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BACHELOR'S DEGREE IN BIOMEDICAL ENGINEERING

BACHELOR THESIS

Sensory electrical stimulation during cycling for the rehabilitation of muscle coordination

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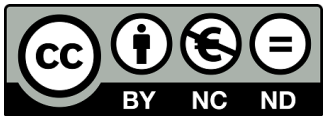

ABSTRACT

Gait (a person's way of walking) is highly complex and involves the musculoskeletal and nervous systems to achieve coordinated movement. Gait is key in daily life, and its impairment greatly reduces the quality of life, personal freedom and self-esteem of affected people.

Gait alterations arise from disruption of neuromuscular coordination mechanisms, either due to injury or degeneration (ictus, spinal cord injury...). The long term goal of the line of research that is started with the work presented in this Thesis is to build and validate a neurorehabilitation platform that uses biologically-inspired mechanisms in order to enhance muscle coordination of walking in neurologically injured patients.

As a first step towards this ambitious goal, this Bachelor's Thesis involved the design, development and technical validation of the neurorehabilitation platform that combines cycling with sensory electrical stimulation. Furthermore, a pilot study was conducted in order to assess the performance and usability of the neurorehabilitation platform. Preliminary results were encouraging, which gives the confidence toward the future applications of the platform.

The present Bachelor's Thesis has developed the necessary setups for performing a variety of experiments related with cycling and electrical stimulation. Future work should focus in further developing the experimental technique used for assessment of muscle coordination, and using the developed neurorehabilitation platform for conducting broader studies with more subjects, both healthy and neurologically injured, with a variety of modalities and diverse stimulation patterns. Eventually, and based on the acquired knowledge about stimulation and neurorehabilitation, a commercial neurorehabilitation device could be created and distributed in order to enable low-cost walking rehabilitation for neurologically injured patients.

Keywords: sensory electrical stimulation; afferent electrical stimulation; somatosensory electrical stimulation; pedaling; cycling; reciprocal inhibition; H-reflex; neuromuscular coordination; neurorehabilitation; stroke; SCI; spinal cord injury

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1. INTRODUCTION

1.1. Motivation

Gait (a person's way of walking) is highly complex and involves the musculoskeletal and nervous systems to achieve coordinated movement. Gait is key in daily life, and its disruption greatly reduces the quality of life, personal freedom and self-esteem of the affected people.

Gait alterations arise from the interruption of the healthy coordination mechanisms of the nervous system, either due to injury or degeneration (due to neurological pathologies like ictus or spinal cord injury...). The motivation of this project is synthesized by the following question:

Can muscle coordination of healthy gait be restored after neurological injury or disease?

1.2. Objectives

A new line of research is to be established with the long term goal of building and validating a neurorehabilitation platform that uses biologically-inspired mechanisms in order to enhance muscle coordination in gait and therefore improve quality of life, independence and well-being of neurologically injured patients. The main scientific hypothesis behind this line of research is that the combination of cycling therapy with sensory electrical stimulation enhances muscle coordination in patients with neurological injuries.

As a very first step towards this ambitious research goal, this Bachelor's Thesis is aimed at designing, developing and technically validating the neurorehabilitation platform and procedures, and enable future researchers to test the posed hypothesis. This main goal is divided in two objectives:

1. Designing and developing a neurorehabilitation platform composed of a robotic cycling ergometer and an electrical stimulator, closely integrated and synchronized.

2. Researching, designing and developing the experimental techniques and procedures to be used for the assessment of the efficacy of the therapy.

A pilot study with healthy subjects is to be conducted in order to verify the achievement of these objectives.

Future works in this line of research should focus on applying these methods on a large sample of healthy subjects, as well as on neurologically injured patients, in order to sci-

entifically prove the main hypothesis. Also, future researchers could use more complex stimulation patterns in order to assess their effect.

1.3. Thesis' structure

Section 1 provides the motivation to perform the work described in this Thesis, as well as the objectives, structure, regulatory and socioeconomic framework.

Section 2 covers the state of the art of the techniques and knowledge related with this Thesis. It allows the reader to acquire the necessary knowledge in order to fully understand the work described and the scientific rationale behind it.

Section 3 describes the used methods and materials. Details of the developed neurorehabilitation platform and experimental techniques are provided.

Section 4 provides the results of the pilot study and a detailed discussion of those results.

Section 5 presents the conclusions on the performance and results of the project.

Section 6 includes the budget and a timeline of the project.

At the end of the document the referenced literature and the appendices can be found.

1.4. Regulatory and Socioeconomic Framework

Regulatory Framework

A pilot study with healthy subjects has been carried out as part of this Bachelor Thesis. All subjects have given informed consent in conformity with the Declaration of Helsinki. The informed consent form can be found in Appendix A.

All used devices are certified as medical devices.

Codes and applications were developed in MATLAB and Simulink under Academic License, which allows academic research with non-commercial purposes.

Socioeconomic Framework

There are a variety of neurological conditions causing gait abnormalities, of which the most prevalent are ictus (also known as stroke) and spinal cord injury. These neuropathologies have a large socioeconomic impact due to expensive rehabilitation, chronic treatments, loss of productive workforce... The prevalence of these neurological conditions is high, at least 9 million new cases per year worldwide (mostly accounting for stroke). Moreover, due to the ageing of society, the incidence of these diseases is expected to increase in the coming years.

Neurological conditions include a large variety of symptoms. Nevertheless, gait alteration or loss is highly frequent and constitutes one of the major rehabilitation goals, as it provides the patient with independence, enables physical activity, increases self-esteem... To sum up, an effective gait neurorehabilitation plays a key role in both physical and psychological aspects of the patient. All these diseases also have a major impact in the lives of those around patients, both relatives and caregivers. Adequate rehabilitation accounts for optimal improvement and recovery of function, which results in increased well-being for the patient and those around. Therefore, gait neurorehabilitation plays a key role in recovering a normal life-style and integrating the patient in society and work.

The financial burden of neurological diseases should not be overlooked. Healthcare for these patients is expensive, as it involves a large amount of professionals, facilities and equipment, and have long recovery times. Moreover, most of these patients develop chronic disabilities, which suppose a large expenditure for healthcare providers.

Additionally, most neurologically injured patients are not able to work again, resulting in a direct income loss for themselves and their families, and a loss for the production capabilities of the society as a whole. In the social aspect, most neurological patients are not independent and therefore place a great pressure in the relatives and caregivers.

Therefore, it is a priority to both reduce the prevalence of these diseases, and improve the rehabilitation strategies in order to achieve optimal recovery or improvement of function. This Bachelor Thesis is part of the research effort that is being placed in the rehabilitation aspect, trying to enhance functional recovery and therefore promoting patient well-being, independence and self-esteem by promoting muscle coordination in gait.

2. STATE OF THE ART

2.1. Electromyography

Electromyography (EMG) is a widely used diagnostic technique to assess muscle activation. It measures the electric potentials generated by activation of muscles, and it is primarily used to diagnose neuromuscular pathologies. It is also used to analyze performance of elite athletes and in research in the fields of neurorehabilitation, biomechanics, neurophysiology, physical medicine...

EMG can be recorded in two different ways: with needle electrodes inserted in the muscle of interest, called intramuscular EMG (iEMG); or with surface electrodes placed on the skin on top of the investigated muscle, what is called surface EMG (sEMG). iEMG provides better signal quality, as well as more specificity, but it is an invasive method. On the other hand, sEMG is less specific (signal from other muscles may be recorded as well) and overall signal quality is decreased as there are several layers of tissue between the muscle and the electrodes, but it is more widespread as it is completely non-invasive.

EMG is recorded with bipolar electrodes (difference of electric potential between two nearby points), and it is in the range of hundreds of microvolts or millivolts. The reference electrode is placed at a point without electrical activity, i.e. a bony area like the ankle or the wrist. EMG signal is first analogically band-pass filtered and amplified (typically 1.000 V/V) before being digitized. Then, depending on the desired application, different digital processing may be applied. The frequency content of EMG lies mostly between 10 and 400 Hz. Although it is common that noise from the power lines (50Hz in Europe or 60Hz in USA) contaminates the signal, usage of digital Notch filters is discouraged as power line frequency lies inside the frequencies of interest of EMG. Therefore, it is recommended to make all efforts to reduce electromagnetic noise before acquisition [1].

In some cases, important features can be extracted directly from the raw signal. In other cases, the information of interest is contained in the evolution over time of the activation of the muscle. Such information is represented by the EMG envelope. In order to obtain it, several steps should be followed. First, EMG signal should be band-pass filtered (lower cutoff frequency 10-20 Hz, higher cutoff frequency 400-1.000 Hz). Then, EMG signal should be rectified, i.e. all points are made positive by applying absolute value, as the information of interest is whether the muscle is active, not whether the EMG data is positive or negative. EMG is acquired through bipolar electrodes, so an inverse positioning of the electrodes (which would also be correct) would yield an inverse signal for the same muscle activation. Thus, it is the magnitude of the signal what truly contains the information of activation, not is sign, and absolute value must be taken. Afterwards, rectified EMG is low-pass filtered (cutoff frequency 5-40Hz) in order to obtain the enve-

lope of the signal, that is, the time evolution of muscle activation. The full procedure is illustrated in Figure 2.1.

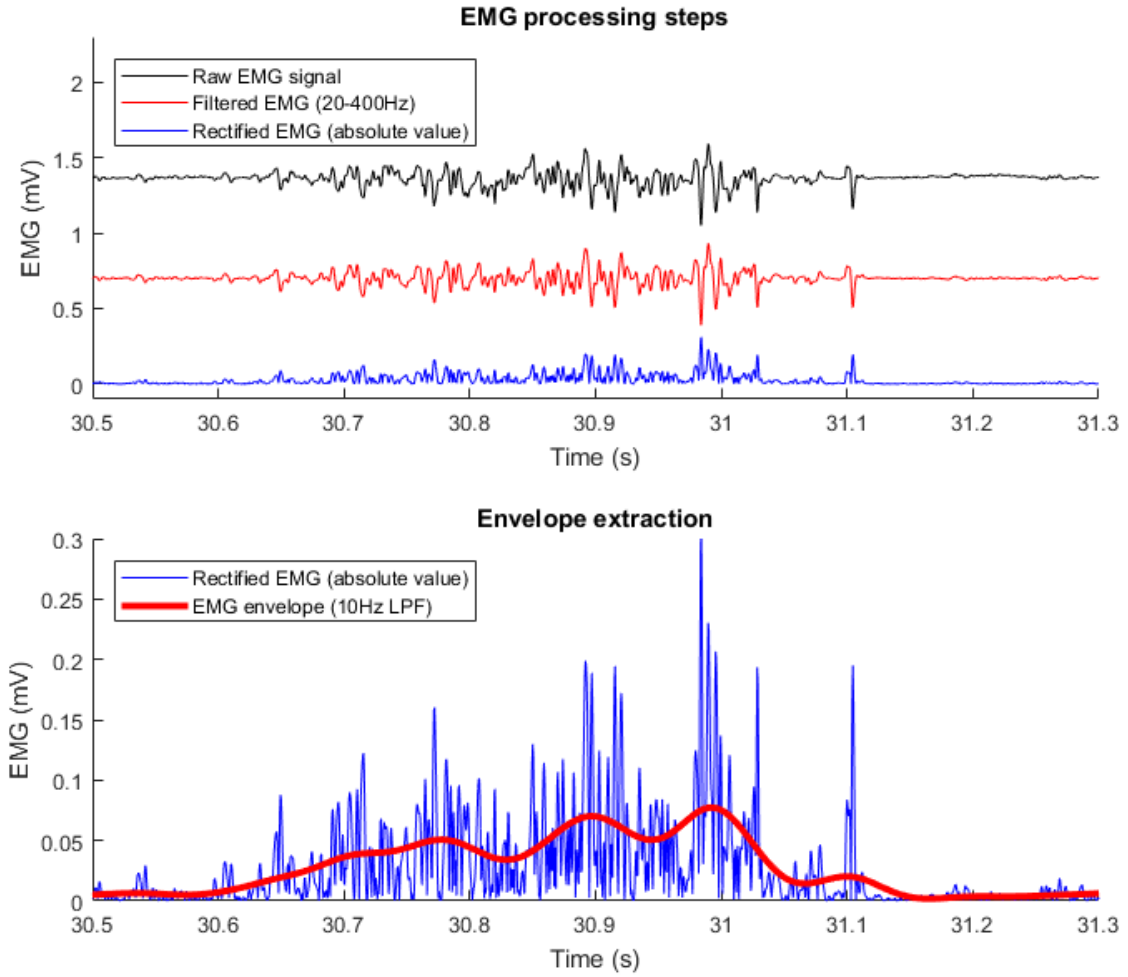


Figure 2.1. *A small section of EMG data (0.8 seconds) is used to illustrate the procedure of obtaining the time evolution of muscle activation (EMG envelope).*
(Upper figure) Raw EMG (black) , band-pass filtered EMG (red) and rectified EMG (blue). Signals have been vertically displaced for ease of visualization.
(Lower figure) Rectified EMG (blue) and EMG envelope (red).

EMG is a technique to measure muscle activity. It is widely used in diagnostics of neuromuscular pathologies as well as in research. Either intramuscular or surface electrodes may be used, the latter being more common as they are not invasive. EMG raw signal can be used for some applications. In other cases, the evolution of muscle activation over time can be extracted, and it is denominated EMG envelope.

2.2. Muscle coordination

Muscle coordination is conventionally assessed from a biomechanical point of view. Also, functional scores evaluating overall motor impairment are widely used in the clinic (Fugl-Meyer score, modified Ashworth scale, 10 Meters Walking Test, Functional Independence Measure...) [2], [3]. Nevertheless, these measures fail to capture the underlying neuromuscular mechanisms of muscle coordination. Those mechanisms are varied: brain cortex control, spinal cord circuits, sensory feedback... How these elements interact among them to generate complex movement patterns and coordination is yet not well understood.

Meanwhile the organization of the brain is highly complex and remains extremely difficult to understand, the spinal cord has lower complexity. Moreover, as the spinal cord participates in all physical behaviours, it forms "the common final pathway" [4] for motor behaviour. Therefore, it is possible to learn about spinal mechanisms by their direct effect on motor behaviour.

Spinal mechanisms have been thoroughly described in the literature. Main spinal mechanisms include presynaptic inhibition (which is involved in cortical control of movement), reciprocal inhibition (related to agonist-antagonist coordination), non-reciprocal inhibition (involving control of muscle stiffness and weight-bearing) and recurrent inhibition (related to stabilization and synchronization of neuron discharge) [5]. Of these mechanisms, this Bachelor's Thesis focuses on reciprocal inhibition for a variety of reasons to be detailed in next section.

2.2.1. Reciprocal inhibition

Reciprocal inhibition, also called reflexive antagonism, is the spinal mechanism by which the muscle of one side of a joint relaxes while the muscle at the other side is being contracted. The muscle being contracted is called the agonist, meanwhile the opposite muscle (which should relax in order to allow movement on the joint) is called the antagonist (see Figure 2.2). Reciprocal inhibition is the neural mechanism underlying coordination between agonist and antagonist muscles and groups of muscles, and therefore plays a major role in movement coordination and in the generation of cyclic movement patterns [6] .

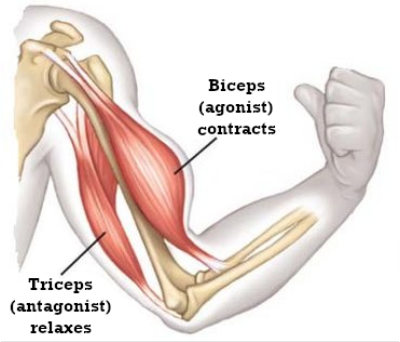


Figure 2.2. *When the biceps (agonists) contracts, the triceps (antagonist) relaxes to allow movement at the joint.*

It has been found that the neural mechanism underlying reciprocal inhibition are located

at the spinal cord. Neurons conveying information from the agonist muscle, called *Ia afferents*, excite *Ia inhibitory interneurons*, which in turn produce inhibition in the *alpha motor neurons* (which are the neurons that directly activate muscles) of the antagonist muscle, therefore relaxing it, or at least decreasing its level of contraction [7] (see Figure 2.3). Reciprocal inhibition has been thoroughly studied and described in the literature, it is present in most muscles of the body and it has the benefits of being relatively simple (involving just one single interneuron) and it is less influenced by supraspinal centers (brain cortex, cerebellum...) than other spinal mechanisms [5].

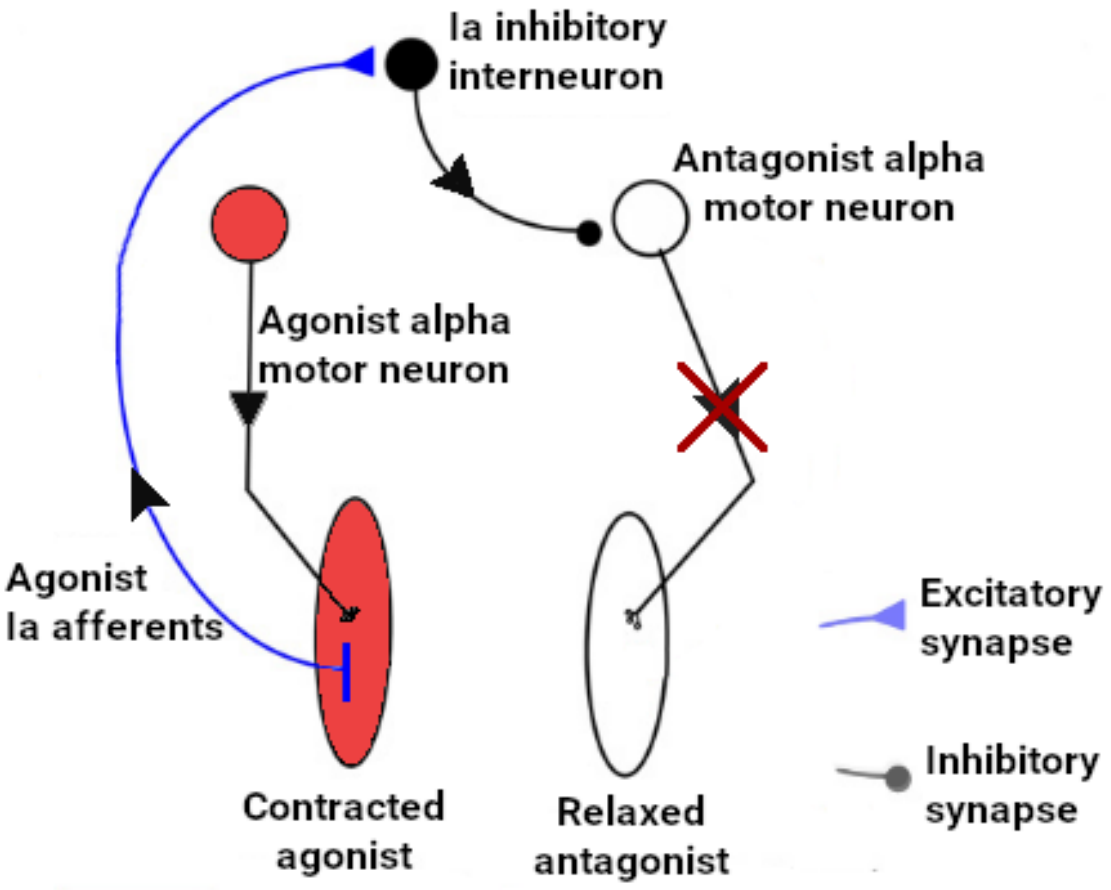


Figure 2.3. *Spinal circuit underlying reciprocal inhibition. Ia afferents from the contracted agonist muscle excite Ia inhibitory interneurons, which in turn inhibit antagonist alpha motor neurons. Thus, the overall effect is that when the agonist contracts, the antagonist relaxes. Modified from [5].*

Reciprocal inhibition plays a fundamental role in movement by regulation of agonist-antagonist coordination [8], [9]. Moreover, reciprocal inhibition has been found to be absent in most neuropathological subjects [10], [11]. Furthermore, patients that keep a higher degree of reciprocal inhibition (closer to that of healthy subjects) have shown better performance in gait ability and functional scores [11]. Studies have measured re-

ciprocal inhibition before and after an intervention to assess whether it does have an effect in spinal cord excitability [12]–[19]. Moreover, they have shown that an increase in reciprocal inhibition correlates with an improvement in overall motor coordination, assessed by functional tests [15], [16], [19], [20].

The Hoffmann reflex (H reflex) has been extensively used, especially in the soleus muscle, for the study of the neural mechanisms at the spinal level, including neural plasticity. The study of H reflex modulation conveys specific information about the plastic changes occurring at the spinal cord [5]. The H reflex was first described in 1910 by Paul Hoffmann, and it is the electrical analogue of the mechanical spinal stretch reflex (also known as knee jerk reflex). The H reflex is elicited by a low intensity electrical stimulation on a peripheral nerve, which may excitate both efferent and afferent nerve fibers. Efferent fibers (descending to the muscle) propagate their action potential until the neuromuscular junction is reached, causing a contraction of the muscle known as M wave. Simultaneously, Ia afferent fibers (ascending to the spinal cord) propagate the action potential towards the spinal cord until they reach the alpha motor neurons and excite them. The axons of the alpha motor neurons form the efferent part of the nerve, and cause a contraction when excited. Therefore, when the Ia afferents are excited by a low intensity electrical stimulus, the action potential travels up from the excitation site to the spinal cord, excites the alpha motor neurons, and travels down the nerve towards the muscle, causing a coordinated contraction known as H-wave (the H reflex) (see Figure 2.4(a)). Both the M and H waves can be recorded with an EMG amplifier with electrodes on the skin above the muscle of interest. As the efferent and afferent pathways have different lengths, M wave can be seen first in time, followed later by the H wave (see Figure 2.4(b)).

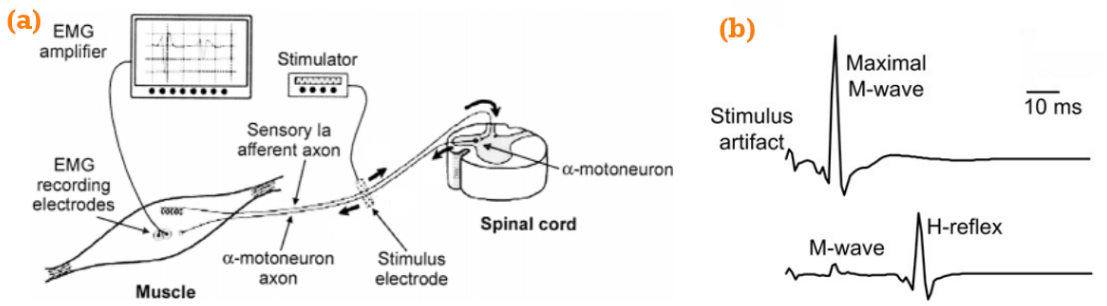


Figure 2.4. *(a) Neural circuit involved in the H reflex. Experimental setup in order to elicit and measure the H reflex. Figure adapted from Palmieri et al. [21].*
(b) EMG recordings containing an M wave and a H reflex. Figure adapted from Knikou et al. [5]

Although theoretically the H reflex can be elicited in any muscle which its peripheral nerve can be stimulated, in reality it is easier to elicit it in some muscles than in others. H reflex of the soleus muscle has been the most widely studied, as it is easily elicited and this muscle plays an important role in gait during the push-off phase. The soleus muscle is situated in the lower posterior part of the lower leg, below the calf muscles, and its

contraction causes plantarflexion (upward movement of the ankle). Therefore, given that soleus is involved in walking and pedaling, and the extensive literature background on soleus H reflex, its modulation by reciprocal inhibition will be used as a way to assess the effectiveness of the therapeutic intervention proposed in this project.

In order to test reciprocal inhibition of the H reflex, the nerve innervating the antagonist muscle has to be stimulated. In the case of soleus muscle, the antagonist muscle is the tibialis anterior. The nerve innervating the tibialis anterior (TA) is the deep branch of the common peroneal nerve (CPN). It is important to note here that the other branch of the CPN, the superficial branch, innervates the peroneal muscles, which are not antagonist of the soleus muscle (see Figure 2.5). Furthermore, it is known that peroneal activation may facilitate soleus H reflex. Therefore, if the branch innervating the peroneal muscles is accidentally activated, it may reduce or even destroy the phenomena of reciprocal inhibition. Special care during experimentation has to be taken to avoid peroneal muscle activation. The spinal circuit involved on reciprocal inhibition of the soleus muscle can be seen in Figure 2.6.

