UNIVERSIDAD POLITÉCNICA DE MADRID

ESCUELA TÉCNICA SUPERIOR
DE INGENIEROS DE TELECOMUNICACIÓN

BACHELOR'S DEGREE IN BIOMEDICAL ENGINEERING

BACHELOR'S THESIS

ASSESSMENT OF PHYSIOLOGICAL MECHANISMS UNDERLYING TREMOR REDUCTION AFTER PERIPHERIAL NEUROMUSCULAR ELECTRICAL STIMULATION

ANDREA FRESQUET MONTER

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ABSTRACT

Parkinson's Disease (PD) is a progressive neurodegenerative disease of the central nervous system that gradually leads to a wide range of motor and non-motor symptoms. Tremor is the most noticeable and biggest issue for both early and advance-stage PD patients, and highly impacts their quality of life and social interactions. In order to treat and reduce tremor in PD patients, pharmacological and surgical approaches are often employed. Nevertheless, there can be significant side effects arising from these therapies, such as the decreased effectiveness in the case of medication, or infection and intracranial haemorrhages in the case of surgical interventions. Moreover, an important percentage of patients are not suitable to undergo surgical approaches. Therefore, there is a palpable need of innocuous, affordable, and effective alternatives to reduce pathological tremor. In this regard, electrical stimulation of the afferent pathways has recently been addressed as a novel approach to reduce pathological tremor and has proven to be a reliable and feasible option to reduce tremor in essential tremor patients. Nevertheless, further research is needed to fully understand and characterise the physiological mechanisms at the spinal level that produce the desired therapeutic effects after or during electrical stimulation. Therefore, the main objective of this Bachelor Thesis is to elucidate some of these physiological mechanisms and use this knowledge to test novel strategies towards tremor reduction in PD patients. To that end, two studies were designed, each of them targeting a specific sub-goal.

It has been suggested that the modulation of Ia afferents through electrical stimulation can inhibit muscle activity of the ipsilateral antagonist. This has been associated with a mechanism known as reciprocal inhibition. In the first study of this Bachelor Thesis, the role of Ia afferent pathways in reciprocal inhibition was further evaluated in healthy subjects by assessing reciprocal inhibition before and after long-period vibration was applied at the wrist tendons. Results provided evidence that stimulation of Ia afferents have – at least to a great extent – an important role in reciprocal inhibition between a pair of antagonist muscles at the wrist joint.

In the second study, electrical stimulation was applied to each of the antagonist muscles in healthy subjects, using two different stimulation strategies, based on online recording of muscle activity. The main objective was to investigate the best stimulation strategy having in mind future improvements towards electrical stimulation techniques for tremor reduction in PD patients. Results were not conclusive regarding the effects of both strategies in terms of reciprocal inhibition. Therefore, more research with bigger sample sizes is needed on this regard.

This Bachelor Thesis demonstrated that Ia afferents have a very important role in reciprocal inhibition of the antagonist muscles at the wrist joint, namely flexor carpi radialis and extensor carpi radialis. It also demonstrated that electrical stimulation of Ia afferents produced alterations in spinal excitability, although the differences between two different stimulation strategies weren't conclusive. A cohort with more participants is required to complement this study and design improved strategies of electrical stimulation towards tremor reduction in PD patients.

KEYWORDS: Electrical stimulation; Ia afferents; Motor threshold; Parkinson's Disease; Reciprocal Inhibition; Vibration.

