscle groups. Such a result is consistent with the idea that cycling coordination can rely on relatively stable motor modules, whose timing and intensity are adapted according to task demands.

Nevertheless, some cadence-dependent changes were also present in the muscle weight matrix. At 15 W, for example, Synergy 4 showed a decreasing contribution of the quadriceps group from 40 to 80 rpm. Since higher cadence at the same power output requires lower torque per revolution, the reduced contribution of VLAT, VMED, and RF may reflect a lower need for strong knee extensor contribution during the propulsive phase. Similar significant differences in quadriceps and hamstring muscles were also observed at 30 and 45 W in Synergy 5, suggesting that cadence-related changes become more evident in specific modules when the mechanical demand increases.

The consistent contribution of GRA in Synergy 3 across the three power outputs is also relevant. Rather than being strongly affected by cadence, this muscle may contribute to a more stable component of the task, possibly related to hip adduction, limb stabilization, or control of the single-leg pedaling trajectory. This interpretation is particularly important because the experimental setup constrained the ankle and involved single-leg cycling. Under these conditions, some muscles may contribute not only to power generation but also to stabilization and control of the limb trajectory.

Overall, these results suggest that, at fixed power output, cadence affects muscle coordination through a combination of temporal and spatial adaptations. The most evident effect was the phase shift of the activation profiles, with earlier synergy recruitment at higher cadence. In contrast, the spatial structure of the synergies was only partially modified, with significant changes limited to specific muscles and synergies. Therefore, cadence appears to modulate the timing and relative contribution of existing coordination modules, rather than inducing a completely different muscle synergy structure.

5.3 Limitations

This study has limitations that should be considered when interpreting the results. The first limitation concerns the data collection process. The semi-recumbent seated configuration, reflected in the characteristics of the chair and the fixed ankle constraint, introduced movement artifacts in some muscles. These artifacts were particularly relevant in signals acquired in the zone near the orthotic braces. Muscles such as MGAS and TFL were the most influenced by this configuration. Although preprocessing was performed to reduce the impact of noise and artifacts, residual contamination may still have affected the quality of some EMG envelopes and, consequently, the structure of the extracted synergies.

Second, the muscle synergies were extracted from the CEINMS adjusted EMG signals rather than fully natural voluntary contractions. Although this is a new approach, it may not fully reproduce the complexity of physiological muscle coordination during unconstrained cycling. As a consequence, the extracted synergies should be interpreted

as representing the specific experimental condition.

5.4 Future work

This thesis is a preliminary investigation of muscle synergies during semi-recumbent cycling. It provides a first step toward their implementation in physiologically inspired FES control strategies.

Future investigations should compare synergies extracted from raw experimental EMG and CEINMS-adjusted EMG, in order to better understand how neuromusculoskeletal modelling influences the resulting synergy structure. In addition, different synergy extraction approaches, such as time-varying or space-by-time models, could be tested to determine whether they better capture the temporal organization of muscle activation during semi-recumbent cycling.

Future work should also evaluate muscle synergies during passive semi-recumbent cycling. In this condition, the ergometer would impose the cycling movement while participants remain relaxed, reducing the contribution of voluntary descending drive, simulating a spinal cord injury condition. This would allow investigation of whether rhythmic, phase-dependent muscle activation patterns can still emerge during passive movement, possibly reflecting the engagement of spinal locomotor circuits such as central pattern generators (CPGs). Muscle synergies extracted during passive cycling could then be compared with those obtained during active cycling in terms of muscle weight similarity, activation timing, and VAF. This analysis may help determine whether future FES controllers should only reproduce voluntary cycling synergies or also exploit residual spinal locomotor mechanisms in individuals with SCI.

Finally, the extracted synergy weights and activation profiles should be translated into FES stimulation patterns. In this context, muscle synergies could help define which muscles should be stimulated, when stimulation should occur within the crank cycle, and how stimulation intensity should be modulated. Integrating these patterns into a neuromusculoskeletal simulation framework and, later, into a closed-loop FES controller could support more efficient, fatigue-resistant, and individualized rehabilitation strategies for people with spinal cord injury.

Chapter 6

Conclusion

In conclusion, this thesis showed that semi-recumbent cycling is characterized by a relatively preserved modular organization of lower-limb muscle activation. Five muscle synergies were sufficient to reconstruct muscle activation patterns across participants and cycling conditions, suggesting that this task relies on repeatable low-dimensional motor modules. Although the spatial structure of the muscle weights was generally maintained, cadence mainly affected the temporal activation profiles, with some synergies showing earlier recruitment at higher cadences. This indicates that changes in cycling cadence are achieved primarily through modulation of the timing and relative contribution of existing coordination patterns, rather than through a complete reorganization of muscle synergies. These findings provide a first step toward the development of synergy-based FES closed-loop control strategies.

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