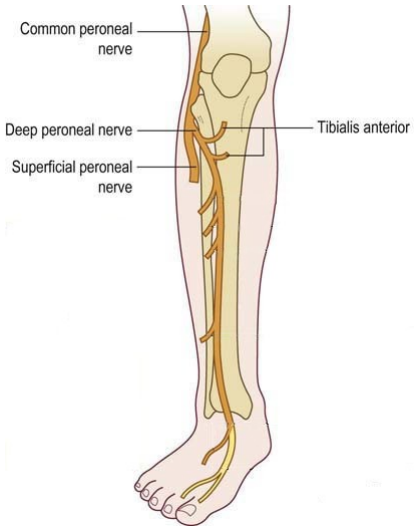


Figure 2.5. *The deep branch of the peroneal nerve innervates the Tibialis Anterior muscle, meanwhile the superficial branch innervates the peroneal muscles.*

In order to modulate the amplitude of the soleus H reflex through reciprocal inhibition, timing of the stimuli is crucial. First, the conditioning stimulus must be delivered exciting the Ia afferents of the CP nerve. The action potential propagates toward the spinal cord and excites the Ia inhibitory interneurons, which in turn inhibit alpha motor neurons of the soleus muscle. Then, after a time interval denominated Conditioning-Test interval (CTi), the test pulse is delivered to the PT nerve in order to elicit the H reflex. For reciprocal inhibition to modulate the soleus H reflex, inhibitory input must arrive just to the alpha motor neurons just before the excitatory input from soleus Ia afferents arrives. Previous research has shown that the optimal Conditioning-Test interval is between 2 and 4 ms [5], [7], [10], [12], [13].

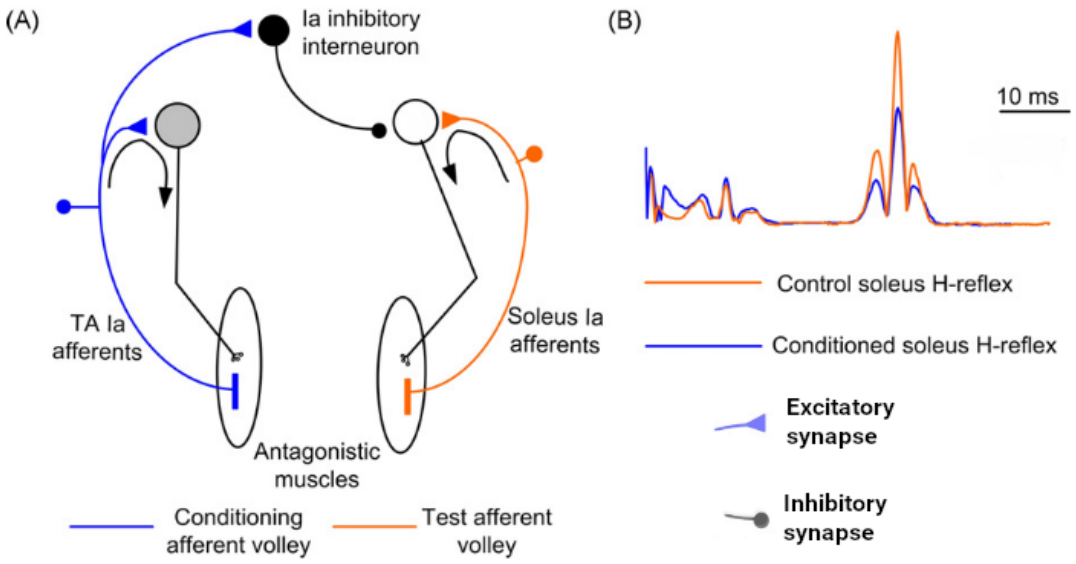


Figure 2.6. *Spinal mechanisms involved in soleus H reflex modulation by reciprocal inhibition. Adapted from Knikou et al. [5].*

Reciprocal inhibition is a spinal mechanism that coordinates agonist-antagonist activation. Moreover, it is fundamental for the generation of cyclic patterns. It is absent in most neurological patients, and a recovery of reciprocal inhibition is associated with an general motor function improvement, as measured by functional tests. Thus, this spinal mechanism will be used in this Bachelor's Thesis as the assessment tool of the effects of the proposed therapy in neuromuscular coordination. Reciprocal inhibition will be measured by its modulatory effects on soleus H reflex

2.2.2. Muscle synergies

Another possible perspective to address the complexity of human movement is through a descriptive model of muscle activations in complex coordinated movements. The human central nervous system is able to control a very large number of muscles, coordinating and modulating the contraction of all of them, in order to perform a wide variety of movements. Nevertheless, how that complex control is performed is yet not fully understood. Moreover, the human musculoskeletal system is redundant, i.e. there are multiple combinations of muscles that can yield the same movement. How the nervous system manages the redundancy and complexity of movement? A theory that has gained support and increasing evidence in the last fifteen years is that the central nervous system uses a set of motor modules, denominated muscle synergies, in order to reduce the complexity of movement control. Under the muscle synergies theory, control of movement is reduced

to modulation of a selection of muscle synergies, without need to control the individual muscles and the fibers that form them. Each synergy is a group of muscles that are activated together and work as a functional unit. Moreover, the same muscle may be included in different synergies (sets of muscles), and its response is a combination of different activation patterns. Each synergy also includes a weight for each muscle, that is, how much that muscle responds when the synergy is activated (see Figure 2.7) [22]. Muscle synergies would not only be co-activated muscles, but are hypothesized to have a structural neurological basis (of which spinal modulatory mechanisms would be part of) and so they are a feasible aim for assessment and neurorehabilitation. Synergies have already been found and studied in grasping, arm reaching, postural balance, walking, cycling...

Muscles synergies are yet not fully validated as a tool for diagnosing the degree of impairment and its underlying causes. Nevertheless, some studies have already pointed to the correlation between dysfunction (as measured by standard functional scales) and the degree of difference between the patient synergies and the healthy reference synergies [23], [24]. Moreover, in the last few years some studies have tried to train muscles synergies as a way to relearn movement [25], [26]–[31] . The results are encouraging, as the neuropathological subjects were able to learn new synergies or relearn the healthy ones, as the same time that they significantly improved their score in functional scales, and they improved more than matched control groups performing standard rehabilitation. However, due to the novelty of this approach, there is still a need for more studies to validate this approach.

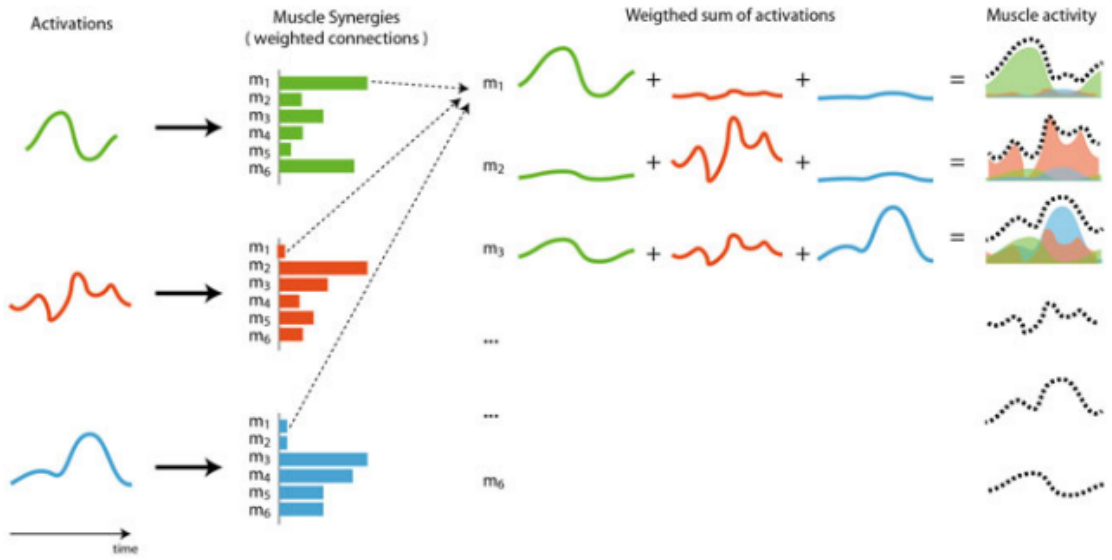


Figure 2.7. *Muscle activation patterns would results from a set of a few activation patterns. Each muscle has a weight for each activation pattern, and its overall activation results from the weighted sum of activation patterns. Adapted from Torricelli et al. [22].*

Muscle synergies are extracted from the envelope of the EMG signal, which describes the degree of activation of a muscle. EMG has to be recorded simultaneously from a representative set of muscles. Also, several repetitions of the movement should be acquired in or-

der to average the results. Then, a mathematical decomposition is performed on the data in order to find the activation patterns and the synergies (weights) associated, which should explain most of the data with a much lower number of synergies than muscles implied in the movement. Most often non-negative matrix factorization (NNMF) is used, which is conceptually analogous to principal component analysis (PCA), but assumes that data and obtained components (synergies and activation patterns) are all non-negative, which is a sensible assumption when considering muscle activation, which cannot be negative. In any case, other decomposition algorithms such as principal component analysis, independent component analysis, or factor analysis, which have also been applied to muscle synergies, yield very similar results to NNMF [32].

The hypothesis of muscle synergies states that the central nervous system controls the complexity of human movement through a set of motor modules (muscle synergies), which are sets of muscles which are activated together. Moreover, it is believed that muscle synergies have a structural neurological basis (which would include spinal modulatory mechanisms) and so it is a feasible neurorehabilitation aim.

2.3. Muscle coordination in neurological patients

Stroke (also known as ictus) is a neurological injury due to an interruption of blood flow in an area of the brain, causing oxygen starvation and therefore producing brain damage. Stroke may cause permanent damage (partial paralysis i.e. hemiplegia, speech difficulties, memory loss...) and even be fatal if not treated. Stroke prevalence is very high: 15 million people worldwide suffer a stroke each year, and 5.8 million die from it [33]. Stroke is the third leading cause of death in the world, and the leading cause of long-term disability [34]. Moreover, due to the ageing of society, stroke prevalence is expected to grow in 30% in upcoming years [35]. Almost 80% of stroke survivors face motor deficits, mostly hemiparesis (weakness in the affected side) or spastic hemiplegia (continuous contraction of the muscles of one side of the body, paralyzing it), which largely affect gait [36].

Spinal cord injury (SCI) is due to trauma in the vertebral column, and results in a loss of motor, sensory and autonomic functions (either complete or partial) below the level of the injury. When the loss is complete, the resulting condition is paraplegia (when only affecting the lower limbs) or tetraplegia (when affecting lower and upper limbs) [37]. It affects between 250.000 and 500.000 people a year, specially young males due to road accidents [38]. However, not all spinal cord lesions cause a complete sectioning of the medulla and therefore residual function remains, in what is called incomplete spinal cord injury (iSCI). It accounts for 40% of all SCI, what means between 100.000 and 200.000 cases per year [39]. SCI and iSCI result in damaged connection between the brain and the muscles, causing either paralysis, weakness or stiffness, and a lack of coordination. Therefore, gait is impeded or greatly difficulted. Nevertheless, modern neurorehabilitation techniques have shown the possibility of regaining gait [40], especially when involving neural plasticity at the spinal cord level, that is, "rewiring" the neural connections. These approaches are more suitable for subjects with iSCI, as they have residual motor and nervous function.

Neurologically injured patients display severe motor deficits due to lack of coordination. Such lack of coordination is also reflected in neuromuscular measures of coordination such as reciprocal inhibition or muscle synergies:

- Reciprocal inhibition is absent in most neuropathological subjects [10], and patients that keep a higher degree of reciprocal inhibition (closer to that of healthy subjects) have shown better performance in gait ability and functional scores [11].
- Neurologically injured patients show a lower number of muscle synergies than healthy subjects, what reflex lack of independent control and co-contraction of naturally antagonistic muscles, what also reflects lack of reciprocal inhibition.

Stroke and spinal cord injury (SCI) are neurological injuries that greatly disrupt motor coordination, causing in most cases a loss of walking ability. These conditions are highly prevalent (specially stroke), with almost 10 million new cases per year worldwide. Moreover, incidence of neurological injuries will increase due to the ageing of society. Neurologically injured patients display a lack of neuromuscular coordination which can be assessed through reciprocal inhition or muscle synergies.

2.4. Rehabilitation of muscle coordination

2.4.1. Neural plasticity in the spinal cord

Traditionally, the adult nervous system was thought to be fixed but for a few specific sites where learning occurred. However, findings in the last decades have shown that changes happen all around the central nervous system (brain and spinal cord) continuously through adult life, in a mechanism called neural plasticity. Plasticity can arise from formation of new connections between neurons (synapses), or potentiation or depression of existing synapses [41], following the Hebbian principle of "neurons that fire together, wire together". This is possible as the nature of neural connections is plastic itself, what enables the formation of new synapses and the modification of existing ones.

Plasticity enables the neural system to continually adapt to new situations and needs. After a neurological injury, the nervous system reorganizes in order to cope with the new situation, as many neural pathways and structures have been disrupted. During the acute (first weeks) and subacute (first months) phases after injury, spontaneous recovery of motor function happens to some extent due to neural plasticity. Thus, if additional therapy is given to patients in these stages, better outcomes have been shown.

A large amount of plasticity is induce by physical activity, both at the spinal and brain levels, and sensory information received through Ia afferents during movement has proven fundamental for this process to happen. Thus, activity-dependent plasticity offers the possibility of re-learning movement coordination for neurologically injured patients, through repetitive movements associated with augmented sensory feedback [42]. This approach can be especially useful for acute or subacute patients. Nonetheless, plastic changes also occur in chronic patients (long time after injury), so enhancement of their neural plasticity by means of physical activity is also possible.

The H reflex can be used to observe plastic changes taking place in the spinal cord. These changes due to neural plasticity may occur as the consequence of direct training (aiming to change the reflex response) or indirect training (aiming to modify something else that the reflex, but modifying it as a side effect) [43]. Experiments in humans use spinal reflexes (such as the H reflex) to observe plastic changes in the spinal cord. Therefore, the study of these reflexes proves as a very valuable tool to study neural plasticity at the spinal cord and its related implications in motor function and dysfunction in neurological patients, as well as to assess training-induced plasticity in healthy and pathological subjects [5].

Neural plasticity is the process by which neural connections are modified so that the neural system can learn and adapt to new situations. A large amount of plasticity is induced by physical activity, and sensory information during movement has proven fundamental for neural plasticity to occur. Activity-dependent plasticity offers the possibility of re-learning movement coordination for neurologically injured patients, through repetitive movements associated with augmented sensory feedback.

2.4.2. Cycling-assisted gait training

Traditionally, neurorehabilitation of locomotion has been performed through manually assisted treadmill training. The patient needs a harness for body-weight support while two or three physical therapists mobilize the legs. This is very labour intensive from up to three highly qualified professionals, what yields low duration of sessions and high cost in human resources.

New locomotion neurorehabilitation techniques have arised over the last twenty years using robotic assistance. Robot-assisted gait training (RAGT) integrates body weight support and robotic mobilization of the legs, and the physical therapist just needs to oversee the therapy, being much less labour intensive and allowing for longer sessions. Moreover, the gait induced is more physiological and reproducible, and measuring muscle activations makes it quantifiable and generates feedback for the patients of their own performance, therefore increasing efficiency. Robot-assisted treadmill training has been shown to have a positive effect, superior to that produced by conventional gait training [44]–[49]. However, the used robotic devices are extremely expensive. The most widespread of these devices is Lokomat (Hocoma AG, Volketswil, Switzerland), which is priced in 250.000 USD. Therefore, only big facilities can afford this equipment, and the amount of patients able to benefit from it will be low, as well as the duration of the rehabilitation sessions will be short and their frequency, low.

Cycling-assisted locomotion therapy has arisen as a suitable alternative to overcome these problems. Locomotion (i.e. walking) and cycling are both cyclic movements of the lower body which share many biomechanical features. Moreover, research has shown that underlying neuromuscular mechanisms are highly similar [50]. Cycling implicates less degrees of freedom and does not need of trunk stabilization, so it is an exercise easier to perform for highly impaired patients. Therefore, in the last years several authors have shown the effectiveness of neurorehabilitation of locomotion through cycling movement using a cycle ergometer [51]–[61]. Patients showed improvement in walking-related functional scores (higher walking speed, longer autonomous walking distances, time to stand up from a chair, etc), and in some studies it was shown that cycling-assisted neurorehabilitation yielded better results than conventional rehabilitation [60], [61]. Also, cycling

exercise has been shown to induce neural plasticity at the spinal cord, producing modulatory effects on spinal reflexes [52], [62]. To sum up, advantages of cycling-assisted gait training are:

- Additional trunk stabilization is not necessary, as patients can perform the therapy seated on their wheelchairs or on conventional seats.

- Patients without enough coordination or strength to perform the pedaling movement can use passive cycling, that is, the cycling movement is performed by the cycle ergometer. Thus, this therapy is suitable for acute patients, which have already been shown to benefit the most from robot-assisted therapy.

- Many researchers have incorporated functional electrical stimulation (FES) to the cycling therapy in order to activate muscles during the cycling therapy and provide augmented sensory feedback to the patient, yielding significantly better outcomes that subjects using just cycling.

- Much less human assistance is needed as the mobilization of the patient is carried out by the device itself, diminishing human resources cost and workload on caregivers.

- Cycling-assisted gait training incorporates the advantages of robot-assisted gait neurorehabilitation (not labour intensive, longer sessions, quantifiable outcomes, repetitive movement) while using much less expensive equipment, as cost of the required rehabilitation devices is much lower. A MOTOmed Viva2 robotic cycle ergometer (as the one used in this Bachelor's Thesis) is valued in 4.600 USD. This pricing makes it very affordable for most rehabilitation clinics and by many individual patients, allowing for home-based rehabilitation, what implies more frequent use and for longer periods of time, therefore enhancing the rehabilitation outcome.

The major drawback of cycling-assisted gait neurorehabilitation is that it does not train locomotion directly. Nevertheless, the aforementioned studies have already shown improvements in locomotion following cycling training in neurologically injured patients, providing supporting evidence that locomotion and cycling share common neural mechanisms and therefore walking can be rehabilitated through cycling exercise. A summary of the discussed locomotion neurorehabilitation techniques can be seen in Table 2.1.

	Human assistance required	Need for trunk stabilization	Movement trained	Cost
Manual treadmill training	Very intensive. Highly specialized therapists. Limited duration of the rehabilitation sessions.	Yes. Body weight support	Locomotion	High cost in human resources.
Robot-assisted treadmill training	Medium. Highly specialized therapist must help in the proper setup of the system.	Yes. Body weight support.	Locomotion	Lokomat is valued in 250.000 USD.
Cycling-assisted gait training	Low. Caregivers with small training. Can be used at home.	No. Patients are seated in their wheelchair or on a conventional seat.	Cycling	MOTOmed Viva2 is valued in 4.600 USD

Table 2.1. COMPARISON OF LOCOMOTION NEUROREHABILITATION TECHNIQUES

Rehabilitating locomotion through cycling offers a promising perspective as the underlying biomechanical and neurological mechanisms are very similar, the associated costs in terms of equipment and human resources are much lower, and it can be applied in earlier stages after trauma (either stroke or SCI), at which chances of improvement are higher.

2.4.3. Electrical stimulation techniques for neurorehabilitation

Functional electrical stimulation

Functional electrical stimulation (FES) uses electrical stimulation in order to achieve functional contraction of muscles. The natural mechanism implies that electrical signals travel down from the brain to the spinal cord and then through the nerves to the muscles. Neurological injuries cause a disturbance at either brain control, spinal communication or coordination, therefore yielding no signal reaching the muscles, or uncoordinated commands that are not able to induce effective, coordinated movement. Nevertheless, if external electric stimulation is applied to the muscles, they are still able to excite and contract. When certain patterns are applied, functional muscle contraction can be achieved. Therefore, muscles can be externally contracted and movement can be controlled, without the need for conscious coordinated control from the subject. This is very useful when the body itself is not able to properly command muscles to move due to a neurological condition. Surface electrodes are usually preferred in rehabilitation as they are non-invasive, although providing less selective stimulation than intramuscular electrodes. Also, biphasic stimulation is conventionally applied in order to balance the charge being injected in the tissue. FES provides augmented sensory feedback as well as causing muscle contraction (as both sensory and effector parts of the nerve are stimulated), therefore providing augmented proprioception (due to muscle contraction) and sensory feedback. FES combined

with cycling exercise has shown possitive rehabilitation outcomes, even outperforming control groups which performed only cycling-assisted training [53]–[58]. However, contractions caused by FES are not fully equivalent to natural ones and long lasting fatigue is rapidly induced [58], [63], constituting the main limitation of FES.

Sensory electrical stimulation

Ia afferent fibers of peripheral nerves (that is, those corresponding to sensory feedback) need lower current intensity in order to become excited than motor fibers, as they present different axon size [5]. Therefore, it is possible to deliver sensory-only stimulation by using low-intensity electrical stimulation applied on nerves with surface electrodes. In order to do so, stimulation intensities should be below the minimum intensity required to cause muscle contraction (denominated motor threshold). This technique in which only sensory augmented feedback is given is denominated sensory electrical stimulation. Sensory electrical stimulation has arisen as a technique which may potentially overcome the fatigue-related problems of FES while maintaining its benefits. Sensory electrical stimulation provides stimulation that augments sensory feedback, but does not produce functional contraction, thus avoiding fatigue. However, proprioceptive feedback (due to muscle contraction) is not augmented, so whether sensory electrical stimulation can achieve similar effects to those of FES is still under research. Sensory electrical stimulation is a very recent field of research, with low number of publications and mostly in the last ten years. Therefore, there is still not enough evidence for or against using this approach for motor neurorehabilitation purposes. Some research articles found some evidence of improvement between sensory electrical stimulation and control groups [64]–[71] while others reported that no significant difference was found [72] [73]. Also, reviews on the topic stress the lack of high-quality randomized trials in order to confirm the effectiveness (or lack of it) of sensory electrical stimulation. Therefore, the question of whether sensory electrical stimulation can be used for motor neurorehabilitation is still open, and specially its effect in muscle coordination.

Finally, a selection of previous works on FES and sensory electrical stimulation applied to spinal neuroplasticity and reflex modulation will be discussed, as they have been the bases of this Bachelor's Thesis.

Piazza et al. [74] proposed to apply sensory electical stimulation at the sole of the feet, in a reflex modulation technique denominated "plantar-conditioned H reflex". The conditioning-test interval is longer that was is used for reciprocal inhibition (25-100ms vs 1-6ms), and therefore the spinal mechanisms involved in this conditioning are different. Healthy subjects, as well as patients with different degrees of spinal cord injury (SCI), were assessed. Before the intervention, healthy subjects presented inhibition of H reflex due to plantar conditioning, meanwhile iSCI subjects presented no modulation of H reflex. Plantar-conditioned H reflex was assessed before and after ten minutes of cycling combined with plantar afferent electrical stimulus. No effect was seen in healthy subjects,

meanwhile large excitability increase was seen in iSCI subjects with moderate impairment, restoring their average H reflex to that in healthy subjects. No effect was seen for severe impairment iSCI or complete SCI patients. Therefore, the conclusion of interest for this Bachelor Thesis is that afferent (sensory) stimulation combined with cycling seems to have an effect in spinal excitability, although only for iSCI patients, not for healthy subjects. Nevertheless, the stimulation provided is different to what is proposed in the present work, so results may or may not be comparable.

Obata et al. [13] studied the effects of electrical nerve stimulation of the tibialis anterior muscle while performing passive stepping on a Lokomat were assessed. Stimulation was given at the minimum level to cause muscle contraction. Ten healthy subjects underwent 30 minutes of passive treadmill training meanwhile receiving stimulus in the common peroneal nerve (which innervates tibialis muscle). Two different interventions were performed: stimulation during activation of the tibialis muscle (in-phase), or activation when tibialis is not active (out-of-phase). Reciprocal inhibition of soleus H reflex was assessed before and after each intervention. Subjects displayed an increase of reciprocal inhibition when stimuli were delivered in-phase with stepping, meanwhile reciprocal inhibition slightly decreased when stimuli were delivered out-of-phase. Moreover, in a previous study it was shown that no changes arised from either passive walking or electrical stimulation alone. Therefore, spinal reflex modulation is produced by passive stepping combined with electrical nerve stimulation. The effects depend also on its synchronization with the natural activation of the muscle. This study also investigated the evolution of the effects over time. The aforementioned changes were maximal five minutes after intervention, decreased at 15 minutes and disappeared after thirty minutes, returning to baseline. As expected, effects from a single session are acute and dissipate over a short time. Nevertheless, it is expected that long-term training could cause permanent effects.

Yamaguchi et al. [12] studied the effects of active pedaling, electrical stimulation and active pedaling combined with electrical stimulation. Twelve healthy subjects participated in the study. In each session, the subject underwent seven minutes of one of the strategies (pedaling, electrical stimulation, or both). Electrical stimulation was given at the common peroneal nerve (innervating tibialis anterior muscle) at the minimum level at which it caused muscle contraction. Reciprocal inhibition of soleus H reflex was measured before and after each intervention. Reciprocal inhibition slightly increased after either pedaling or electrical stimulation alone, and significantly increased after the combined intervention.