RESUMEN

La enfermedad del Parkinson es un trastorno neurodegenerativo del sistema nervioso central que conlleva la aparición gradual de una amplia gama de síntomas motores y no motores. Tanto para pacientes con Parkinson de inicio temprano como avanzado, el temblor es el síntoma motor que mayormente supone un problema para dichos pacientes, además de representar un enorme impacto en la calidad de vida y en las interacciones sociales. Para tratar y reducir el temblor en pacientes con Parkinson se utilizan en mayor medida terapias farmacológicas y quirúrgicas. Sin embargo, importantes efectos secundarios pueden surgir a raíz de dichos tratamientos, ya sea una disminución de la eficacia en el caso de los medicamentos o infecciones y hemorragias intracraneales en el caso de intervenciones quirúrgicas. Además, un porcentaje importante de pacientes no son aptos para someterse a dichas intervenciones. De este modo, alternativas inocuas, asequibles y efectivas son necesarias para la reducción del temblor patológico. En esta línea, un enfoque novedoso es la estimulación eléctrica de las vías aferentes, que ha demostrado ser una opción fiable y viable en la disminución del temblor patológico en pacientes con temblor esencial. No obstante, se requiere más investigación para comprender completamente los mecanismos fisiológicos que ocurren a nivel espinal y que dan lugar a los efectos terapéuticos durante o después la aplicación de estimulación eléctrica. Por lo tanto, el objetivo principal de este Trabajo de Fin de Grado (TFG) es aclarar algunos de estos mecanismos fisiológicos y utilizar este conocimiento para probar nuevas estrategias de reducción de temblor en Parkinson. Para ello, dos estudios fueron diseñados, cada uno con un subobjetivo.

Previamente ha sido demostrado que, a partir de la modulación de las vías aferentes Ia, la actividad del músculo antagonista ipsilateral es inhibida, lo cual ha sido asociado a un mecanismo llamado inhibición recíproca. En el primer estudio de este TFG, la función de las fibras aferentes Ia en la inhibición recíproca fue evaluada antes y después de la aplicación de una vibración prolongada en los tendones de la muñeca. Los resultados obtenidos demostraron el papel de las vías aferentes Ia en la modulación de la inhibición recíproca entre los músculos antagonistas de la muñeca.

En el segundo estudio, se aplicó la estimulación eléctrica a cada uno de los músculos antagonistas de la muñeca (flexor radial del carpo y extensor radial del carpo), utilizando dos estrategias de estimulación diferentes basadas en el registro *online* de la actividad muscular. Su objetivo principal era investigar la mejor estrategia de estimulación teniendo en cuenta futuras mejoras en las técnicas de estimulación eléctrica para la reducción del temblor en pacientes con Parkinson. Los resultados no fueron concluyentes en cuanto a los efectos de ambas estrategias en la inhibición reciproca, por lo que un mayor tamaño de muestra sería necesario.

Este TFG ha conseguido demostrar que las vías aferentes Ia tienen un papel muy importante en la inhibición recíproca de los músculos antagonistas de la muñeca. Además, también demostró que la estimulación eléctrica de dichas aferentes produce cambios en la excitabilidad espinal, aunque las diferencias entre las dos estrategias de estimulación no fueron concluyentes. Por ello, un mayor número de participantes sería necesario para completar este estudio y diseñar mejores estrategias de estimulación eléctrica con el fin de reducir el temblor en pacientes con Parkinson.

PALABRAS CLAVE: Estimulación eléctrica; Vías aferentes Ia; Motor umbral; Enfermedad de Parkinson; Inhibición Recíproca; Vibración

ACKNOWLEDGMENTS

First and foremost, I would like to thank my family for their endless support and help. You represent one of the pillars that keeps me standing. None of this would have been possible without you. Thank you for believing in me.

I can’t thank my mentor, Alejandro Pascual Valdunciel, and my tutor, Filipe Oliveira Barroso, enough for their constant encouragement, help, and patience during this past year. Without your feedback and guidance this couldn’t have been possible. Thank you for sharing your knowledge with me and giving me this opportunity.

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Many thanks to all the volunteers that took their time and participated in these studies. The progress of this investigation is also because of you. I will be forever grateful.

Lastly, I would like to thank my best friends for sharing a life with me and having my back during all these past years.

More beautiful days are about to come. Thank you.