FES-cycling already has a strong research body supporting its efficacy in inducing neural plasticity at the spinal cord. However, FES induces high muscular fatigue, a limitation that has not been solved yet. Sensory electrical stimulation does overcome this limitation, but whether it does enhance neural plasticity is still uncertain. Selected works related to the topic yield the following conclusions:

- ***Sensory electrical stimulation combined with cycling seems to have an effect on spinal excitability for iSCI patients.***
- ***Electrical nerve stimulation combined with passive walking produces significant effects on reciprocal inhibition of soleus H reflex. Effects of the intervention changed completely from delivering stimulation in phase (in which RI increased, denoting improvement in neuromuscular coordination) with respect to delivering stimulation out of phase (in which RI decreased, what could be considered a negative effect). Therefore, timing of the stimulation and synchronization with the natural activation of the muscle is fundamental.***
- ***Pedaling plus electrical stimulation was able to induce spinal plasticity and an increase in reciprocal inhibition in healthy subjects.***

3. MATERIALS AND METHODS

The Bachelor's Thesis has two main objectives:

1. Designing and developing a neurorehabilitation platform composed of a robotic cycling ergometer and an electrical stimulator, closely integrated and synchronized.

2. Researching, designing and developing the experimental techniques and procedures to be used for the assessment of the efficacy of the therapy.

The materials, methods and procedures developed for achieving each of these objectives are detailed in this section.

3.1. Neurorehabilitation platform design and development

A neurorehabilitation platform based in cycling and sensory electrical stimulation has been designed and developed by the author of this Bachelor's Thesis. The diagram can be seen in Figure 3.1.

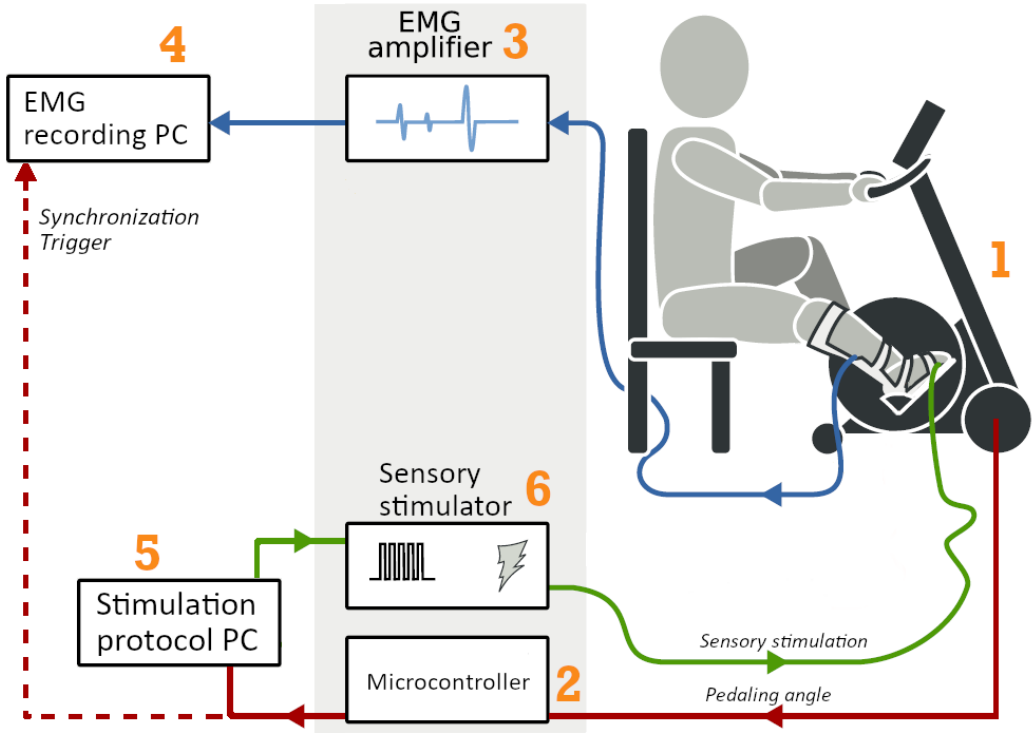


Figure 3.1. *Diagram of the designed and developed neurorehabilitation platform. Some elements of this diagram have been adapted from Piazza et al.[74].*

The goal of the developed platform is to provide the subject with sensory electrical stimulation in order to promote muscle coordination by means of neural plasticity at the spinal level. Instead of delivering continuous stimulation throughout the cycle, the designed neurorehabilitation platform enables to stimulate at specific phases of the pedaling cycle. Therefore, it enables to use customized bio-inspired stimulation patterns based on the natural muscular activation patterns. The platform comprises the following elements:

1. *Rehabilitation cycle ergometer*
2. *Microcontroller*
3. *EMG amplifier*
4. *EMG recording PC*
5. *Stimulation protocol PC*
6. *Sensory stimulation device*

Each of them will be described in detail for reproducibility and future reference. Also, some design choices and elements of interest will be discussed:

1. Rehabilitation cycle ergometer

The rehabilitation device used is the cycle ergometer MOTOmed Viva2 (RECK-Technik GmbH, Betzenweiler, Germany), a commercial product that enables three modalities:

- Active training: the user performs the movement against a configurable pedaling resistance.
- Passive training: the device internal engine performs the movement.
- Motor-supported training: as an intermediate of the previous modes, the device internal servomotor assists the patient to perform the pedaling movement.

The choice of using cycling for neurorehabilitation of gait is supported by the following reasons:

- First, pedaling can be a very useful tool for neurorehabilitation of gait. Pedaling is a cyclic movement that uses the same neuromuscular mechanisms, but without the weight-loading and balance implications of gait, therefore being much easier to perform for motor impaired patients.
- Second, it can be used in passive/motor-supported mode by patients with very low residual strength or very low muscular coordination. These modes could be used by patients in the post-acute phase, i.e. soon after the stroke or injury, when plastic changes are more likely to occur.

- Third, it is a low-cost clinical neurorehabilitation device, when compared to robot-assisted gait training, and it can even be afforded for home-based, personal use. Moreover, patients can be seated in a conventional chair or wheelchair, and no weight-bearing devices are needed. Therefore, when compared with other gait neurorehabilitation devices like Lokomat, this device can be used by a broader segment of the target population of patients, and it is affordable both for clinical and personal use.

This neurorehabilitation device has already been used in other research projects in the Neural Rehabilitation Group [74], [75] and in works in other research centers [51], [54], [55], [57], [58], [76].

Real time pedaling angle reading

A magnetic encoder (AS5048, AMS AG, Premstaetten, Austria) is attached to the rotation axis of the MOTOMed plus a microprocessor (STM32F302K8, STMicroprocessors, Geneva, Switzerland) that reads the encoder and sends the pedaling angle value via USB, UART and CAN communication protocols. This implementation was done previously to the start of this work. Nevertheless, UART communication does not allow to change the sampling frequency at which the microprocessor read the encoder (while it is possible through USB and CAN). Also, when plotting angles received simultaneously through USB and CAN, the former has a delay greater than one second with respect to the latter. When USB is tested alone, the same large delay is present. It is assumed to be due to wrong implementation at the microprocessor level.

Angle data read through CAN communication visually appeared to be real time. Nevertheless, additional tests were performed in order to obtain a precise measure of the delay existing in the data, as real time reading of the pedaling angle is fundamental in order to deliver stimulation to the subject at precise time intervals. In order to ensure real time reading, one part of a magnetic switch was placed in the mobile part of the cycle ergometer while the other was placed at the static part, in a way that the magnetic switch closed when a certain "threshold" pedaling angle was reached. Thus, the magnetic switch enabled for analogical, fully real time detection of the threshold angle being reached, using hardware interruptions on a Arduino UNO microprocessor (Arduino LLC, Italy). The threshold angle was carefully measured by very slowly turning the wheel and checking the angle at which the magnetic switch closed. Then, the magnetic switch was used as a hardware interruption while pedaling angle data was read via CAN communication. By measuring the time delay between the magnetic switch interruption and the threshold angle being read, it was possible to know that the delay between the real angle and what was received in the Arduino UNO via CAN communication was always below five milliseconds, either for slow, medium and fast pedaling speeds (see Figure 3.2). Therefore, for our desired application, reading the pedaling angle via CAN communication can be considered fully real time.

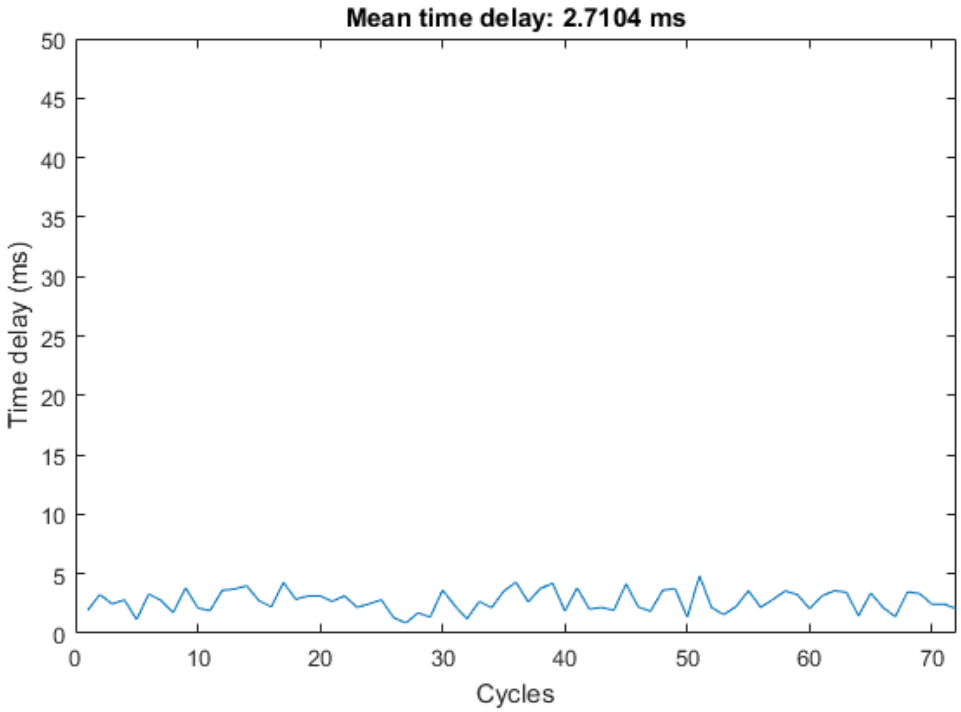


Figure 3.2. *Communication delay for more than 70 pedaling cycles, that were performed at different pedaling speeds. Delay in between the threshold angle being reached and the threshold angle being received through CAN communication is in all cases below 5ms.*

2. Microcontroller

An Arduino UNO microcontroller (Arduino LLC, Italy) performs two different functions in the neurorehabilitation setup:

- Receives the pedaling angle reading via CAN communication, and sends it to the stimulation protocol PC.
- Sends simultaneous synchronization signals to the EMG amplifier and the PC.

In order to enable the Arduino UNO to receive CAN messages, a CAN shield (SparkFun Electronics, Boulder, CO, USA) is installed on the Arduino, taking advantage of the capability of modular extensions of this microcontroller. Also, a Protoshield UNO (Microbot, Borgo Carso, Italy) is used to solder a BNC output to one of Arduino UNO digital output pins, in order to send the synchronization signal to the EMG amplifier.

3. EMG amplifier

The EMG amplifier used in this setup is Trentadue (see Figure 3.3) (OT Bioelettronica, Milano, Italy). This device is able to amplify and digitize up to 32 EMG channels, and record activity from up to 16 individual muscles. It also enables auxiliary inputs for

additional purposes, like the BNC synchronization input from the microcontroller used in this application.

At earlier stages of the project, the EMG amplifier Quattrocento (OTBioelettronica, Milano, Italy) was used. This device enabled the acquisition of 400 EMG channels plus auxiliary inputs. Also, the quality of the signal was slightly improved with respect to Trentadue. Nevertheless, it was decided to substitute it by Trentadue as the latter is small (5.9 x 9.5 x 2 cm), lightweight (110g) and enables wireless communication with the computer, therefore simplifying the setup by reducing the amount of wires and bulky devices. Moreover, the Quattrocento acquired four hundred EMG channels when only a few were needed, and in some occasions the EMG recording PC would get overloaded and data would be lost.

The EMG data is sent over a Wi-Fi network created by Trentadue, and it can be received in a computer either using the manufacturer's software OTBioLab or using the MATLAB scripts provided by the manufacturer for custom applications.

Figure 3.3. *Trentadue EMG amplifier (OT Bioelettronica, Milano, Italy). Image extracted from Trentadue User Manual.*

EMG signal is acquired using disposable Ag/AgCl surface electrodes (22x35mm) (Vermed, VT USA) on top of the investigated muscles. Although the quality of EMG signal is higher when acquired with intramuscular electrodes, they are highly invasive. Therefore, surface electrodes are used, placed on the skin directly over the muscles of interest. In order to minimize skin impedance, it is previously cleaned with alcohol.

4. EMG recording PC

An Acer Aspire E17 computer (Acer Inc, New Taipei, Taiwan) is used in order to acquire and store the signal from the EMG amplifier. A custom Graphical User Interface (GUI) has been designed and developed by the author of this Thesis using AppDesigner in MATLAB R2017b (MathWorks, Natick, MA, USA). The GUI performs the connection and reception of the EMG data using the MATLAB scripts provided by the manufacturer of the EMG amplifier. The GUI displays the electromyographic data in real time, allowing for detection of noise, artifacts or wrong connections in the different EMG channels. At the end of the recording, data is stored in a MATLAB data file.

5. Stimulation protocol PC

A Lenovo ThinkPad L470 computer (Lenovo Group, Hong Kong, China) runs Simulink release 9.2 (running on MATLAB R2018b). A Simulink model (see Figure 3.4) has been developed by the author of this Thesis in order to receive the pedaling angle data from Arduino UNO, using Instrument Control Toolbox of Simulink, and send the corresponding stimulation commands to the stimulation device, using the custom library provided by the stimulator manufacturer.

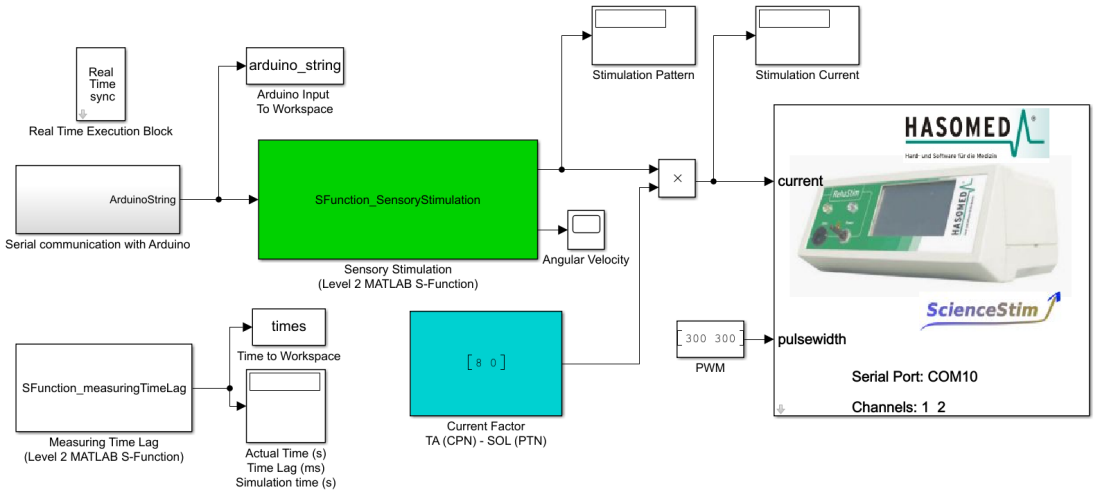


Figure 3.4. *Simulink model developed for real time sensory stimulation based on the pedaling angle and the designed stimulation strategy.*

The Simulink model receives, previous to execution, the designed stimulation strategy as a function of the pedaling cycle. The stimulation strategy, based in natural muscle activation, has to be enclosed as two MATLAB data files (.mat) named "*H.mat*" (for the activation vectors) and "*W.mat*" (containing the weight coefficients for each muscle, i.e. the muscle synergies).

The core of the Simulink model is *SFunction_SensoryStimulation*, a Level 2 MATLAB S-Function. This function receives the current angle, loads the files containing the de-

sired stimulation strategy, and generates the corresponding output value to be sent to the stimulator at each iteration. Output of this function is always in the [0,1] range, so it is later multiplied by a current factor specific for each muscle. Before therapy is started, the minimum intensity to cause muscle contraction (motor threshold) for each muscle is found. Then, each muscle's current factor is determined so stimulation never overcomes its motor threshold and stimulation remains only sensory, without causing any contraction nor fatigue.

Manufacturers of the sensory stimulation device used in this project provide a custom-made library for Simulink-device communication. Also, it incorporates a RealTime Simulink block which enforces an iteration time specified by the user, as long as the computer does not get overloaded. A custom function to monitor execution time during therapy and ensure real time execution has been included in the Simulink model.

Performing EMG recording in one computer and the stimulation protocol in another separate computer has been chosen in order not to overload the latter, what would cause the stimulation to be lagged. This would seriously compromise the efficacy of the therapy, as the delivered stimulation would not coincide with the desired bio-inspired stimulation patterns, in which proper timing in the stimulation delivery is crucial.

6. Sensory stimulation device

RehaStim (HASOMED GmbH, Mageburg, Germany) is an 8-channel biphasic stimulator. It incorporates the Science Mode, which allows the stimulator to be controlled from a PC via USB communication, defining the desired current amplitude and pulse width, as defined in Figure 3.5.

The Simulink model (which includes the RehaStim block) in the stimulation protocol PC sends commands to the device, which then performs the desired stimulation. Pulse width is fixed at 300 μs, and current amplitude is varied as required by the stimulation strategy, as a function of the pedaling cycle. This device has been designed for FES applications. Nevertheless, by lowering the intensity of the stimulation and placing the electrodes on top of nerves instead of muscles, it can be used in order to provide sensory stimulation. First, the minimum current amplitude that triggers contraction (denominated motor threshold) in the stimulated muscle is investigated. It is known that sensory afferent fibers have thicker axons than efferent fibers causing contraction, and therefore are excited at lower stimulation intensities. Thus, the maximum current amplitude to be reached during the therapy is set at 70% of motor threshold. At this level, subjects reported feeling the stimulation but not being uncomfortable. Also, it was seen that using higher stimulation amplitudes (around 90% of motor threshold), even when not causing contraction, induced fatigue in the subjects.

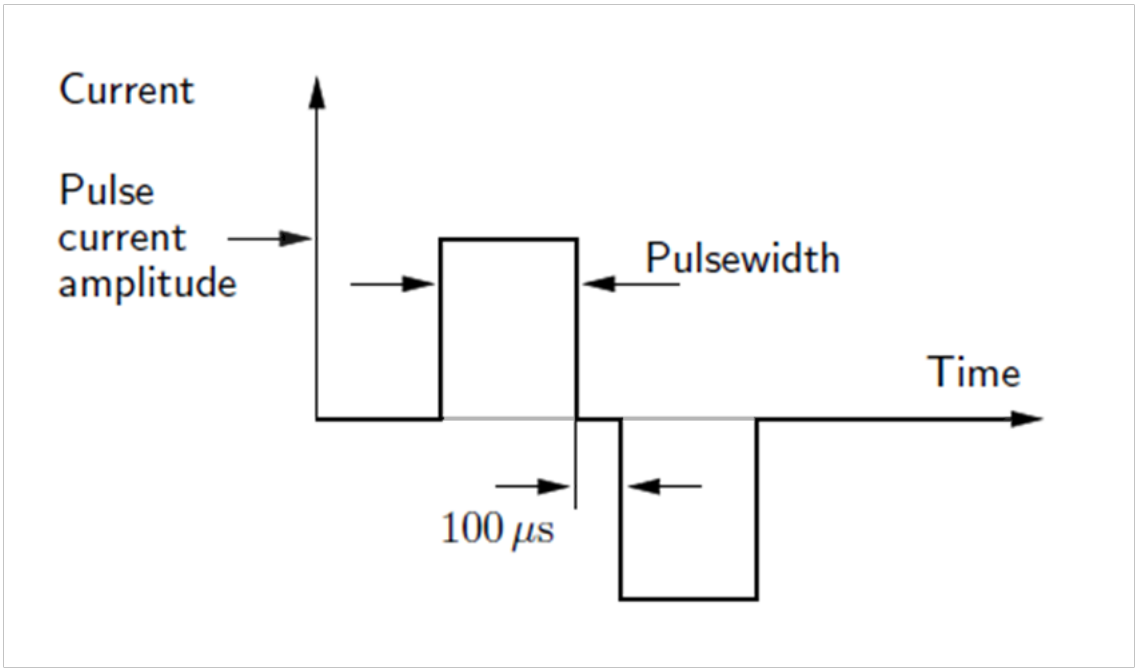


Figure 3.5. *RehaStim delivers biphasic pulses. The main stimulation parameters are pulse width and pulse current amplitude. Extracted from RehaStim Science Mode manual.*

Real time design

Delays in communication between devices and in execution of the commands is a major source of concern in this project, and special attention and care has been devoted to ensure real time execution and proper stimulation timing. First, a real time block has been included in the Simulink model. Additionally, a custom function has been designed in order to check the difference in between real and stimulation time, and allows to visually check if any major delay is being generated during the execution of the stimulation protocol. It has been especially useful in order to detect whether the computer is able or not to work at a given iteration time. This is highly dependent on the computational power of the stimulation protocol PC. In this setup, iteration time is adjusted to 20ms (50Hz), at which the used computer performs well and no delay is generated. Nevertheless, it is not possible to know if the stimulation itself is delivered at the appropriate times, as delays may arise in the communication between Simulink and the stimulator, or in the execution of the stimulation in the stimulator device itself. To solve this limitation, EMG data needs to be stored and later analyzed together with pedaling angle data, in order to carefully ensure that stimulation is delivered in the appropriate moments.

Offline signal synchronization

After EMG and pedaling angle signals have been separately recorded and stored, they can be analyzed together. Nevertheless, it is needed to know the time correspondence between both signals, that is, which EMG data sample was generated simultaneously with

a given angle data sample. Synchronization triggers have been included to enable time alignment of both signals. Each ten seconds, the microcontroller includes a characteristic feature in the pedaling angle message that sends to the stimulation protocol PC. The computer detects it and saves that time point as an "angle trigger point". At the same time, a synchronization square pulse is sent through a BNC wire to the EMG amplifier and it is read in a specific auxiliary channel. These square pulses can be easily detected in post-processing and labeled as "EMG trigger points" (see Figure 3.6). Afterwards, when analyzing the results, it is known that angle and EMG trigger points exactly correspond to each other. Signals are split into ten second segments, limited by consecutive triggers. Thus, homologous segments of angles and EMG data are known to be aligned in time. Moreover, to ensure that the alignment is unique and correct, a characteristic "initial trigger" is delivered to the EMG amplifier when angles start to be recorded.

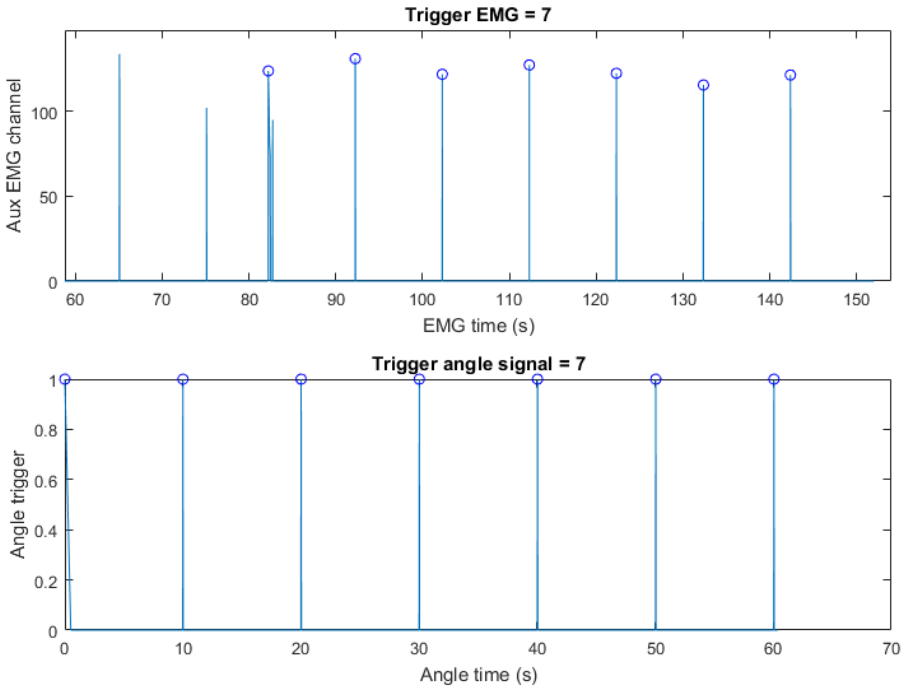


Figure 3.6. *Synchronization triggers in both signals: EMG and angle. In the upper figure it is possible to see that although several triggers were received prior to the start of the recording, the characteristic initial trigger (double peaked) can be detected by the algorithm making the alignment unique.*

However, angle data is sampled at a lower frequency than EMG (50Hz and 2.000Hz, respectively). Thus, angle data is resampled in order to have the same amount of data points than EMG for the homologous 10 seconds segments. Resampling has been done by assigning the value of the previous known point, in order to resemble that the stimulation Simulink model keeps one angle value until the next angle sample is received. The advantage of having synchronization signals is that it is possible to align the angle and EMG recordings in the off-line analysis, notwithstanding the communication delay between the Arduino and the stimulation protocol PC, or the EMG amplifier and the EMG recording PC.

Generation of bio-inspired stimulation strategy

Thanks to signal synchronization, it is possible to align angle and EMG data. Moreover, it is possible to map EMG data as a function of pedaling angle. Thus, a personalized, bio-inspired stimulation pattern can be defined for each individual subject. In order to obtain the natural activation patterns of a subject, EMG and angles data has to be acquired during pedaling (without stimulation) for a few tens of cycles. Normally, one minute is acquired. First, EMG signal has to be filtered, rectified (absolute value is taken) and low-pass filtered in order to obtain the EMG envelope. Envelopes could be understood as overall muscle activation. Then, it is possible to average envelopes along all cycles and obtain a general activation pattern for each muscle as a function of the pedaling cycle. The full procedure is illustrated in Figure 3.7.

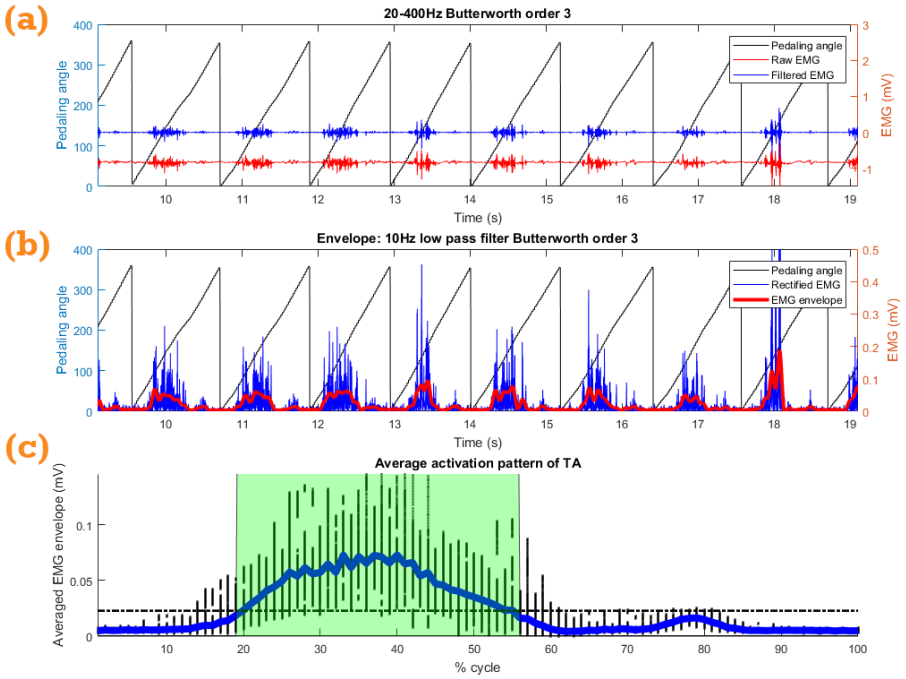


Figure 3.7. *The full procedure for obtaining the activation pattern of a subject is illustrated. All data shown corresponds to Tibialis Anterior muscle for one subject. First of all, data has to be aligned according to the synchronization procedures already described.*
(a) Then, raw EMG data (red) is band-pass filtered (blue).
(b) Afterwards, data is rectified (blue) and envelopes (red) are obtained by low-pass filtering, so high frequency components are eliminated.
(c) Finally, envelopes are expressed as a function of angle (angle is converted to cycle percentage). Black dots represent the values of the envelopes of all cycles for that specific portion of the cycle. The blue line represents the average activation profile. The horizontal black line represents the average value of activation of the muscle, and values above it are considered to be "active". The shaded green area represents the cycle interval in which the muscle is active. The personalized stimulation strategy will be designed based on this naturally active area, stimulating only when the muscle is active.

Ensuring appropiate stimulation

In order to assess whether the timing of the stimulation is correct, the EMG acquired during the stimulation therapy can be analyzed. Electrical stimulation induces a sudden depolarization of the tissue near the electrodes. When stimulation is delivered to the subject, recording electrodes situated near stimulation electrodes register a stimulation artefact, which consists of a high amplitude peak. The size of such artifacts is much larger than the signal arising from the physiological activation of the muscles. These artifacts therefore obscure the intrinsic activity of the muscle, but also enable a careful inspection of the delivered stimulation. First, pedaling angle and EMG data needs to be aligned, as already explained. Then, it is possible to compare the area in which stimulation pulses should be given, according to the defined stimulation strategy, and when those pulses are actually given. Several measures can be derived:

- Onset lag, that is, the time difference between the angle sample at which stimulation should begin (according to the defined stimulation strategy) and the time at which the first stimulation pulse is delivered. This metric is obtained for all cycles, in order to check both its magnitude and its evolution over time (in case a delay adds up, there would be a continuous drift in this quantity).
- End lag: similarly to the previous, it is the time difference between the end of the desired stimulation and the actual last stimulation pulse.
- Correlation: all cycles are added up in order to obtain an average stimulation profile. Linear correlation of such profile with the desired stimulation pattern is obtained. Also, cross-correlation is obtained in order to check if there is a constant lag.

The importance of proper, real-time stimulation should not be disregarded, as it can constitute a major pitfall in experimental setups of this type. Lack of attention to this issue could lead to time-lagged stimulation strategies, what may highly reduce the effectiveness of the therapy.

Application possibilities

The designed setup and codes are already enabled to stimulate up to eight different muscles (as it is the maximum number of stimulation channels available in the stimulator) and record electromyographic data from up to thirty two channels (maximum number of channels in the EMG amplifier). Moreover, personalized stimulation patterns, modulated both in amplitude and time, can be designed for each individual patient. Therefore, the system is enabled for a variety of applications:

- Both FES and sensory afferent stimulation can be delivered, depending on whether amplitude of delivered pulses is above or below the motor threshold.

- Muscle synergies can be extracted, as a significant number of independent muscles can be recorded (up to 16).
- Any combination of personalized stimulation patterns can be delivered (to up to eight different muscles). Therefore, it is suitable for stimulation patterns inspired in muscle synergies and delivered to several different muscles.

3.2. Testing spinal reflex excitability

The second objective of the Bachelor Thesis is to select and develop the experimental technique to be used as an assessment of the efficacy of the therapy. The chosen technique is to observe plastic changes in reciprocal inhibition through modulation of the soleus Hoffmann reflex. Experimental procedures have been developed by the author of this Thesis, based on the available literature, in order to assess H reflex modulation by reciprocal inhibition.

Experimental procedure for evocation of soleus H reflex

In order to elicit the soleus H reflex, the posterior tibial nerve (PTN) must be stimulated (1-ms rectangular pulse) with a monopolar electrode (the cathode) placed in the popliteal fossa (posterior part of the knee, see Figure 3.8(a)). The anode must be placed over the patella (anterior part of the knee). The electrodes should be fixed by adhesive tape around the knee, exerting some pressure, in order to improve stability of evoked reflexes and to reduce possible discomfort [77] . To record the soleus H reflex, EMG signal of the soleus muscle must be recorded. Electrodes must be placed over the soleus muscle, on the medial face of the lower leg, at 2/3 of the line between the medial condylis of the femur and the medial malleolus (see Figure 3.8(b)). The reference electrode is placed at the ankle of the subject.

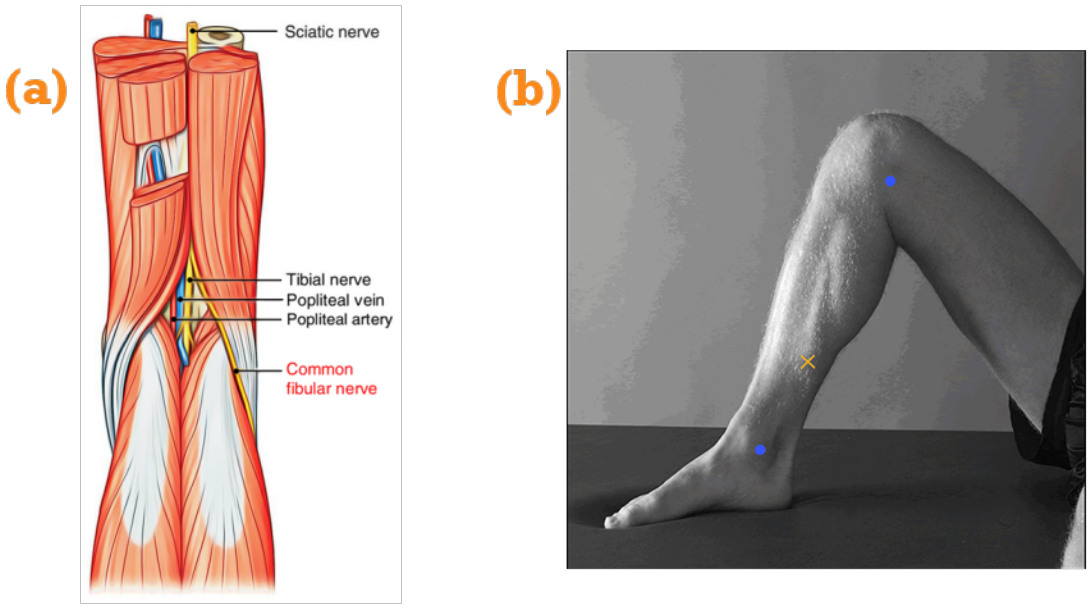


Figure 3.8. *Stimulation and recording sites for soleus H reflex. (a) Anatomy of the popliteal fossa. Edited from https://www.earthslab.com/anatomy/popliteal-fossa/ . Electrode positioning for soleus EMG recording. Extracted from [1]*

The amplitude of the M wave and of the H reflex are measured as the peak-to-peak amplitude in the intervals [8-20] ms and [25-50] ms after the stimulus (see Figure 3.9).

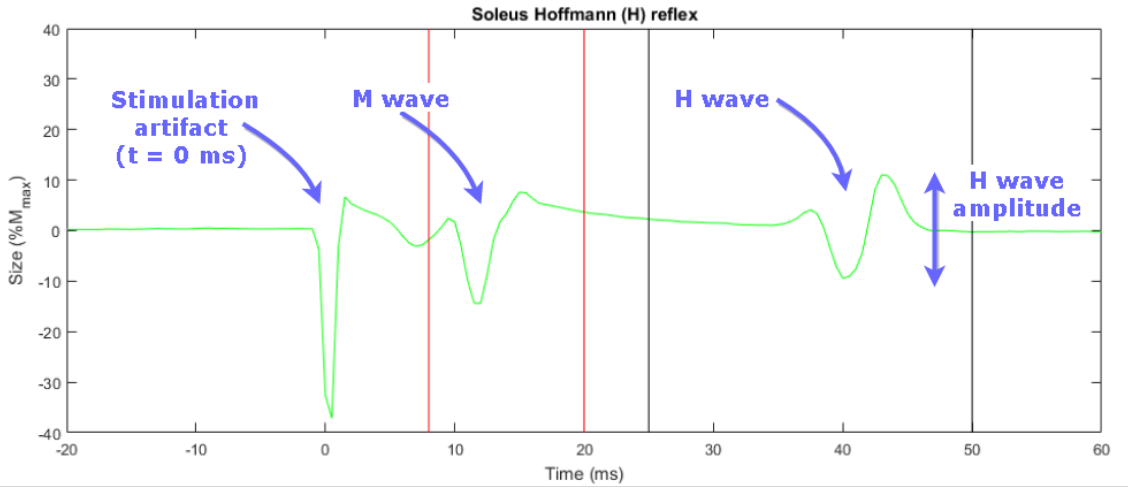


Figure 3.9. *Example soleus H reflex. M wave can be found in the 8 - 20 ms interval meanwhile H wave is found in the 25 - 50 ms interval.*

Many factors may influence the amplitude of the H reflex: posture, attention, site of stimulation, intensity of stimulation, muscle length, muscle strength... In order to obtain an stable H reflex, subjects are asked to sit comfortably of a chair, with semi-flexion of the hip (100^{o}), bending of the knee (45^{o}) and ankle at 110^{o} plantarflexion. Also, subjects are asked to fully relax their legs during testing. Soleus EMG signal has to be carefully monitored during all the experiment to ensure that the soleus muscle is fully relaxed.

First of all, EMG signal has to be thoroughly checked. When the subjects are fully relaxed, low electromagnetic noise should be observed in the signal. Then, the subject is asked to successively contract the different muscles and activation in the corresponding EMG channels must be observed. Then, a few stimulation pulses are given at a intensity in which a small visible contraction is produced, and the EMG signal is inspected for the H reflex. Common problems in this stage are:

- Incorrect EMG signal: It may be produced by incorrect electrode positioning, electrode failure, connection wires failure, or wrong setup of the EMG amplifier. In case there are problems with one or more of the EMG channels, all these factors must be carefully checked.

- High amplitude of 50Hz noise: This problem arises from incorrect isolation. Ensure that the reference electrode is properly connected, and that the EMG amplifier is not in contact with any device connected to the power plug.

- No H reflex is elicited: Although infrequent, this issue may arise. First, it should be checked the positioning of the soleus EMG recording electrodes, and the subject should perform a soleus contraction, to ensure that the EMG signal is properly recording the activity of the soleus muscle. Next, if must be checked that the stimulus causes a visible soleus contraction. If still neither the M or the H waves can

be seen, the positioning of the stimulation electrodes must be changed until reflex activity can be seen in the soleus EMG.

- The stimulation artifact has a long decay time (>30ms), obscuring the M and H waves: This problem arises from a too large skin-electrode impedance. If this issue is detected, the recording electrodes must be checked and replaced if necessary.

The magnitude of the H reflex is not constant across subjects, due to a variety of factors: muscle size, electrode-skin impedance, amount of fat between the muscle and the skin... In order to normalize the results and allow in-between subject comparison, amplitudes are given as a percentage of the maximal M wave amplitude, M_{max}. M_{max} represents all alpha motor neurons being activated simultaneously. Therefore, normalization using this value is expected to give the fraction of alpha motor neurons being activated by the H reflex, and allowing for comparison between subjects. Thus, after the EMG has been thoroughly checked, and the presence of the H reflex has been verified, the supramaximal stimulation intensity has to be found and M_{max} has to be measured. High stimulation intensities, although rarely painful, can be unpleasant. Therefore, it is necessary to carefully increase the stimulation until the M wave does not further increase in size, instead of directly using high intensities in order to obtain M_{max}, in order to reduce discomfort for the subject.

Finally, the stimulation intensity at which we desire to stimulate has to be found, so that it elicits H wave amplitudes around 20% M_{max}. To that end, stimulation intensity should be put to zero and then successive pulses should be given, gradually increasing stimulation intensity. At some point H reflex will start to be elicited. Then, stimulation intensity should be slowly increased until the desired H reflex amplitude is reached.

Experimental protocol for testing reciprocal inhibition of soleus H reflex

In order to test reciprocal inhibition of the H reflex, the nerve innervating the antagonist muscle has to be stimulated. In the case of soleus muscle, the antagonist muscle is the tibialis anterior (TA). The nerve innervating this muscle is the deep branch of the common peroneal nerve (CPN). It is needed to appropriately stimulate the deep branch of the CPN without stimulating the superficial branch. If the superficial branch innervating the peroneal muscles is accidentally activated, it may reduce or even destroy the phenomena of reciprocal inhibition, as peroneal muscles facilitate soleus activation. In order to only stimulate the deep branch of the CPN, bipolar electrodes should be used near the head of the fibula, towards the anterior part of the knee. The cathode (negative) electrode must be placed close to the TA muscle belly, and the anode should be placed 2 - 3cm above the cathode. The conditioning stimulus is a 1 ms rectangular pulse. The conditioned stimulus intensity is given at 1.5 times the minimum stimulus intensity that causes visible contraction from the tibialis muscle, as maximal reciprocal inhibition is observed at this stimulus intensity [5]. Trial stimulations should be given to the CPN in order to carefully check the

correct positioning of these electrodes, and that only the deep branch is being stimulated. To do so, the stimulus intensity in the CPN must be incremented until the M_{max} is reached in the tibialis anterior muscle, and check that there are no M nor H waves in the peroneal EMG signal. Therefore, EMG signal from tibialis anterior and peroneal muscles must be acquired. The peroneus longus muscles is anatomically very close to tibialis muscle, so crosstalk may occur (TA signal being recorded in peroneus longus electrodes). Thus signal is to be acquired from peroneus brevis, which is also innervated by the superficial peroneal nerve and it is further from tibialis muscle. Additionally, it should be ensured that stimulation delivered at the popliteal fossa to the posterior tibial nerve (PTN) to elicit soleus H reflex, should never excite the CPN, because this could provoke reciprocal inhibition even in the test stimulus. Thus, reciprocal inhibition would also be present in test reflexes and there would be no difference between test and conditioned reflexes. Therefore, stimulus intensity at the PTN should be incremented until the M_{max} is reached in the soleus muscle, and check that M and H waves are absent in tibialis and peroneal muscles. To achieve this, a punctual electrode (much more localized stimulation is delivered) is used for the cathode and placed at the medial side of the popliteal fossa, meanwhile the anode is a surface electrode placed below the patella. The punctual electrode is held by the experimenter throughout each recording, applying a constant pressure against the fossa popliteal and slightly medial. The placement point of the punctual electrode is to be marked for repeatability. The Conditioning-Test interval (CTi) is fundamental, as it enables the activation of CPN Ia afferents to reach the spine first and produce inhibition in the soleus H reflex pathway. In the present protocol, the first time reciprocal inhibition is assessed in a session, different CTi times are assessed, from 1 to 6 ms, plus test reflexes (three times each, in randomized order). Test pulses are those in which no CPN stimulation is given. Then, the CTi yielding the highest amount of reciprocal inhibition is chosen for the rest of the session. Modulation of the H reflex depends crucially on the size of the test H reflex. A control H reflex size around 20% M_{max}, in the rising section of the H reflex curve, should be maintained. Moreover, when assessing reciprocal inhibition, conditioned and test pulses are given in a randomized sequence. In this way, biasing of the data is avoided, and it can be monitored that the size of the test H reflex remains stable during the full measurement. One sample test and one sample conditioned soleus H reflexes, acquired successively, can be seen in Figure 3.10.

At this point it is important to note that the H reflex is a physiological measurement and a huge amount of factors have an influence on it: posture, attention, muscle tension, age, disturbances in the surroundings... Therefore, it is impossible to get a completely identical sequence of H reflexes even when the experimental conditions are constant and carefully monitored. Therefore, what is used is the mean of the amplitude of a set of H reflexes elicited at the same stimulus intensity and in the same conditions. This variability is already taken into account when analyzing the results and applying statistical tests for significance.

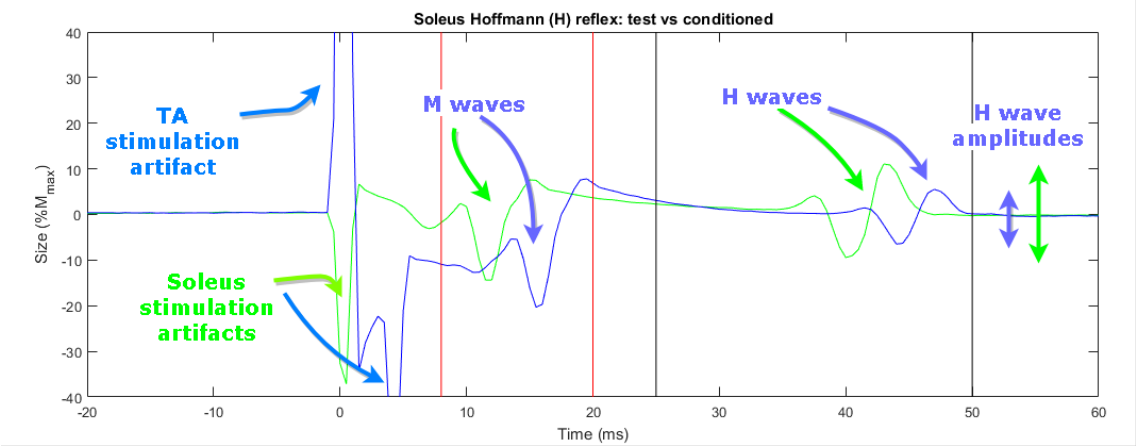


Figure 3.10. *Two successively evoked H reflexes, one test and one conditioned, are displayed here. In green, the test H reflex, in which only the soleus stimulation artifact (at posterior tibial nerve) can be seen. After approximately 10ms the M wave appears, and after approximately 40ms the H wave appears as well. In blue, the conditioned H reflex can be seen. The automatic detection algorithm detects the tibialis stimulation as t=0. Conditioning-Test interval was 4ms in this case, so soleus stimulation appears at t=+4ms. As it is this second stimulus what generates the H reflex, all the signal displayed is delayed in 4ms, what also makes visualization of data easier. This conditioned H reflex was taken just 5 seconds after the test H reflex at the same conditions and stimulus intensity. It can be observed that conditioned H reflex is noticeably smaller, due to reciprocal inhibition.*

In order to ensure that stimulation is constant, constancy of M wave should be monitored when it is present. If significant variations of M wave or test H reflexes occur throughout a recording, each section with significant different means should be treated as different sample sets. In order to minimize the effect of random variations, ten reflexes of each kind are taken. This responds to a balance between having as much data as possible, and keeping experimental time within reasonable limits. Between consecutive pulses, an Inter-Stimulus Interval (ISI) is left for the muscle and nerves to return to normal conditions, and avoid homosynaptic depression, another spinal mechanism that inhibits successive reflexes. Although ten seconds are necessary to fully ensure that it is not playing any role, usually less time is needed for most of it to be vanished (homosynaptic depression is maximum at 1-2 seconds ISI). Again, a compromise with reasonable experimental times has to be met. For this reason, five seconds is the InterStimulus Interval used most often in the literature [10], [12], [13] and that will therefore be used in this experimental procedure.

Experimental setup for testing spinal reflex excitability

A setup has been developed by the author of this Thesis in order to test spinal reflex excitability, evoking soleus H reflexes and modulating them through reciprocal inhibition.

The diagram of the setup can be seen in Figure 3.11.

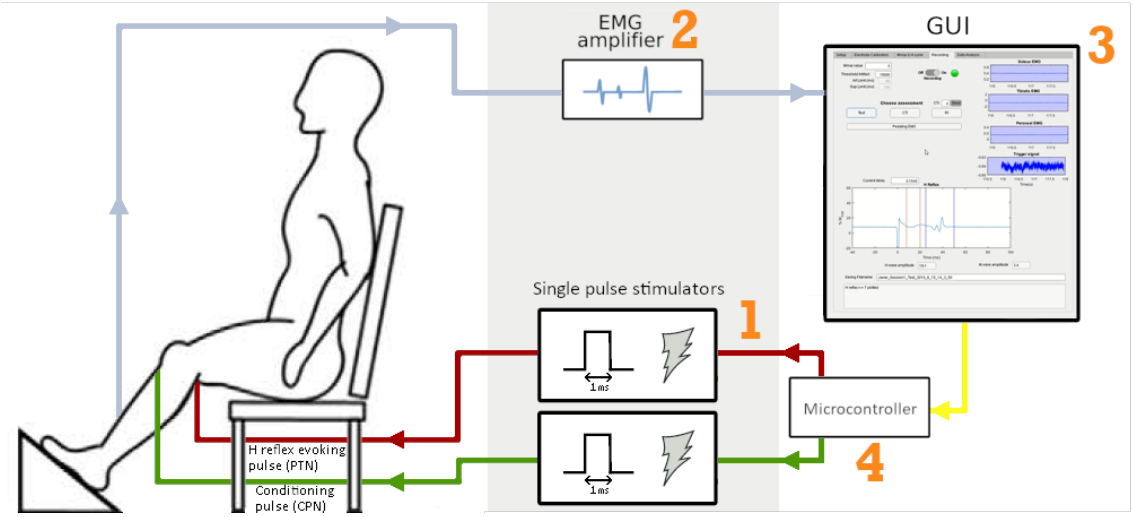


Figure 3.11. *Diagram of the developed setup for spinal reflex excitability testing.*

The setup comprises the following elements:

1. Single pulse stimulators

Two Digitimer DS7A (Digitimer Ltd, Hertfordshire, UK) were used. They are direct current stimulators, in which the time-width and current intensity of the stimulation can be controlled. Moreover, they include a BNC trigger input for precise timing of the delivery of the stimulus. One of the stimulator devices is used to stimulate the PTN to elicit the soleus H reflex, meanwhile the other stimulator is used to deliver the conditioning stimulus to the CPN.

2. EMG amplifier

Trentadue (OTBiolettronica, Milano, Italy) is used. It is able to acquire 32 EMG channels (16 independent muscles) simultaneously. The reasons behind choosing this device are the same that those for using it in the neurorehabilitation platform.

3. Graphical User Interface

A custom Graphical User Interface (GUI) has been designed using AppDesigner in MATLAB R2017b (MathWorks, Natick, MA, USA). A detailed description of its features will be given in next section. The GUI runs on a Acer Aspire E17 (Acer Inc, New Taipei, Taiwan) computer.

4. Microcontroller

Arduino UNO (Arduino LLC, Italy) is used in order to precisely control the timing of the stimulations. It gives the trigger to the stimulator in charge of giving the conditioning stimulus and, after the prescribed time Conditioning-Test interval (CTi), it gives the trigger to the stimulator in charge of stimulating the soleus H reflex. The maximum error of the timing in between stimulus, which is fundamental for obtaining reciprocal inhibition, was measured to be of 16 microseconds. Even in the case of the shortest CTi used, 2ms, the relative timing error is of 0.8%. Moreover, it was possible to check accurate delivery of the pulses in the EMG signal, as stimulation pulses generate large amplitude artifacts, and so the time delay between them can be checked for consistency.

Custom GUI

A graphical user interface (GUI) has been developed for this Bachelor Thesis in order to ease the application of the described protocols. The GUI has been coded using the AppDesigner tool of MATLAB (version 2017a) (MathWorks, Natick, MA, USA). The GUI performs the following functionalities:

- Setup of EMG acquisition parameters (sampling frequency, number of channels, muscle in each channel...).
- Connects to the chosen EMG amplifier and to the Arduino microcontroller that controls of the stimulation pulses.
- Plots the EMG data in real time for all the channels.
- Enables real time visualization of H reflexes, what is key in electrode placement and calibration.
- Enables acquisition of reciprocal inhibition data, with real time calibration and monitoring of the data.
- Analyzes the obtained data . Contains custom routines for analyzing each kind of recording:
  - Test: only test H reflexes are evoked, without conditioning. It is aimed to check stability of soleus H reflex.
  - CTi: a series of pulses is given, using pseudorandom Conditioning-Test interval (CTi) (test, 1ms, 2ms, 3ms, 4ms, 5ms, 6ms). Three reflexes of each kind are obtained and averaged.
  - Reciprocal inhibition: reciprocal inhibition is tested by evoking test and conditioned reflexed in a pseudorandom order, to avoid biasing of the data. Also, constancy of M wave amplitude and test H wave amplitude can be monitored

in order to ensure stability. The analysis also obtains statistics as mean values and standard deviations, and p-value of two-sample t-test between test and conditioned samples.

- Saves the data for future reference and analysis.

Moreover, the GUI prompts messages in order to guide the experimenter through the protocol. It also notifies errors, and tells the most common causes and solutions in order to fix them. This GUI is a useful tool for the experimenter, and is also designed for ease of future experimenters, taking into account that this work is to be continued by people other than the author of this Bachelor's Thesis. A capture of real time data acquisition with the custom GUI can be seen in Figure 3.12.

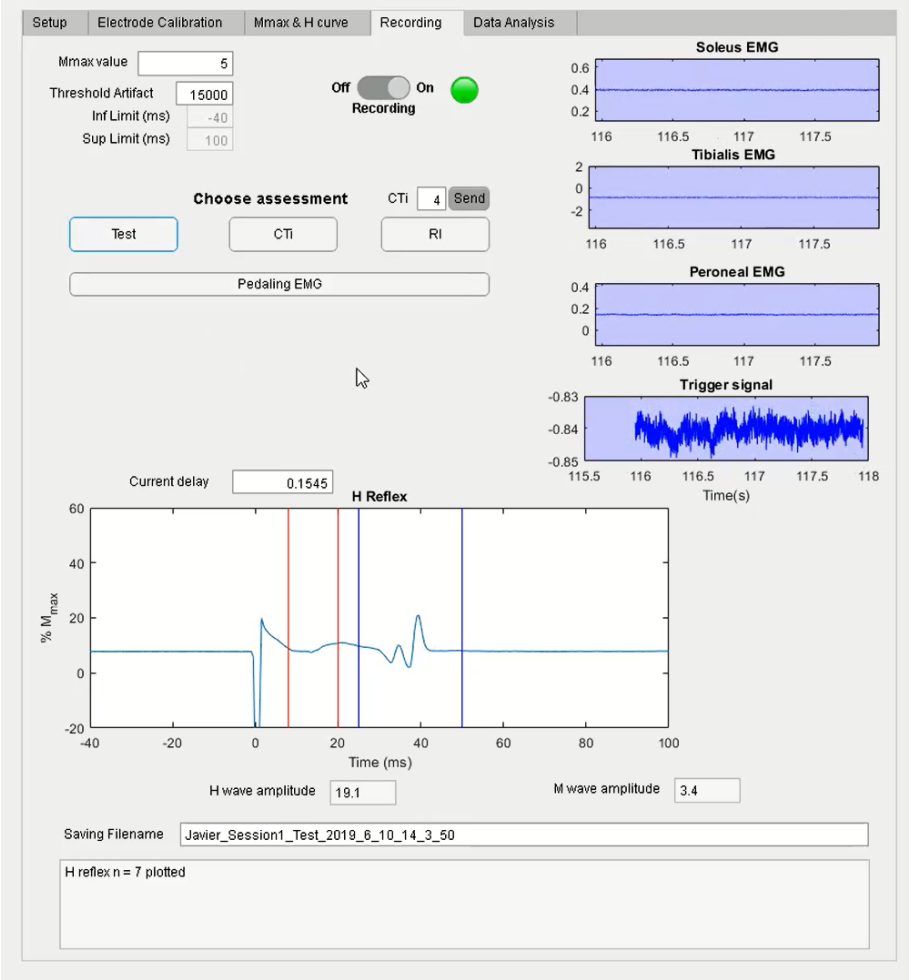


Figure 3.12. *Data acquisition with the custom GUI. EMG can be monitored in real time, signals can be sent to the Arduino microcontroller in charge of synchronizing stimulation pulses, and obtained H reflexes can be monitored in real time.*

Reflex modulation analysis

In order to assess the effects of pedaling combined with sensory afferent stimulation, reciprocal inhibition of soleus H reflex is to be assessed, before (PRE) and after (POST) the intervention. Each set of measures consists in twenty reflexes, of which ten are conditioned (a previous pulse is given to the common peroneal nerve in order to create reciprocal inhibition) and the other ten are test (that is, only the posterior tibial nerve innervating the soleus is stimulated). An interstimulus interval of 5 seconds is to be used. Soleus reflexes obtained from some subjects may be highly unstable, that is, there is a major change in amplitude from one reflex to the next one, even if all stimulation conditions remain constant. This is the reason for using test reflexes, in order to verify the constancy of the response. Also, the test reflexes should be allocated throughout all the set of measures, so that the stability of the set of measures over time can be assured. Thus, the order of conditioned and test pulses is pseudorandomized, in order to have test reflexes distributed all along the set of measures, and to avoid biasing of the data. Moreover, several sets of measures are to be taken from each patient and condition (PRE/POST), so that some data sets can be discarded if they happen to be inestable.

Direct analysis

All the sets of measures have to be first analyzed for:

1. **Presence of outliers**: The measured reflex amplitude is expected to follow a normal distribution, and its mean value is the data of interest. Thus, points which are two standard deviations below or above the mean are considered outliers and are eliminated from the analysis.

2. **Stability**: Stability (constancy) of the test reflexes is evaluated, in terms of the standard deviation of their amplitude. Also constancy of M waves amplitudes is analyzed.

3. **Normality**: As said previously, reflex amplitude is assumed to follow a normal distribution. This assumption is tested by means of Shapiro-Wilk test. If reciprocal inhibition occurs, test and conditioned reflexes follow different normal distributions, so normality is tested separately.

Following this analysis, data sets were discarded attending to these various reasons:

- Test H reflex instability: attending to personal experience obtained through experimentation, standard deviation of test H waves of stable sets is below 3% M_{max}, that of acceptable sets is below 6 and unstable samples have standard deviations of H measures around 6 or higher. Thus, sets with σ_{Htest} above 6 were discarded.

- M wave instability: attending to experience obtained through experimentation, standard deviation of M waves of stable sets is below 0.5% M_{max}, that of acceptable sets is below 1.5 and unstable samples have standard deviations of M measures higher than 1.5. Thus, sets with $\sigma_M > 1.5$ were discarded.

- Sets not fulfilling a normality distribution were discarded, i.e. a result of 1 in the Shapiro-Wilk test, therefore rejecting the null hypothesis of following a normal distribution.

It is important to note that, at this stage, no analysis of the results themselves has been carried out, in order to perform the decision of which data is suitable completely dependent on the stability of the samples and not in the results they would provide. After selection attending to stability has been carried out, the remaining samples are tested for the presence of reciprocal inhibition. The rationale of statistical testing is that conditioned and test reflexes follow normal distributions with unequal means. Normality of distributions has already been assessed with Shapiro-Wilk test. Now, paired-sample Student t-test is used in order to test for the presence of statistically significant difference between the means of the test reflexes (μ_{Htest}) and the conditioned reflexes (μ_{Hcond}). The test is two-sided with significance level P<0.05. In case there is no PRE or POST set which achieves significance at $P < 0.05$, the significance level may be lowered to $P < 0.1$. The reciprocal inhibition (RI) in each data set is calculated as described by Yamaguchi et al. [12], where μ_x represents the mean of the corresponding set.

$$RI = \frac{\mu_{Htest} - \mu_{Hcond}}{\mu_{Htest}} \cdot 100\%$$

If there is more than one set taken either before or after the intervention in which significant reciprocal inhibition is found, the final PRE reciprocal inhibition value is the average of all the PRE values, and the final POST value is the average of all the POST values.

Analysis of variance

However, data acquired from some of the subjects may not be stable enough to be directly analyzed, and most data sets are to be discarded due to high variance (lack of stability). Nevertheless, in some data sets closer inspection reveals that some areas of the data are stable while others are not, and therefore selecting only those areas would provide data with the desired stability.

In order to extract that useful, stable information from the overall unstable set, a method based on analysis of variance has been developed by the author of this work. Following this method, all possible data subsets (portions of the full data set) are to be assessed. However, any subset should contain a minimum length of ten samples (including both test and conditioned) in order to be able to compute reciprocal inhibition in a statistically meaningful way. Additionally, all samples have to be consecutive in time, as the purpose

is to detect and extract time spans during which the soleus H reflex is stable (both H_{test} and M waves have low variance) and therefore measures of reciprocal inhibition are reliable. In order to evaluate which subset of the data presents better stability, the quantity "*Total Variability*" has been defined as the sum of the standard deviations of the test reflexes and of the M waves. The standard deviation (σ) of these quantities has been used in the previous section in order to assess whether a set of measures is stable or not. Thus, it seems natural to use them in order to define this metric.

In the previous section it has defined that acceptable sets have standard deviations of test reflexes below 5 % M_{max}, and stable sets have it around 3 or below. Similarly, sets with acceptable stability have an standard deviation of M waves below 1, and stable sets have it below 0.5. Therefore, it is not reasonable to penalize a subset with standard deviation of 0.2 with respect to another with 0.1, as both are really stable subsets of data. Thus, a minimum value of 3, 0.5 and 0.5 is assigned to σ_{Htest}, σ_{Mtest} and σ_{Mcond}, respectively, when computing *Total Variability*. Furthermore, length of the subsets should also be taken into account. A longer subset is more statistically meaningful than a shorter one, so it is preferable as long as both have similar stability. This is even more relevant when the subset is short. Therefore, length has been introduced in the metric, in a logarithmic way in order to favour increases in length of short subsets more than those of long subsets, and avoid the metric to fall in small local minima. To include all these terms, an objective function f has been defined as:

$$f = Length\ Bonus - Total\ Variability + K$$

- *Length Bonus* has been defined to quickly increase near the minimum defined length, ten samples, and then increase more slowly as length keeps increasing (see Figure 3.13).

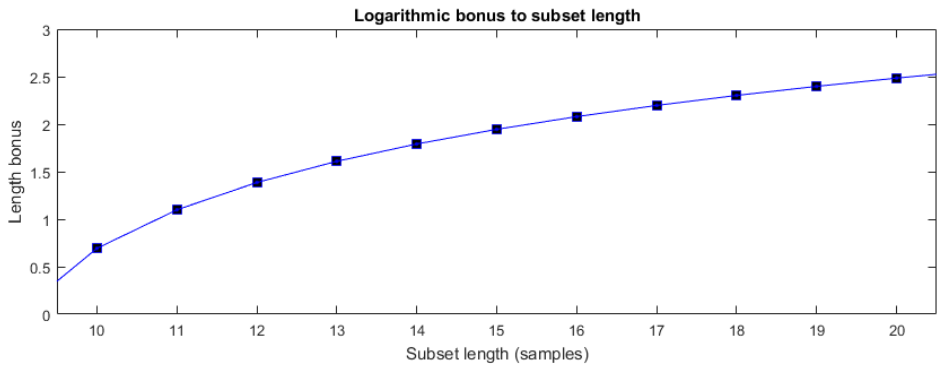


Figure 3.13. *Logarithmic length bonus. The used formula is Length Bonus = ln(length - minimum length + 2). Length Bonus will have a value of 0.6931 for length = minimum length = 10 , then 1.099 for length = minimum length + 1 = 11 (increase of 0.406), and so on. Finally, the increase from length 19 to 20 is of only 0.087, as increasing from 19 to 20 samples is not so relevant statistically*

- Following the previous discussion about minimum values for variability,

$$Total\ Variability = max(\sigma_{Htest}, 3) + max(\sigma_{Mtest}, 0.5) + max(\sigma_{Mtest}, 0.5)$$

  Therefore, *Total Variability* ranges from 4 (for a stable set) to any higher value for a less stable sample, which would produce a higher Total Variability that will then be subtracted, leading to a lower value in the objective function *f*.

- A constant $K = 20$ has been added to the function in order to have positive values and enable easier visualization.

The most stable subset will be the one that maximizes this objective function. A function iterated through all possible combinations of first and last samples (as long as they fulfilled a minimum length of ten samples) and computed the value of the objective function for each subset. The results of applying this method to one example data set can be seen in Figure 3.14.

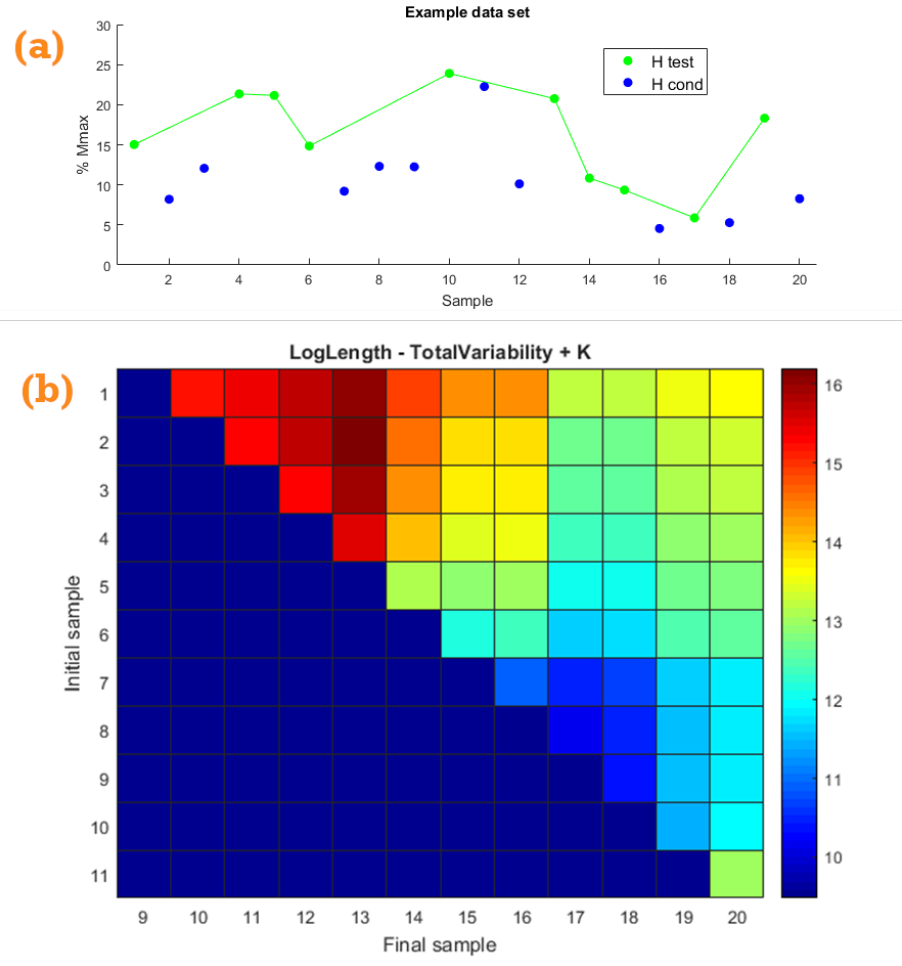


Figure 3.14. *Heatmap representation of the variance of an example data set.*
(a) In the data set, although variable, it can be seen how the H_{test} size remains in the range 15-25% until sample 13. Then, suddenly, it becames unstable and drops below 10%. This lack of stability produces a large variability value.
(b) When the proposed objective function is applied to the example data set, the objective function f = LogLength - TotalVariability + K is calculated for all possible subsets of consecutive samples. Each position represents a different subset of data, spanning from sample "Initial sample" to sample "Final sample". Elements below the diagonal would contain less than 10 samples and are therefore disregarded. The maximum value (in dark red) is found in position (2,13), what indicates that the most stable subset according to the objective function f is the subset spanning from sample 2 to sample 13. Looking to the original data set (a), it is easy to see that actually from sample 14 onwards the set starts to be unstable. Therefore, the objective function has successfully (and automatically) detected the subset with the lowest variability. Also, it suggests to eliminate sample 1, as it is somehow lower that the other H_{test} values. This behaviour is expected due to the construction of the function.

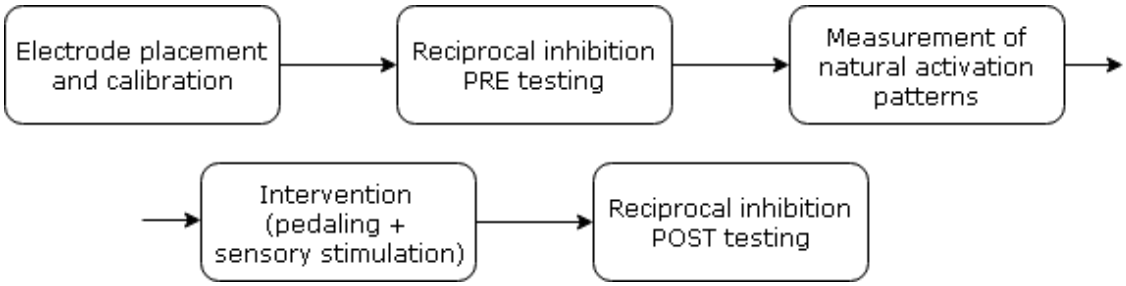
Using such a function enables automatization of the process of selecting the most stable subset of each unstable set. Previous to the development of the method, several unstable data sets have been manually inspected to find the most stable subsets, and the objective function has been developed and tuned by testing on those data set (learning sets). Afterwards, it has been tested in other data sets, yielding equal or better results than manual subset selection. Moreover, it does not imply visualization of data by the experimenter, so biasing is avoided i.e. the experimenter deciding which areas are more suitable not only based on variability but also on the expected reciprocal inhibition. Thus, using the defined function for analysis of variability carries several benefits:

- Time optimization, as it is not needed to analyze each data set manually.
- More accuracy, as the defined function may reveal stable subsets not seen by the experimenter.
- It is completely blind with respect to the final result of reciprocal inhibition. All bias from the experimenter is avoided.

After the unstable subsets have been checked for subsets with maximal stability, direct analysis of the selected subsets is carried out, as described in the previous section: outliers are eliminated, subsets that still have a high variability are discarded (unstable data sets may not have any stable subset), normality of data is tested, and finally significant reciprocal inhibition is tested.

3.3. Pilot study

A pilot study with healthy subjects is to be performed in order to assess the performance and usability of the designed neurorehabilitation setup. Additionally, some preliminary results concerning whether the proposed therapy is effective in inducing neural plasticity at the spinal cord in healthy subjects are to be obtained. These results, although obtained in healthy subjects, can provide useful information, as several studies have shown that the same intervention produced spinal plasticity in both healthy and neurologically injured patients [17]–[19], [62]. The general protocol for the experiments is the following:



In order to validate the developed neurorehabilitation platform, EMG signal acquired during the therapy is to be used, in order to acquire different metrics that characterize the accuracy in time of the delivered stimulation. Also, a brief survey is to be filled by each subject evaluating the ease of use of the platform, whether the stimulation is unpleasant, whether it increases their sense of fatigue...

Additionally, changes in reciprocal inhibition in healthy subjects are to be assessed. The hypothesis is that the proposed intervention of cycling plus afferent stimulation is able to induce neural plasticity to enhance coordination between muscles, what would mean that reciprocal inhibition is increased, both in healthy subjects and neurologically injured patients. The expected outcome is that reciprocal inhibition is increased immediately after the intervention (POST measurement) with respect to its baseline value before intervention (PRE measurement). Previous studies indicate that baseline values of reciprocal inhibition are around 20%, and it is increased to 30-40% after intervention [10], [12], [13], [19].

The designed intervention is an in-phase, ON-OFF sensory electrical stimulation to only one muscle. Before the intervention, the natural activation pattern of the Tibialis Anterior muscle is to be obtained, and the muscle is considered to be active in the pedaling phases in which its EMG activation is above its own average. Then, during the intervention, sensory electrical stimulation is to be delivered at the same time that the Tibialis Anterior muscle is naturally active. Although the developed platform has been designed to support complex stimulation patterns in several muscles simultaneously, it has been decided to use a simpler stimulation strategy (one muscle, ON/OFF) in order to reduce the number of factors affecting the outcome. Thus, results would only concern cycling plus sensory electrical stimulation, without adding additional variables.

Each experimental session is composed of the following sections:

- **Electrode placement and calibration**: careful and precise positioning of electrodes is fundamental for achieving reciprocal inhibition.
- **PRE Reciprocal inhibition testing**: Reciprocal inhibition is tested prior to the intervention.
- **Measurement of natural activation patterns**: EMG signal is recorded for one minute before the intervention, in order to obtain the biological cycling pattern of each subject. This cycling pattern is to be used to design the intervention itself: stimulation is to be delivered at moments in which the muscle is active (average activation above the mean), and not stimulated for the rest of the cycle. The chosen muscle was Tibialis Anterior muscle, which is the antagonist of soleus muscle.
- **Intervention (pedaling + sensory stimulation)**: The intervention consists of ten minutes of active pedaling with fixed pedaling resistance. Subjects are instructed to maintain a cadence around 45rpm, and are given visual feedback about their actual cadence at each point of time. EMG is recorded during the intervention for posterior analysis of the stimulation. While the subject is pedaling, sensory stimulation is delivered to the common peroneal nerve innervating the tibialis anterior muscle. The stimulation is to be delivered simultaneously to the biological activation of the muscle, extracted from the pedaling before the intervention. This bioinspired strategy provides augmented sensory feedback for tibialis activation, specifically customized for each subject. The intensity of the stimulation should be 70% of the minimum value required for causing muscle contraction. Thus, the stimulation is only sensory and caused no contraction. The subjects reported that this level of sensory stimulation was noticeable but not uncomfortable and did not interfere with execution of pedaling.
- **POST Reciprocal inhibition testing**: Reciprocal inhibition is also tested right after the intervention, in order to find whether the proposed therapy has any plastic effect on the spinal modulatory mechanisms.

The detailed protocol followed during the sessions can be found in Appendix B.

4. RESULTS AND DISCUSSION

This Bachelor's Thesis has two objectives, covering the first steps towards the development of a neurorehabilitation platform based on pedaling plus sensory electrical stimulation:

- Designing and developing a neurorehabilitation platform composed of a robotic cycling ergometer and an electrical stimulator, closely integrated and synchronized.
- Researching, designing and developing the experimental techniques and procedures to be used for the assessment of the efficacy of the therapy.

The fulfilment of the objectives are assessed in the next sections, based on the pilot study that has been conducted.

4.1. Performance of the neurorehabilitation platform

The development of the neurorehabilitation platform is the main outcome of the project, as it constitutes the basis for future neurorehabilitation applications that include sensory electrical stimulation and that may include complex stimulation patterns based on muscle synergies. Performance of the neurorehabilitation platform has been assessed through a pilot study with five healthy subjects. A picture of the developed neurorehabilitation platform can be seen in Figure 4.1.

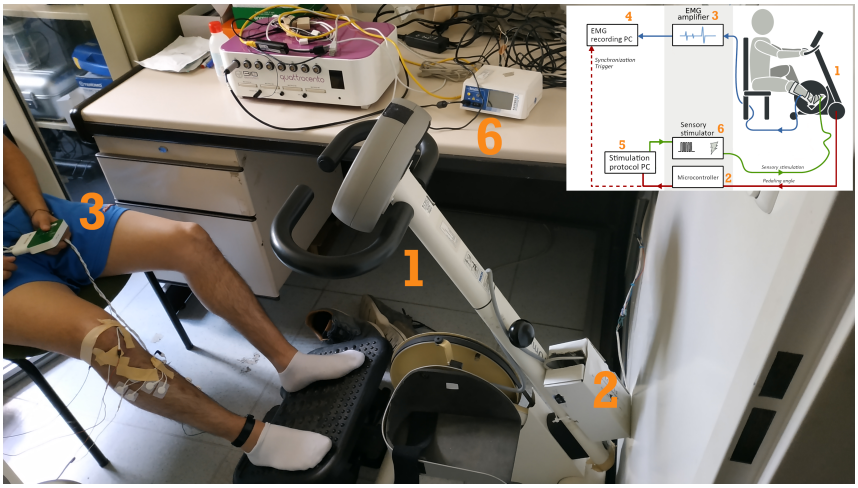


Figure 4.1. *Picture of the developed neurorehabilitation platform. The diagram of the design can be seen in the superior right corner. Elements of the design have been indicated in the picture with the corresponding numbers. Elements 4 (EMG recording PC) and 5 (Stimulation protocol PC) are out of sight.*

Real time constitutes the key feature of the system. If not properly achieved, stimulation would be delivered at different times than what is expected. Therefore, it would not be possible to properly deliver bio-inspired strategies based on natural patterns of muscle activation. In order to check whether the developed neurorehabilitation platform stimulated at the desired times, different metrics (onset lag, end lag and desired-real stimulation correlation) will be analyzed, as detailed in the Materials and Methods section. First, it is necessary to align the data (see Figure 4.2). This is possible due to the simultaneous synchronization triggers delivered to both systems.

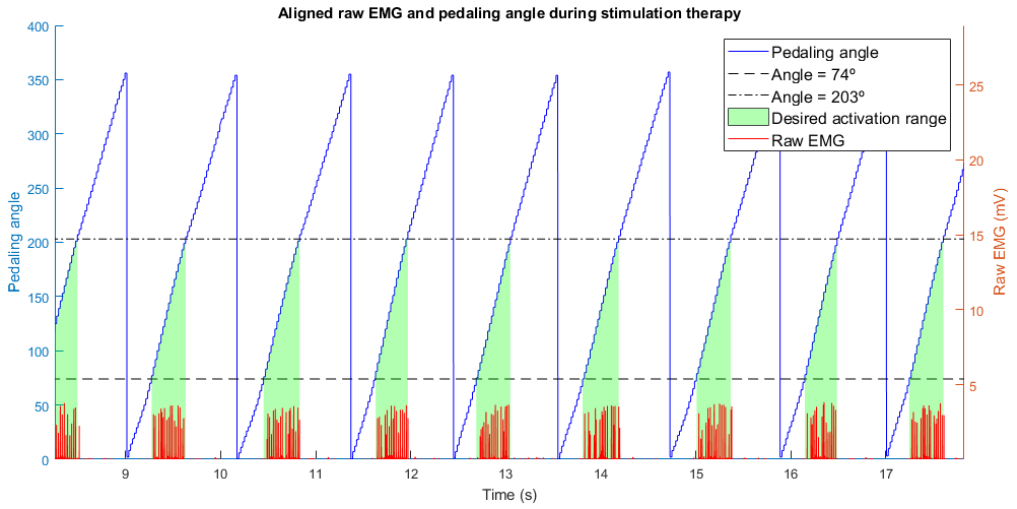


Figure 4.2. *Pedaling angle, which is used for real time stimulation, and EMG data are aligned and displayed. The peaks in the EMG recording are stimulation artifacts, which are much larger than intrinsic muscle activity. In the figure, it can be seen how stimulation is always delivered at a specific portion of the pedaling cycle.*

As it can be seen in Figure 4.3, there is some delay between the angle at which the stimulation should start and the moment when the first stimulation pulse is actually delivered. This is to be denominated "onset lag". Similarly, "end lag" is defined as the delay from the time the stimulation range ends until the last stimulation impulse is given. This values have been obtained for each cycle and are represented in Figure 4.4.

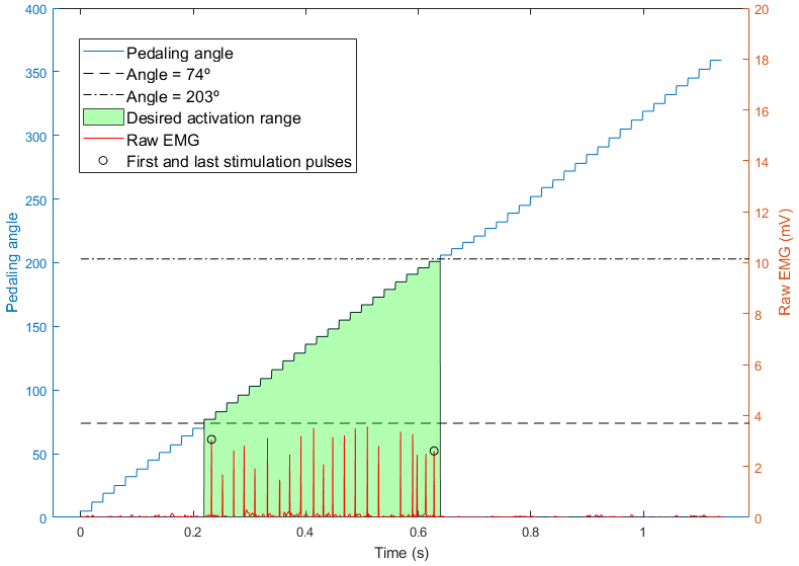


Figure 4.3. *Just one cycle is presented for closer inspection. The predefined stimulation range is plotted as a function of the pedaling angle. Although the time between consecutive impulses is somehow variable, it can be seen that stimulation is delivered within the defined angle limits (74º and 203º).*

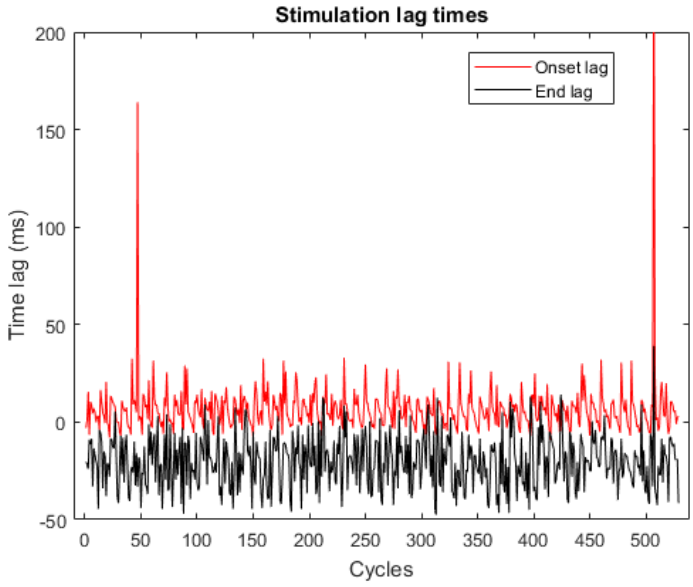


Figure 4.4. *Onset lag and end lag values for all cycles for one of the subjects, corresponding to 10 minutes of pedaling therapy. Except for two specific cycles which present a high onset lag (stimulation was delayed 150-200ms for uncertain reasons), all the other cycles (more than 500 cycles) present a onset lag lower than 30ms, with an average value of 6.7 ms. End lag is negative for most cycles, with a mean of -20.2 ms. A negative value indicates that the last impulse is given before the defined stimulation range ends, so actually it is delivered within the established limits.*

Onset lag and end lag have been analyzed and yield small values, in the order of tens of milliseconds. This was a real concern, as it is necessary to consider that information that is obtained in the encoder has to be sent to the microprocessor, then to the computer and finally to the stimulator, which must execute the commanded stimulation. Any of these devices or the communication protocols between them could potentially slow down the flow of information and disable real-time execution.

Furthermore, it can be observed that onset and end lags do not vary over time, that is, there is no drift. That means that delay does not get accumulated over time, and time lags are similar between the beginning of the stimulation and at the end, ten minutes later. This can be visually checked in Figure 4.4, but it was also statistically tested by performing one way ANOVA test for each variable subdivided in sets of fifty cycles. No significant difference was observed in between any of the subsets for any of the subjects on either variable.

Finally, rectified EMG data was averaged across all cycles in order to obtain an average stimulation profile. Measuring linear correlation of the average stimulation profile with the defined stimulation range yields a correlation coefficient around 98% for all subjects. The results for one subject can be seen in Figure 4.5. Cross-correlation was also tested, yielding that the best correlation is achieved with no shift.

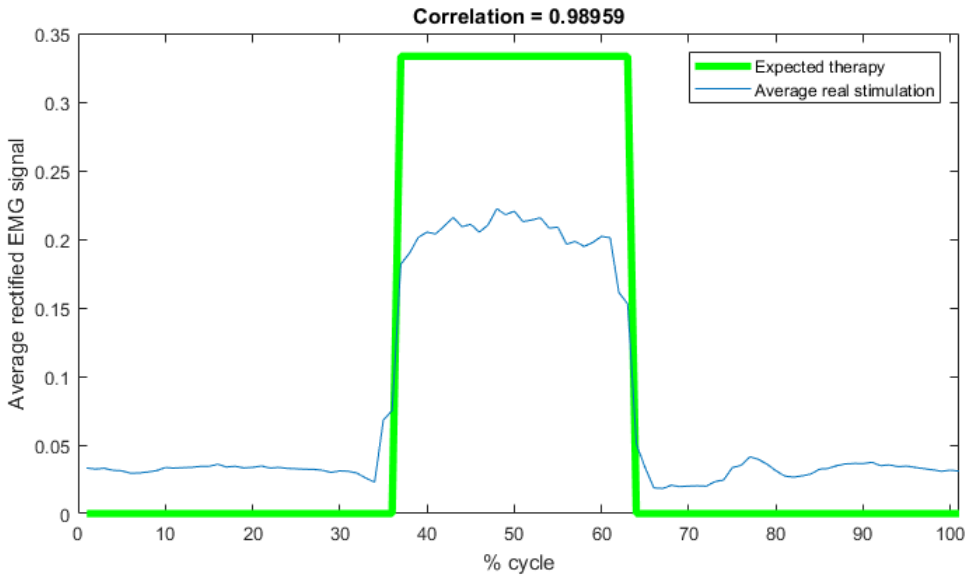


Figure 4.5. *Recorded EMG (rectified) has been averaged through all cycles. It can be seen that stimulation falls strictly inside the prescribed limits, yielding a correlation value of 98.959% with the input therapy.*

Therefore, by analyzing the stimulation data, it was confirmed that the delays are within very reasonable limits and that real-time therapy execution is possible in the designed setup. Moreover, it is possible to calibrate the system and ensure proper behaviour by performing a short therapy application on a single healthy subject and analyzing the recorded data to obtain these metrics.

4.2. Usability and comfort of the developed neurorehabilitation platform

The developed neurorehabilitation platform has also been tested in terms of usability and comfort. Furthermore, use of sensory afferent stimulation aims to overcome muscle fatigue induced by FES. Thus, subjects have been also asked to evaluate their self-perceived fatigue and to which extent they attribute it to sensory electrical stimulation. All subjects filled a survey after the experiment concerning these aspects. Results are displayed in Table 4.1.

	Subject data			Reciprocal inhibition testing	
	Neurological injury	Physical leg injury	Previously used electrical stimulation	Discomfort related to supramaximal M_{max} elicitation	Discomfort related to H reflex elicitation
Subject 1	No	No	Yes	4 / 10	3 / 10
Subject 2	No	No	No	9 / 10	7 / 10
Subject 3	No	No	Yes	5 / 10	2 / 10
Subject 4	No	No	Yes	1 / 10	1 / 10
	Usability of the neurorehabilitation platform. Fatigue due to sensory electrical stimulation.				
	Ease of use of the neuro-rehabilitation platform	Discomfort due to sensory electrical stimulation during pedaling	Fatigue due to pedaling plus sensory electrical stimulation	Self-perceived fatigue increase due to sensory electrical stimulation	Maximum therapy duration that could sustain
Subject 1	4 / 5	2 / 5	2 / 5	3 / 5	45 minutes
Subject 2	5 / 5	3 / 5	1 / 5	1 / 5	30 minutes
Subject 3	5 / 5	1 / 5	3 / 5	2 / 5	45 minutes
Subject 4	5 / 5	1 / 5	1 / 5	1 / 5	>1 hour

Table 4.1. SURVEY OF USABILITY, COMFORT AND FATIGUE

Concerning discomfort related to reciprocal inhibition testing, no subject reported pain at any moment. Only one subject reports discomfort higher than 5 over 10. It is important to note that this subject is the only one who never received electrical stimulation before. In the experience of the author, electrical stimulation produces more discomfort the first times it is experienced, as it is a new sensation. Then, tolerance to the sensation largely increases with exposure.

Concerning comfort and ease of use of the neurorehabilitation platform and the associated therapy, all subjects reported high ease of use (mean 4.75 over 5) and low discomfort due to sensory electrical stimulation (mean 1.75 over 5). About fatigue due to the therapy (cycling plus stimulation), low levels have been reported (mean 1.75 over 5). Moreover, subjects reported low fatigue increase due to sensory electrical stimulation (mean 1.75 over 5). All subjects confirmed that could sustain the therapy for longer periods of time. When enquired about the maximum amount of time they would suggest for this therapy, an average duration of 45 minutes per session was given.

4.3. Reciprocal inhibition modulation of the H reflex

The second objective concerns the selection of an appropriate, validated method for assessing muscle coordination. Reciprocal inhibition and its modulatory effect on the soleus H reflex is the chosen method. It is necessary to mention that it is a neurophysiological measure and therefore proper methodology is fundamental for correct conditioning and modulation of the reflex. A large amount of time and effort has been devoted to testing and refining the experimental setup and procedure. Significant reciprocal inhibition was defined as a significant statistical difference between the mean of test reflexes and the mean of conditioned reflexes. Student t-test were carried out with significance level $P < 0.05$.

In the pilot study, five healthy subjects participated. It was possible to obtain significant reciprocal inhibition in four of those subjects, while in the fifth subject it was not possible within the prescribed experimental time and the session had to be aborted. Thus, the fifth subject was discarded. Due to the methodological complications related to the technique, achieving reciprocal inhibition in four out of five subjects can be considered as a good rate of success. However, many of the data sets had high variability. The variances of each data set, the results of testing for the presence of reciprocal inhibition and the obtained values for each subject can be found in Appendix C. For each subject, the data set which yielded the most significant reciprocal inhibition is to be displayed and discussed here:

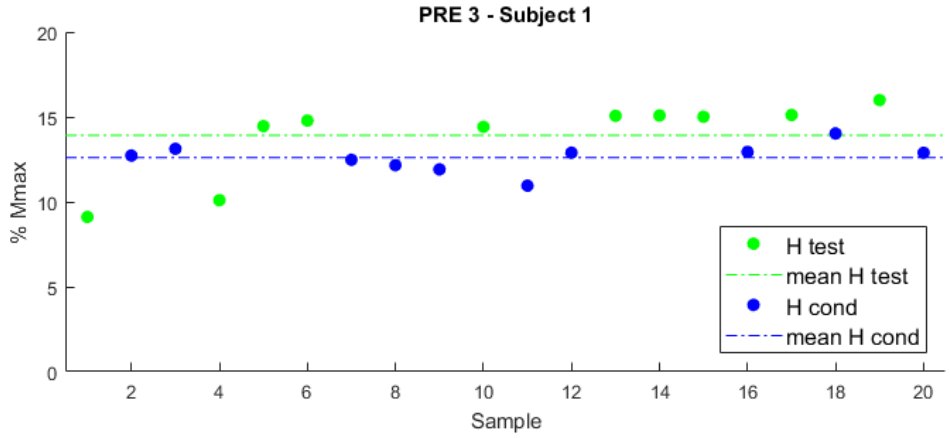


Figure 4.6. *Reciprocal inhibition example from subject 1. Variability of samples of subject 1 leaded to apply analysis of variance. For this data set, analysis of variance yielded that the first five samples should be disregarded, as the amplitude of H_{test} is significantly lower for samples 1 and 4 than for the rest of the recording. Stability of the remaining samples is very good ($\sigma_{Htest} = 0.49$). Student t-test for significant reciprocal inhibition yields a value of 3.267 E-05, and RI = 16.41%.*

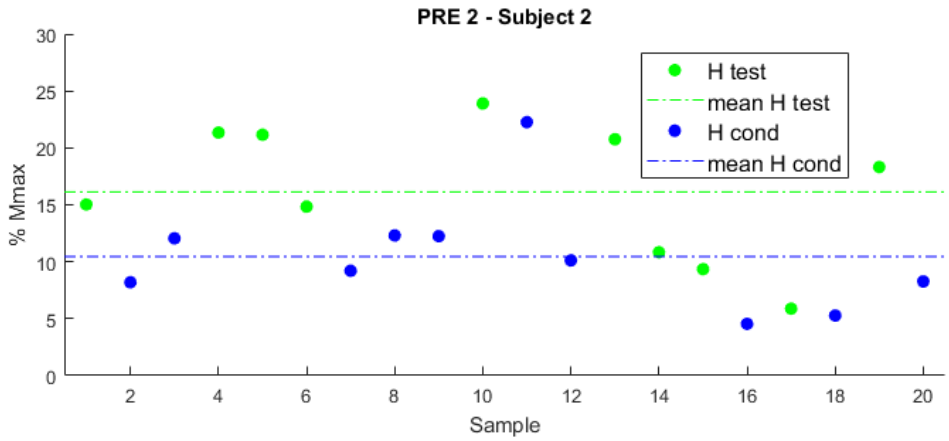


Figure 4.7. *This data set presents much larger variability. Nevertheless, H_{test} in the 2-13 subset yielded an acceptable variance of H_{test} (σ_{Htest} = 3.345). Sample 11 has been removed as an outlayer. Finally, the subset yields RI = 47.60% with p-value = 0.0012.*

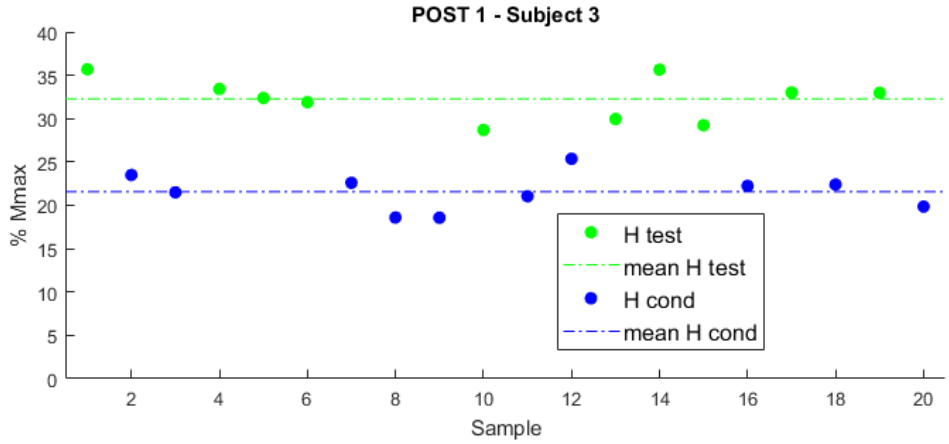


Figure 4.8. *Data for subject 3 is stable and no analysis of variance has been performed. Although there is some variability in H_{test}, it is a normal amount of variability (σ_{Htest} = 2.43). Most importantly, there is no drift in amplitude, and all H_{test} remain around the mean for the full recording. Reciprocal inhibition calculation for the full set yields RI = 33.22% with a p-value of 5.115 E-09.*

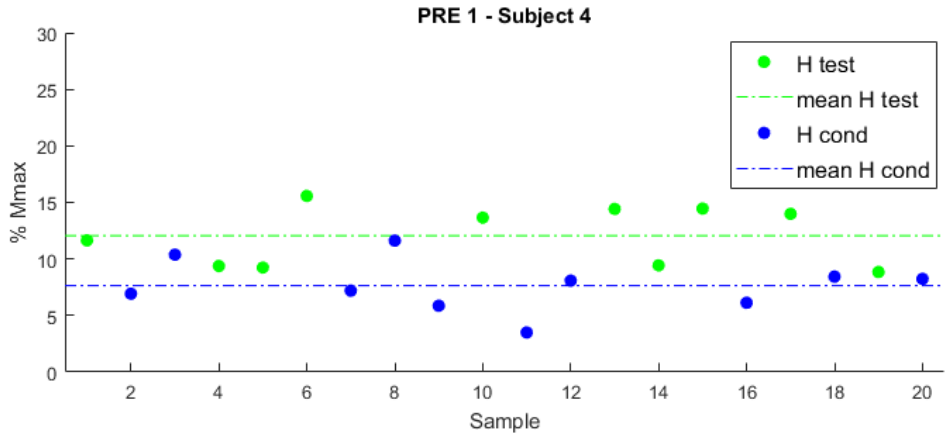


Figure 4.9. *Several data sets from subject 4 had to be discarded due to extremely large variability that could not be overcome even through analysis of variability. Nonetheless, other data sets could be directly analyzed as they presented low variability. Variability of this data set is good (σ_{Htest} = 2.63). It yielded RI = 36.69% with p-value = 8.7 E-04*

It is fundamental to note that selection of subsets by analysis of variance has been performed only based on the σ_{Htest}, σ_{Mtest} and σ_{Mcond}, as explained in the Materials and Methods section, and never taking into account the reciprocal inhibition results, in order to avoid biasing of the results.

Due to the high variability of the data, in most cases it was not possible to maintain H_{test} in the 15-25% M_{max} range, as indicated in the literature. It is unknown to which extent this may have affected the results.

Therefore, the technique has been correctly performed to a large extent, enabling the acquisition of significant reciprocal inhibition in four out of five subjects. However, variability is still too high, so the technique needs to be further tested and refined in order to lower the variability of the data.

4.4. Effects of sensory electrical stimulation on spinal plasticity

The Bachelor's Thesis objectives were to design, build and validate the neurorehabilitation platform, as well as the experimental procedures needed to assess the effects of the proposed therapy. In addition to the fulfilment of those objectives, it was possible to obtain some preliminary results on the effectiveness of cycling plus sensory electrical stimulation applied on healthy subjects. Either direct analysis (when data sets had low variances) or analysis after selecting subsets of minimal variance was carried out for all four subjects in which significant reciprocal inhibition was found. The results for all four analyzed subjects can be seen in Figure 4.10.

According to previous studies, reciprocal inhibition previous to the intervention is typ-

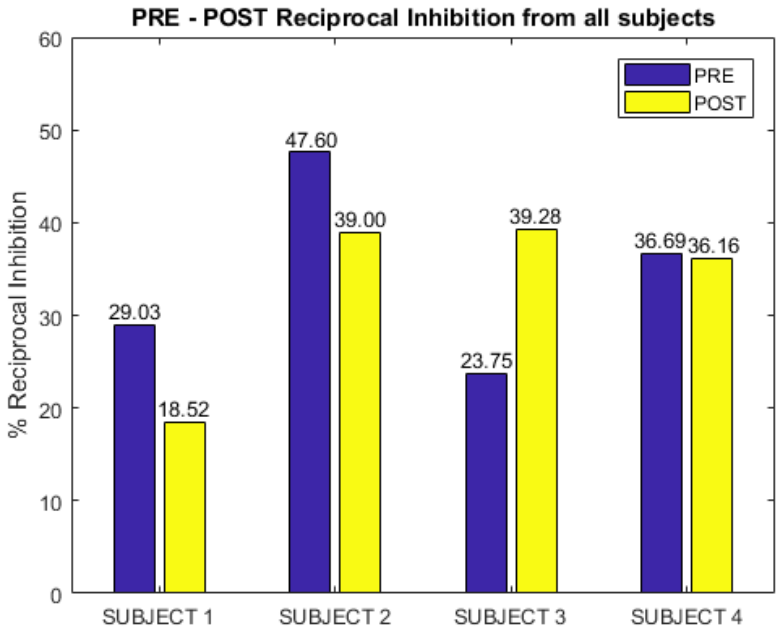


Figure 4.10. *Results of Reciprocal Inhibition before (PRE) and after (POST) the intervention of pedaling with sensory electrical stimulation, for the four subjects analyzed.*

ically around 20% (baseline) and it increases to 30-40% after intervention. [10], [12], [13], [19]. However, if pedaling plus sensory afferent stimulation does not have any effect of spinal excitability, reciprocal inhibition before and after the intervention would yield baseline values (around 20%). However, the results obtained in the pilot study (see Figure 4.10) are highly heterogeneous and do not coincide with any of the two situations (either the therapy does or does not have an effect) described above:

- In subject 1, there was a decrease in reciprocal inhibition. The PRE value was somehow high, and the POST value corresponds to a value corresponding to baseline.
- Subject 2 presents a decrease in reciprocal inhibition. Nevertheless, the PRE value is extremely high, what may suggest that there may be some kind of methodological error either in the acquisition or processing of the data.
- Subject 3 behaves as expected, according to previous studies. It is also important to note that for this subject the data was the most stable, and direct analysis could be performed. Moreover, two data sets were available for PRE condition and another two for POST, each pair having similar values. Therefore, data from this subject is the most reliable of all the acquired data.
- In subject 4, reciprocal inhibition is maintained equal before and after the intervention. However, PRE value is high according to the literature.

It is also important to note that for subjects 2, 3 and 4, the obtained value for POST

reciprocal inhibition (35-40%) is very homogeneous, and similar to what is reported in the literature. This may suggest that sensory electrical stimulation may actually be producing increased values (35-40%) of reciprocal inhibition. It is the initial values what are the most heterogeneous across different subjects and differ from what is reported in the literature.

Nevertheless, the sample size (n=4) is too small to draw any solid conclusion. Further studies involving a larger amount of subjects are required in order to know if sensory electrical stimulation does produce an effect on reciprocal inhibition.

5. CONCLUSIONS

The Bachelor's Thesis has developed the neurorehabilitation platform and experimental techniques to be used in this line of research, aimed for the neurorehabilitation of neuromuscular coordination of walking in neurological patients. A complete setup has been designed and built, integrating different technologies and equipment in order to deliver the desired stimulation at specific points of the pedaling cycle. Real time execution, what is key in this application, has been verified through the data obtained in the pilot study. All the described work has been carried out by the author of this Bachelor's Thesis.

The developed setups, procedures and codes already support stimulation of up to 8 individual muscles plus any kind of stimulation pattern (modulated both in amplitude and time) for all of them. Also, it is able to extract EMG data of up to 16 individual muscles and extract their natural activation pattern. Therefore, it is already enabled to extract and stimulate complex, personalized patterns, that could be based on the natural activation patterns of the subject and/or the muscle synergies hypothesis.

Subjects of the pilot study reported ease of use of the neurorehabilitation platform, low discomfort associated with the sensory electrical stimulation, and low fatigue due to the therapy. The latter is specially relevant as the purpose of applying sensory electrical stimulation is to overcome fatigue induced by functional stimulation. Also, subjects reported that they could perform the therapy in sessions of up to 45 minutes.

This neurorehabilitation platform also aims to have much lower cost than current solutions in order to enable access for a wider segment of the target population and even enabling home use. Used devices are relatively inexpensive and easy to use when compared to current solutions like Lokomat. Moreover, cycling has proven to be a good alternative for gait neurorehabilitation and it is easier to perform for most patients and at earlier stages after injury, when neurorehabilitation is most effective. Also, it would greatly reduce the amount of highly specialized human resources that each patients needs.

Secondly, the necessary setup for testing reciprocal inhibition in Hoffmann reflex has been developed. Modulation of spinal reflexes is well established in the literature and widely used for assessment of neural plasticity at the spinal cord. However, it is a neurophysiological measurement and thus it implies methodological difficulties. Nonetheless, the technique has been developed to a large extent and reciprocal inhibition has been obtained in four out of five subjects. However, variability of data is still high, what is assumed to be the origin of some results which do not adjust to what is described in the literature. Therefore, work should be carried out in order to further refine the technique.

It is also relevant to note that the developed GUI greatly facilitates the tasks of the experimenter and has been key for obtaining proper reciprocal inhibition. It enables connection

to the EMG acquisition device, real time monitoring of EMG data, real time display and monitoring of obtained reflexes, storage of data and data analysis. It has proven very valuable during the performed pilot study, and it will be an important tool also for future researchers. Furthermore, the code is available, commented and easily modifiable. Thus, it can be tuned for other specific applications which may have some special requirements and procedures.

The results of the pilot study validate the performance of the platform and the methodologies and setup enabling to obtain reciprocal inhibition, as just discussed. Additionally, the pilot study provided some preliminary results concerning the effects of cycling plus sensory afferent stimulation on coordination. Data obtained before the intervention (PRE) is highly heterogeneous. Nevertheless, data obtained after the intervention (POST) is very similar in three of the four subjects and very close to the values reported in the literature. Data for subject 3 is the most stable and moreover it is consistent with what is reported in the literature. In this subject, a clear increase in reciprocal inhibition following cycling plus sensory electrical stimulation was seen. Nevertheless, high variability, heterogeneous results and low number of subjects make impossible to draw any solid conclusion. Thus, further refinement of the methodology for obtaining reciprocal inhibition needs to be performed in order to achieve higher stability and homogeneity, and a study with a larger sample size should be carried out.

Finally, all the developed codes and applications have been thoroughly commented and documented for ease of use by future researchers who will continue this line of research.

5.1. Limitations of the current work

Main limitations of this work are related with the high variability when measuring H reflex modulation led to heterogeneous, non-conclusive results concerning the efficacy of the proposed intervention. Furthermore, H_{test} levels could not be maintained around 20 % M_{max}, as indicated by the literature. It is unknown how this may have affected the results. Moreover, the sample size in the pilot study is too small to draw any kind of conclusion

5.2. Future work

The present Bachelor's Thesis has developed the necessary setups for performing a variety of different experiments and techniques related with electrical stimulation in pedaling. Therefore, it opens a variety of work lines in order to find and assess effective therapies for improving coordination of neurological patients:

- First of all, variability of the recordings of reciprocal inhibition should be addressed,

in order to be able to acquire stable, reliable data.

- A study with bigger sample size should be performed in order to assess the effects of pedaling plus sensory afferent stimulation and generate a clear conclusion about its effectiveness.
- Studies to test the effects of pedaling plus sensory electrical stimulation against pedaling plus FES stimulation. Both spinal plasticity and muscle fatigue should be measured.
- Studies to test the effects of sensory electrical stimulation when combined with either active pedaling (the subjects does effort for pedaling) or passive pedaling (the gear inside the cycle ergometer does the work).
- Studies to test the effects of different timings (synchronously with muscle activation, in the opposite phase or delayed with respect to natural muscle activation) or more sophisticated activation patterns on reciprocal inhibition. These can be personalized complex bio-inspired patterns extracted from the intrinsic subject muscular activation, and using the muscle synergies hypothesis.
- Studies should be carried out both in healthy subjects and in neurologically injured subjects.

Finally, after the effectiveness of different stimulation techniques and stimulation patterns have been thoroughly tested, a commercial neurorehabilitation device based on the developed system could be created and distributed in order to enable low-cost walking neurorehabilitation for neurologically injured patients.

6. BUDGET AND PROJECT TIMELINE

This project had expenses of two types: first, use of technical equipment (hardware and software) required to develop the project. Second, human resources cost should be included as the student, the tutor and several other researchers have been involved in the development of the work. Costs associated to technical equipment can be seen in Table 6.1, and costs associated to human resources can be seen in Table 6.2.

Technical Equipment	Cost per unit (€)	Total time of use	Depreciation time (months)	Number of units	Total cost (€)
MOTOmed viva2 cycle ergometer	4.150	3 months	48 months	1	260
Arduino UNO microcontroller	24	6 months	24 months	2	12
Trentadue EMG amplifier	10.000	3 months	48 months	1	625
Quattrocento EMG amplifier	27.950	20 days	48 months	1	398
Stimulation protocol computer	640	3 months	24 months	1	80
RehaStim stimulation device	4.260	3 months	48 months	1	266
Digitimer DS7A single pulse stimulator	3.888	3 months	48 months	2	486
Personal computer	700	9 months	36 months	1	175
Stimulation electrodes	2	Single use	-	30	60
EMG recording electrodes	1.5	Single use	-	50	75
MATLAB Academic License	0	9 months	12 months	1	0
				Total cost	2.473 €

Table 6.1. TECHNICAL COSTS OF THE PROJECT

Also, a timeline of the project has been designed attending to the time devoted to each of the tasks (see Table 6.3). The chronological time allocation is specified, as well as how much of the total project time (estimated in 650 hours, not including the process of making this report) has been devoted to each of the tasks.

Human resources	Hours	Cost / hour (€)	Total cost (€)
Student	650	15	9.750
Tutors	30	40	1.200
Other senior researchers nvolved in the project	20	40	800
		Total cost	11.750 €

Table 6.2. HUMAN RESOURCES COSTS OF THE PROJECT

BACHELOR'S THESIS PROJECT TIMELINE		Sept	Oct	Nov	Dec	Jan	Febr	March	April	May	June	% TOTAL TIME
Literature review												10%
Design and development of the rehabilitation platform	Encoder communication											10%
	Stimulator and Simulink model											10%
	Ensure real time performance											10%
	Integrate EMG in the platform											5%
Development of reciprocal inhibition experimental technique and setups												35%
Pilot study (including analysis of the results)												20%

Table 6.3. TIMELINE OF THE DEVELOPMENT OF THE PROJECT

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APPENDIX A

HOJA INFORMATIVA

Este documento contiene la información sobre el experimento en el que usted va a ser voluntario. Por favor, léalo atentamente y pregunte al experimentador cualquier duda que le pueda surgir.

Objeto del experimento

Este experimento se realiza en el marco del Trabajo de Fin de Grado (TFG) titulado provisionalmente "*Afferent stimulation rehabilitation platform - Neurophysiological study and rehabilitation potentials*" y habiendo sido realizado en el NEURAL REHABILITATION GROUP (NRG), Instituto Cajal (CSIC) durante el curso 2018/2019.

El experimento tiene dos objetivos:

- Valorar los efectos de la estimulación aferente en sujetos sanos en la coordinación de los músculos del segmento inferior de la pierna.

- Validar el funcionamiento de la plataforma de rehabilitación desarrollada a este efecto durante el mencionado Trabajo Fin de Grado.

Participantes

Los criterios para ser seleccionado en este estudio son:

- Persona adulta, de cualquier sexo.
- Ausencia de lesión o enfermedad neurológica.
- Ausencia de cualquier otra lesión que impida o restrinja los movimientos de marcha y pedaleo.

Descripción del experimento

El experimento consiste en la realización de 10 minutos de pedaleo en una bicicleta estática mientras se proporciona estimulación eléctrica de baja intensidad en la pierna (la "intervención").

Para valorar los efectos de la intervención, se realizarán medidas electro-fisiológicas (reflejo de Hoffmann en el músculo sóleo) antes y después del pedaleo. Dichas medidas se realizarán proporcionando pulsos eléctricos a los nervios tibial y peroneal, y midiendo la respuesta muscular en los músculos de la pierna mediante electromiografía (EMG). Dichas estimulaciones y mediciones se realizarán con electrodos superficiales. Por tanto, el experimento es completamente no invasivo.

El experimento consta de dos sesiones, a ser realizadas en días diferentes, de duración aproximada de 1 hora 45 minutos. Adicionalmente, si la persona voluntaria acepta, se realizará una tercera sesión.

Electrodos superficiales y estimulación eléctrica

Los electrodos superficiales (tanto de estimulación como de medición) son pequeños dispositivos que se colocan sobre la piel y suministran corriente eléctrica de baja densidad de forma focalizada en el punto en el que son colocados.

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La estimulación eléctrica de baja intensidad que será suministrada es inocua e indolora, aunque para alguna personas la sensación que produce es molesta. La estimulación eléctrica produce activación de los músculos y nervios cercanos, por lo que el sujeto puede experimentar leves contracciones involuntarias de músculos cercanos. Esto es un efecto normal y totalmente transitorio, no dejando ningún efecto duradero después de la propia estimulación.

Posibles efectos adversos

La estimulación eléctrica puede causar picor e irritación en la piel a algunas personas, así como molestias durante la estimulación. En caso de sufrir cualquiera de estos síntomas, comuníqueselo al experimentador.

El participante podría fatigarse debido al ejercicio de 10 minutos de pedaleo. En cualquier caso, se pretende evitar efectos de fatiga muscular, por lo que se ruega que se seleccione una velocidad y resistencia cómodas y que comunique al experimentador si se produce fatiga durante el desarrollo del experimento.

Beneficios de la experimentación

El presente estudio busca explorar nuevas terapias para pacientes con lesiones y/o enfermedades neurológicas que afectan a su capacidad motriz (lesión medular, ictus, parálisis cerebral...).

Usted, como voluntario sano, no percibirá beneficio alguno al realizar este experimento.

Tratamiento de datos

Se guardará estricta confidencialidad conforme con la normativa vigente (Ley Orgánica de Protección de Datos de Carácter Personal 15/1999, de 13 de Diciembre). Se le garantizan los derechos de acceso, rectificación, cancelación y oposición. Para ejercer los mismos, diríjase por escrito al investigador. Tanto a los datos como a los resultados sólo tendrán acceso los investigadores y serán guardados y archivados de forma confidencial tras la finalización del mismo. Los resultados serán presentados como parte del Trabajo Fin de Grado en el que se enmarca este experimento, así como pudiendo ser publicados en diferentes conferencias y revistas médicas. En cualquier caso, no se aportaran datos personales.

Precauciones antes y después del experimento

No es necesaria ninguna preparación previa a la prueba, ni debería notar ningún efecto posterior a ella. Si observa cualquier problema o cambio y cree que pueda estar relacionado con el experimento, contacte al experimentador.

Voluntariedad

Su participación en este estudio es completamente voluntaria y es libre de abandonarlo en el momento que desee y sin necesidad de aportar ninguna justificación.

APPENDIX A

HOJA DE CONSENTIMIENTO INFORMADO

"Afferent stimulation rehabilitation platform - Neurophysiological study and rehabilitation potentials"

Yo, D/ Dña. _____ , participante del presente estudio, en pleno uso de mis facultades, y de forma libre y voluntaria, **expongo que he sido debidamente informado/a por _____ mediante una entrevista a fecha (día _____ , mes _____, año _____), previa al comienzo del estudio al que se refiere el presente consentimiento, y que doy mi consentimiento informado para entrar a formar parte del presente estudio**.

Afirmo que he recibido explicaciones tanto de forma verbal como escrita, sobre la naturaleza y propósitos del protocolo, beneficios, riesgos, y medios con que cuenta el laboratorio y el Instituto Cajal para su realización, habiendo tenido ocasión de aclarar las dudas que me han surgido al respecto.

Por lo que MANIFIESTO que he entendido y estoy satisfecho de todas las explicaciones y aclaraciones recibidas sobre el proceso citado.

Entiendo que este consentimiento puede ser revocado por mí en cualquier momento, antes y durante la realización del protocolo experimental.

Podrá ejercer sus derechos sobre sus datos, así como informarse sobre cualquier otra cuestión relativa al experimento, sus objetivos, etc. contactando a:

Javier García Ordóñez
javier.g.ordonnez@gmail.com
678854194

Y, para que así conste, firmo el presente documento en,

Madrid, a día..... de..... del 20.....

Firma del participante: Firma del Experimentador:

El consentimiento podrá ser revocado durante el experimento por parte del voluntario en cualquier momento sin necesidad de dar ninguna explicación.

APPENDIX B

EXPERIMENTAL PROTOCOL

Day before

- Charge Trentadue.
- Charge Quattrocento.

Before the experiment

- Connect Arduino to the two Digitimers, and place the other Arduino in the ergometer box and connect it to the CAN port.
- Ensure that there are enough stimulation and recording electrodes for the next experiments.
- Connect the angles measuring Arduino's trigger to the EMG device in charge of recording the pedaling EMG.
- Place Rehastim, as well as related wires, on the table and charge it.

General experiment outline

- Electrode placement and calibration
    - **Clean with alcohol** and shave the area.
    - Place EMG electrodes, following SENIAM recommendations (see Annex). Place reference band at the ankle.
    - Connect Arduino and Trentadue to the GUI. Check quality of EMG signal. Perform successive contractions to check all channels. **Fix wires with tape**.
    - Ensure that the subject sits in proper posture for H reflex testing: 100° hip angle, knee 45° from maximum extension, 110° ankle plantarflexion.
    - **Ask the subject to fully relax, close eyes and not to move at all**.
    - Place the stimulation electrodes (**with gel**) of CPN and ensure that no activation is found in peroneal muscles even at TA Mmax. **Fix electrodes with tape**.
    - Place the stimulation electrodes (**with gel**) of PTN and ensure that only the soleus muscle gets activated. Check that there is no TA activation even at SOL Mmax. Ideally, gastrocnemius should not be stimulated either. **Fix electrodes with tape**.
- RI calibration
    - Calibrate MT for TA.
    - Calibrate SOL Mmax.
    - Find test stimulation intensity at which H reflex of size 15%Mmax is elicited.
    - Perform a test measure, to check constancy of H reflex.
    - Perform CTi test. Once a specific CTi is selected, send it to Arduino.
- Perform RI (PRE) measurement.
    - Mark a point (with a marker) the point at which the punctual electrode has been placed in the popliteal fossa, for repeatability after the pedaling therapy.
- Ask the subject to start pedaling. Start the EMG recording and then run the OnlyAngle Simulink model for 60 seconds.
- Extract the TA activation pattern from the data from previous step.

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- Connect the stimulation electrodes of CPN to Rehastim and find the MT of TA for continuous afferent stimulation. In the therapy model, set stimulation intensity at 70% of this value.
- Ask the subject to start pedaling. Start the EMG recording and then run the Therapy Simulink model for 600 seconds (10min).
- Repeat the RI measurement:
    - Ensure that the subject sits in proper posture for H reflex testing: 100° hip angle, knee 45° from maximum extension, 110° ankle plantarflexion.
    - **Ask the subject to fully relax, close eyes and not to move at all**.
    - Check that high stimulation in CPN does not stimulate peroneal muscles, and high stimulation in PTN does not stimulate TA.
    - Calibrate MT of TA.
    - Calibrate Mmax.
    - Find test stimulation intensity at which H reflex of size 15%Mmax is elicited.
    - Record RI POST (twice if needed).
- Store all recorded data.

STIMULATION ELECTRODE LOCATION AND PROCEDURE

When stimulating the CPN (TA):

- Place the stimulation electrode (cathode, black) between the fibular head (lateral side of the leg) and TA muscle belly.
- Place the anode (red) electrode (**with gel**) 3 cm above.
- Search for the location of the stimulation electrode where TA Mmax is elicited (high intensity) without generating an M wave in the peroneal muscles. Note that when placing the electrode closer to TA muscle belly (or even onto the muscle belly), higher intensities will be needed when compared to stimulation at the fibular head.

When stimulating the PTN (soleus):

- Place the anode (red) electrode (**with gel**) in the anterior side of the leg, below the patella and medial.
- Place the stimulus electrode in the fossa poplitea, on the inside from the center of the knee.
- Increase stimulation intensity until Mmax of soleus is reached, and ensure that TA is not stimulated (no M nor H wave).
- Also, gastrocnemius should not be stimulated at this point.
- **Fix electrodes with tape**.

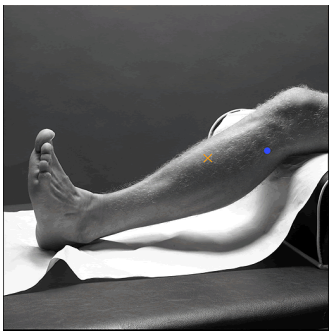
APPENDIX B

ANNEX - SENIAM EMG ELECTRODE LOCATION

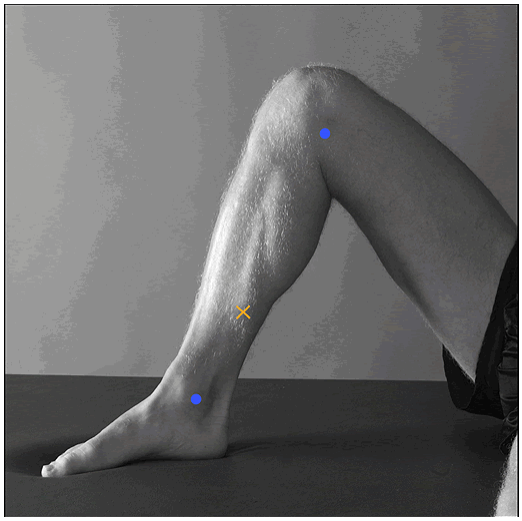
Tibialis Anterior

Ask subject to perform dorsiflexion (without extension of great toe).

Electrodes: Place at 1/3 on the line head of the fibula - tip of the medial malleolus.



Soleus



Sit with knee 90º flexed. The experimenter should press the knee downwards while asking the subject to lift the heel from the floor.

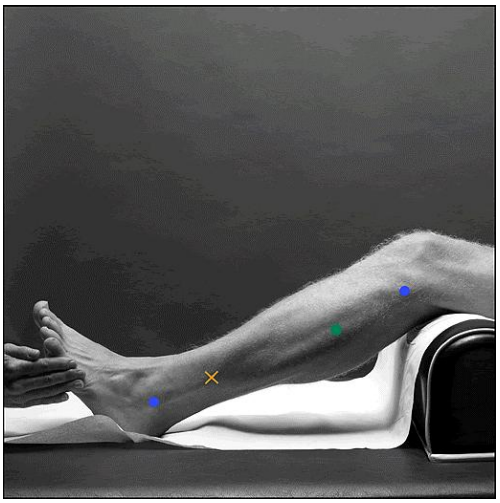
Electrodes: Place at 2/3 of the line medial condylis of the femur - medial malleolus.

Peroneus Brevis / Longus

Ask the subject to rotate the leg medially, and support it above the ankle. The subject should everse the foot and perform plantar flexion, while the experimenter applies pressure in the opposite sense (towards dorsiflexion and inversion of the foot).

Electrodes Peroneus Brevis: Place at 1/4 of the line tip of the lateral malleolus - fibular head, anterior to the tendon of the peroneus longus (orange cross).

Electrodes Peroneus Longus: Place at 3/4 of the line lateral malleolus - fibular head (green spot).

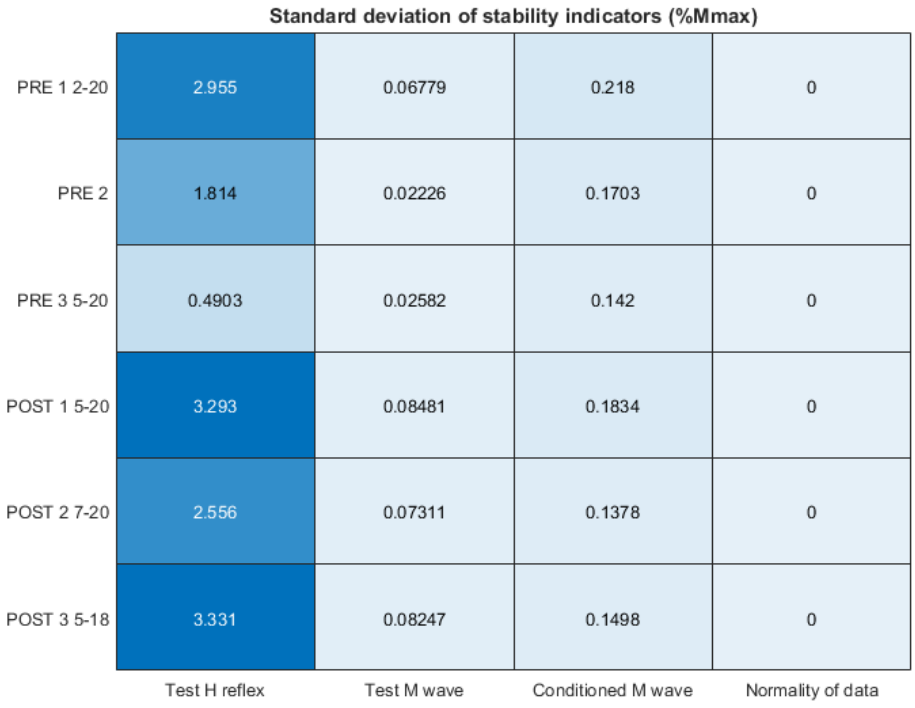


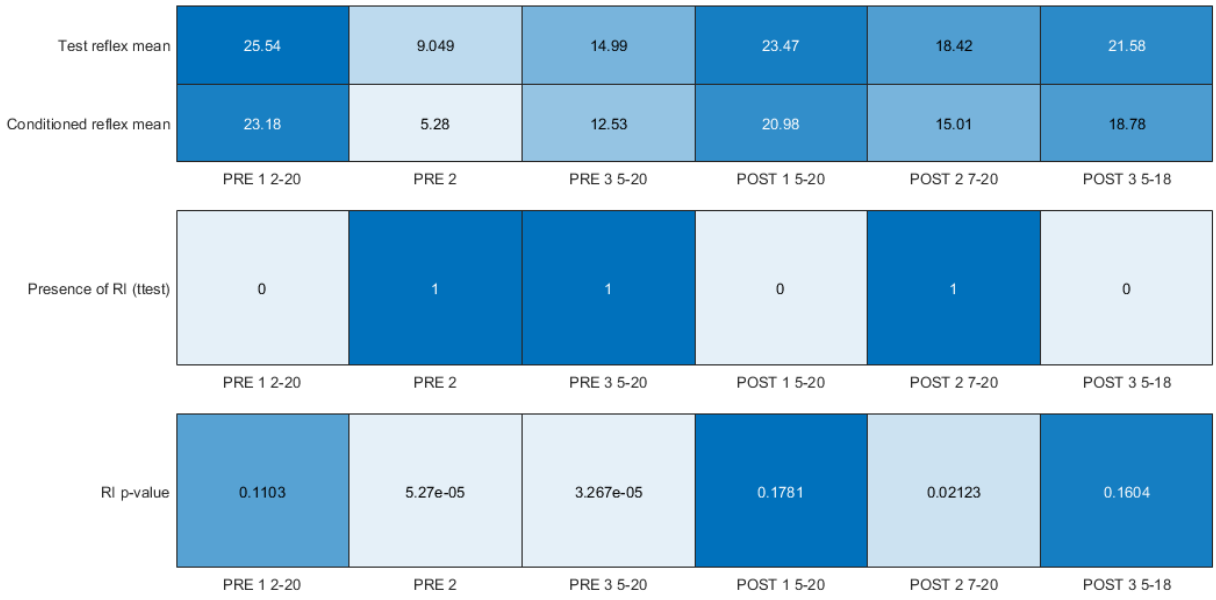
Remarks: peroneus brevis is mainly covered by digitorum lateralis and other muscles. It may be difficult to access and have crosstalk.

- Extracted and adapted from SENIAM (http://seniam.org/) -

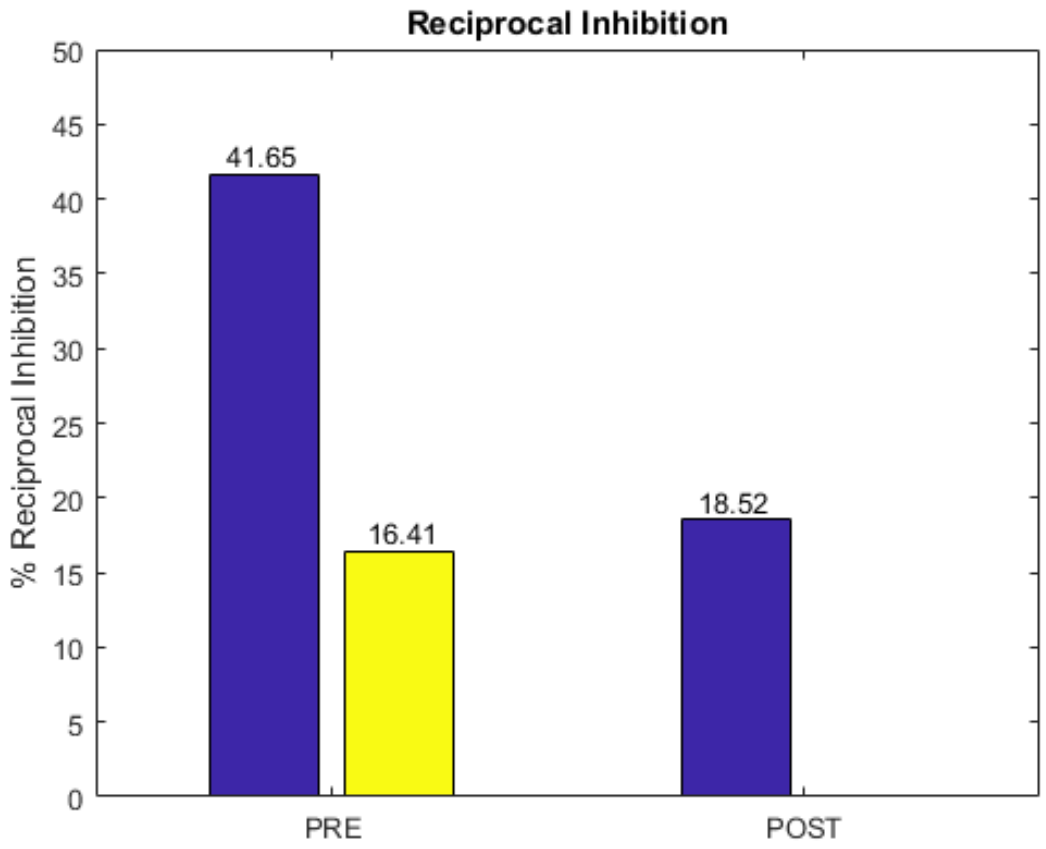
APPENDIX C

SUBJECT 1





APPENDIX C

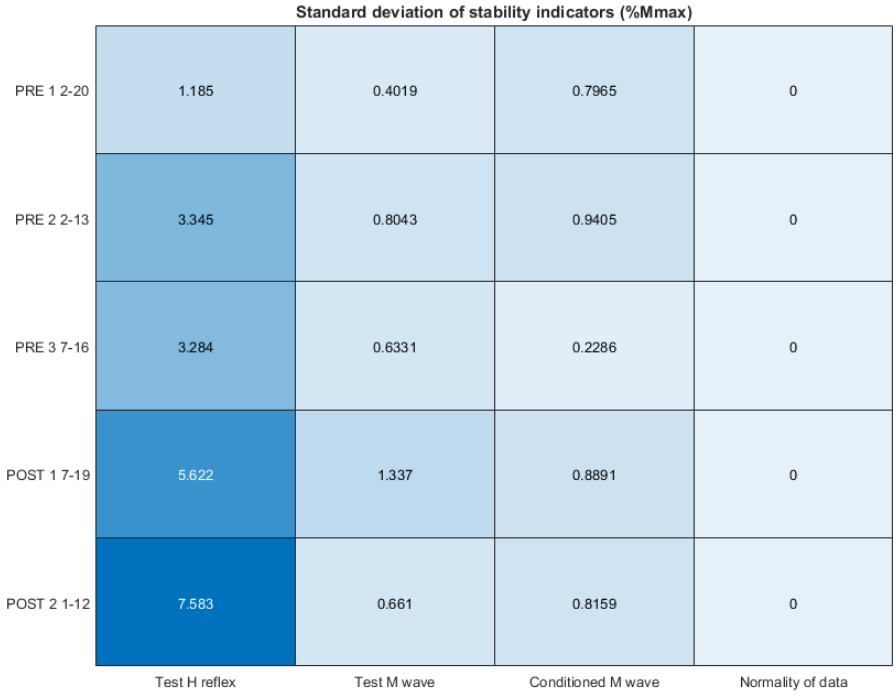


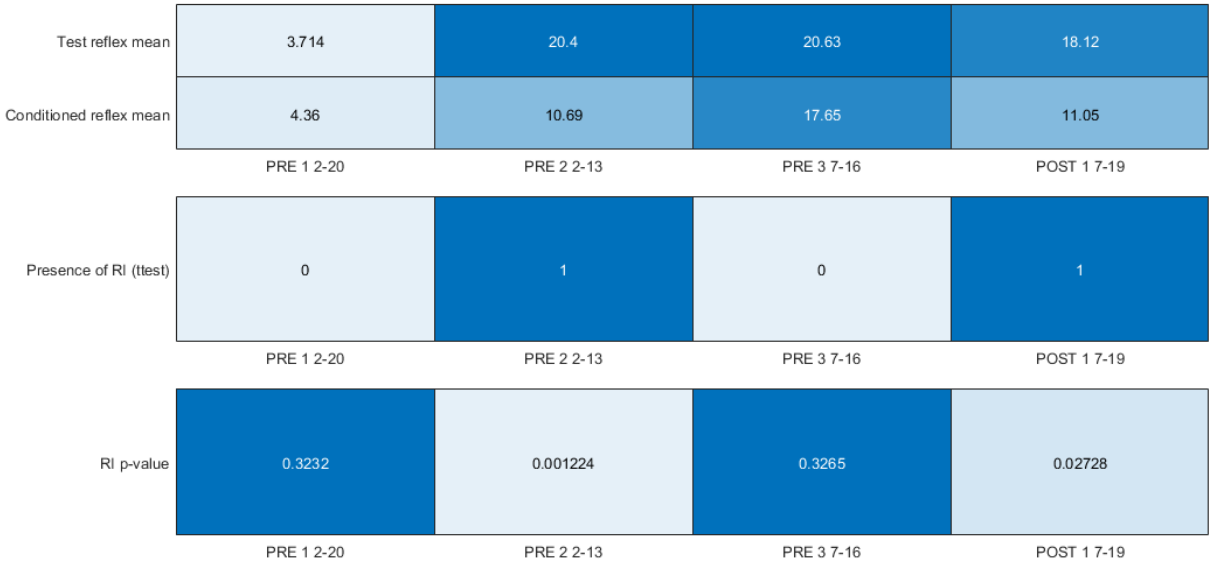
Average PRE RI = 29.03 %

Average POST RI = 18.52 %

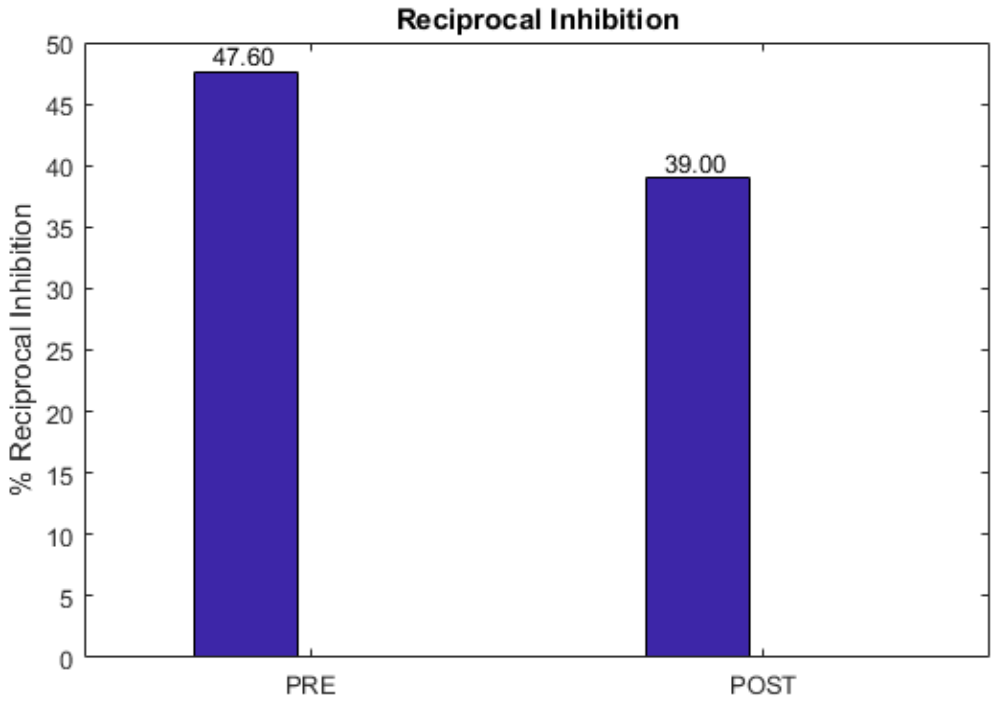
APPENDIX C

SUBJECT 2





APPENDIX C

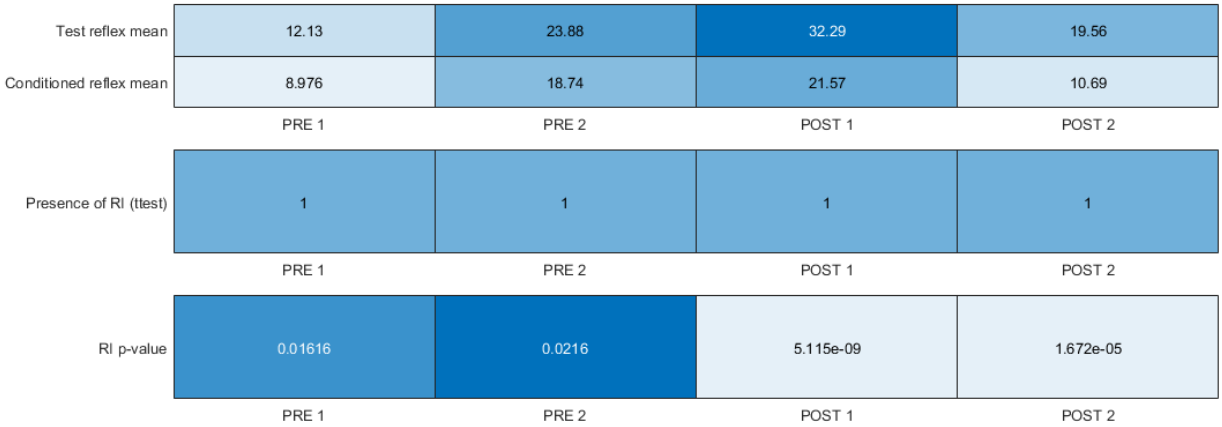


Average PRE RI = 47.60 %

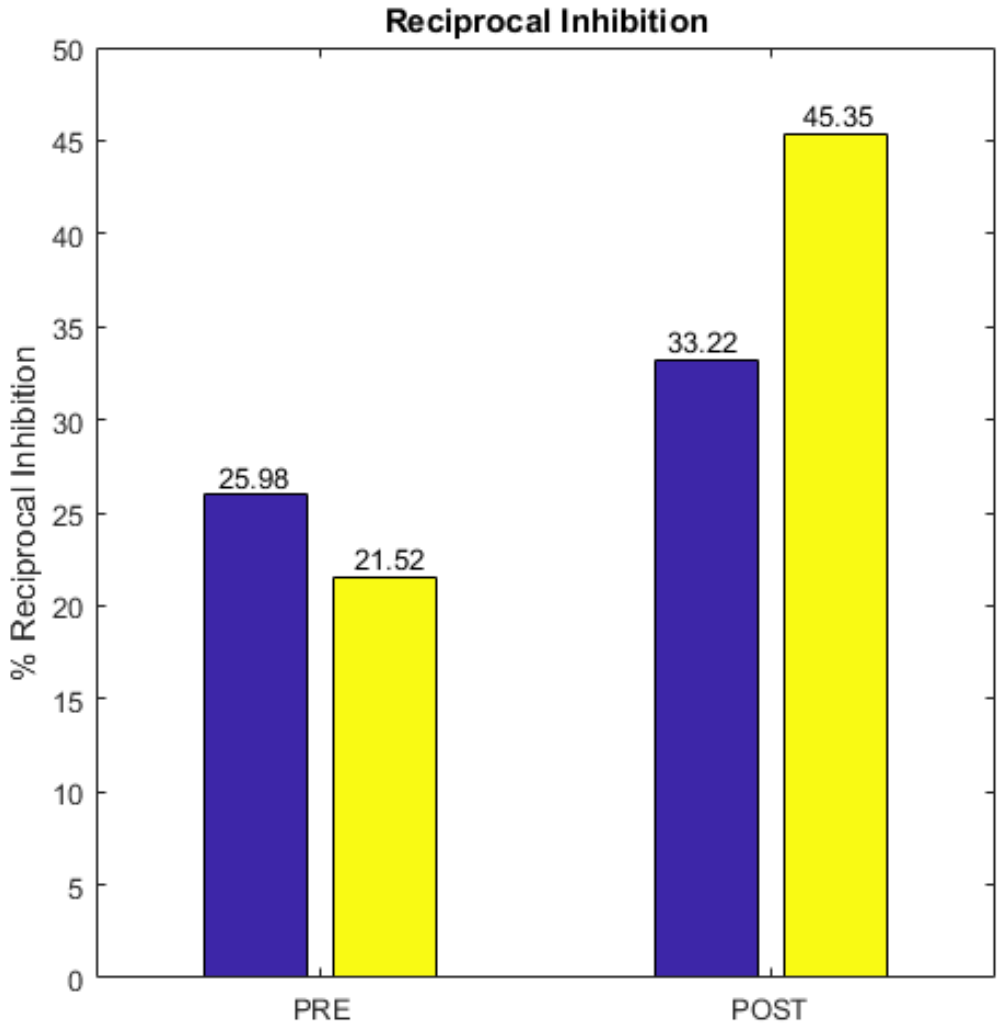
Average POST RI = 39.00 %

APPENDIX C

SUBJECT 3



APPENDIX C

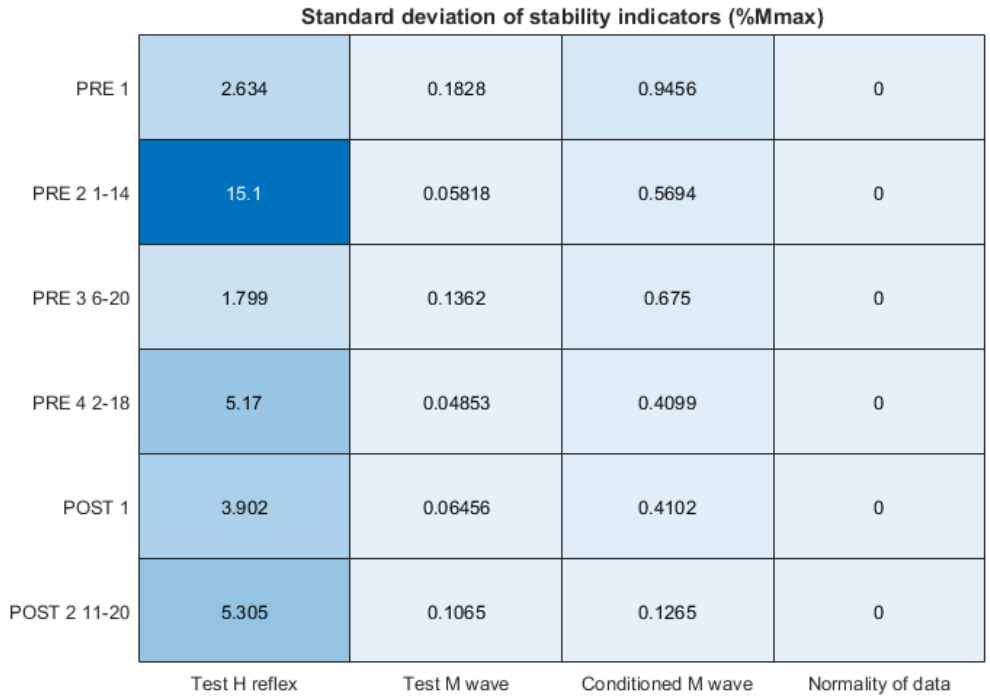


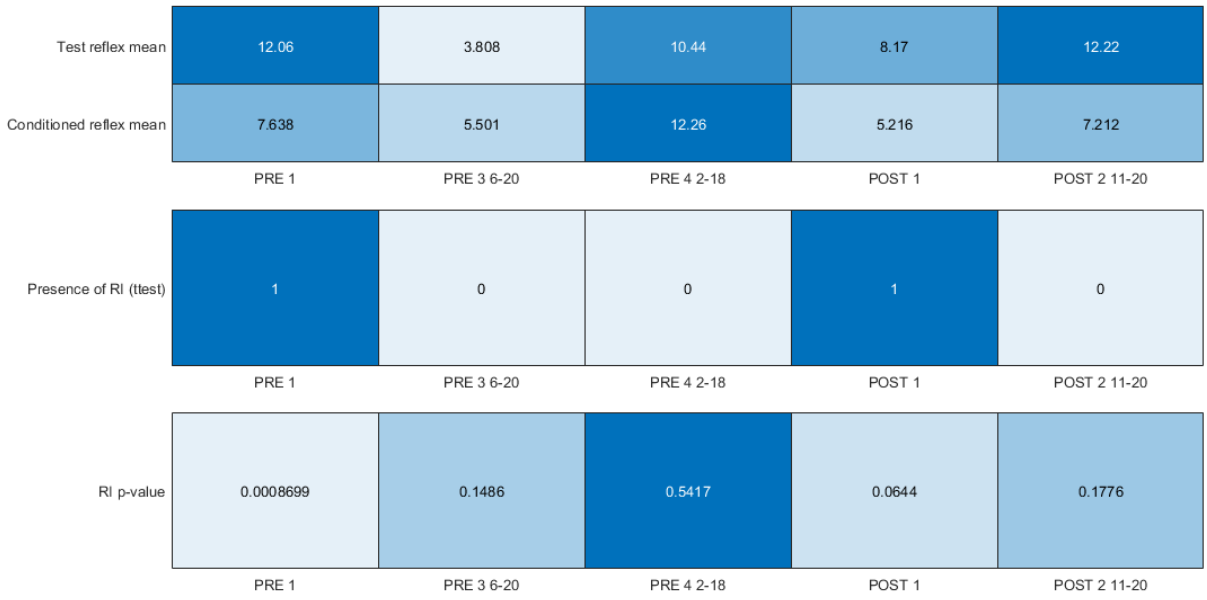
Average PRE RI = 23.75 %

Average POST RI = 39.28 %

APPENDIX C

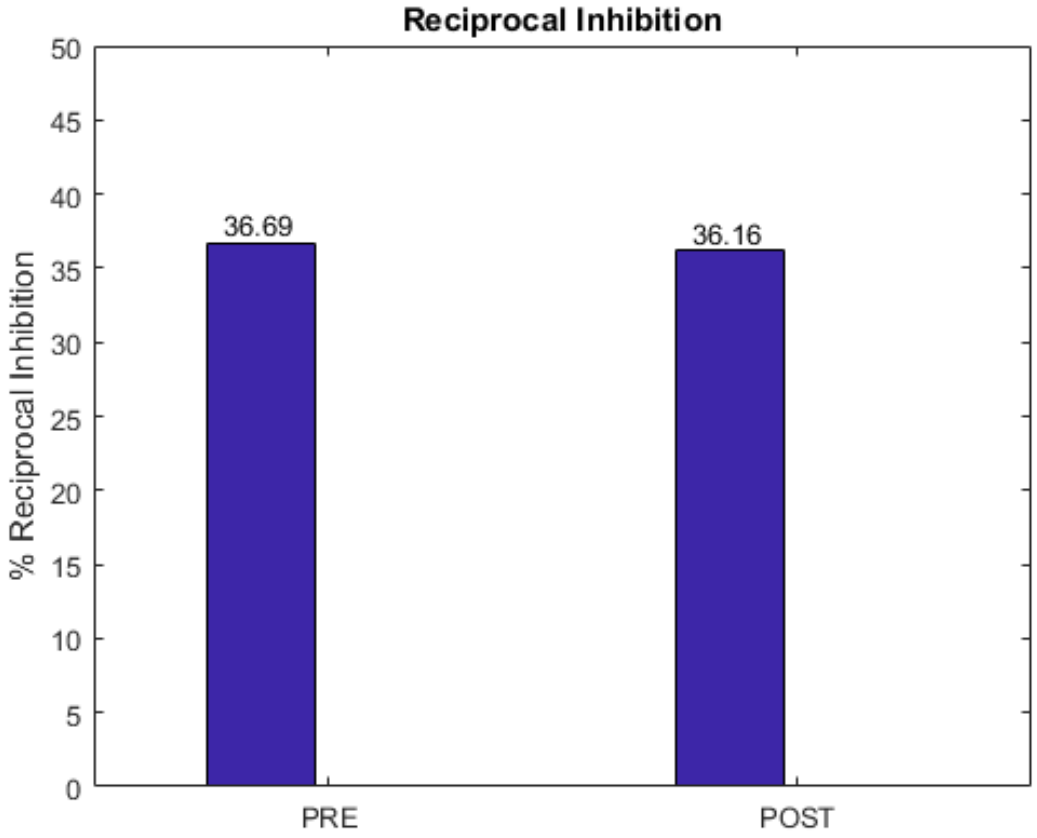
SUBJECT 4





APPENDIX C

Level of significance was lowered to P < 0.1 in order to have at least one significant set in POST.



Average PRE RI = 36.69 %

Average POST RI = 36.16 %