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LIST OF ABBREVATIONS

ADL	Activities of the Daily Living
BCI	Brain Computer Interface
BHNS	Bidirectional Hyper-Connected Neural Systems
BR	Brachioradialis muscle
CNS	Central Nervous System
CSIC	Consejo Superior de Investigaciones Científicas
DBS	Deep Brain Stimulation
DRIFTS	Dynamically Responsive Intervention for Tremor Suppression
ECR	Extensor Carpi Radialis
EMG	Electromyography
ET	Essential Tremor
FCR	Flexor Carpi Radialis
FES	Functional Electrical Stimulation
GBD	Global Burden of Diseases, Injuries, and Risk Factors Study
GPi	Globus Pallidus internal
GUI	Guided-User Interface
IP	In-Phase stimulation strategy
LRRK2	Leucine-Rich Repeat Kinase 2
LB	Lewy Body
LP	Lesioning Procedures
MRgFUS	Magnetic Resonance-guided Focused Ultrasound
MRI	Magnetic Resonance Imaging
MT	Motor Threshold
MVC	Maximum Voluntary Contraction
NRG	Neuro-Rehabilitation Group
PD	Parkinson's Disease
PES	Peripheral Electrical Stimulation
OOP	Out-Of-Phase stimulation strategy
RF	Radiofrequency
RMS	Root Mean Square

SATS	Selective and Adaptive Timely Stimulation
SD	Standard Deviation
sEMG	Surface electromyography
SRS	Stereotactic Radiosurgery
STN	Subthalamic Nucleus
TFG	Trabajo de Fin de Grado
U.S.	United States

1. INTRODUCTION

1.1. MOTIVATION

Parkinson's Disease (PD) is a progressive neurodegenerative disease of the central nervous system (CNS) that gradually leads to both loss of motor and of non-motor features, being the most common and fastest growing movement disorder [1, 2]. The Global Burden of Diseases, Injuries, and Risk Factors Study (GBD) investigated in 2015 the global epidemiology of PD from 1990 to 2015 and showed that its incidence boosted up globally to 6 million cases [3]. This sharp increase is significantly fuelled by the aging of the world's population, contributing to longer disease duration and thus higher prevalence. Consequently, due to the general increase of longevity, the number of PD patients is expected to increment to 12 million by 2040. Additional factors such as neurotoxic chemicals and industrial substances, including solvents, pesticides, and heavy metals could pick up these incidence rates to over 17 million also by 2040 [4]. In economic terms, this increase will translate into a total of $79 / €71.850 thousand million by 2037 only in the United States (U.S) [5].

The core diagnostic features of PD centre upon four cardinal motor signs: rest tremor, rigidity, bradykinesia, and postural instability [6]. This Bachelor Thesis focuses on the tremor component of the disorder, for it is the most common and noticeable symptom of PD. Tremor is principally localised in the upper limb, followed by the lower limb, the jaw, and the lips [7]. The estimation of PD patients with tremor varies from 79% to 90%, yet a prospective study in deceased PD patients found that 100% of patients had tremor in the course of the disease [6, 8].

When asked about what the most bothersome symptom of PD was, a survey of 75 participants ranked tremor as the most distressing feature [9]. Similarly, in another study involving 265 participants, shaking was reported by a total of 32.6% of early PD patients as the most troubling symptom. In the case of 173 advanced PD patients, it was listed in the top 10 most bothersome features, being the only motor feature in the ranking. Therefore, these observations reveal the distress tremor produces when PD is first diagnosed and as it progresses [10]. Thus, tremor adversely interferes with the activities of the daily living (ADLs) both at home and at work, including dressing, holding paper material, writing/typing, eating, or talking; but it is also worth mentioning that for many PD patients not being able to carry out these tasks normally is a significant cause of social anxiety and embarrassment [11]. According to a survey, more than 50% of the participants declared feeling ashamed of their motor symptoms for not being able to follow certain established social rules: 'I feel ashamed about the way I'm sitting here talking, totally different. (. . .) Well, I don't talk any more when I'm in a large group, because the words come out so awkwardly, don't you think?'. Therefore, the combination of the perception of rule-breaking behaviour and the impossibility of concealing tremor, results in the withdrawal from the public world to the private one: 'I used to like going out for dinner with the children, but I don't dare anymore. Then, I'm sitting there, fumbling, being all clumsy. You can do that at home, but not in a restaurant' [12]. Moreover, 58% of PD patients declared feeling left out of things (29%), noticing others being uncomfortable with them (28%) or avoiding them (19%) at least once [13].

First-line treatments to reduce tremor in PD patients are pharmacological approaches. Levodopa is the most used medication for treatment of PD motor symptoms by compensating the deficit or inappropriate dopamine imbalance [1]. However, advanced-stage PD patients revealed their profound concern about the ineffectiveness of the medication due to a beforehand reappearance of motor symptoms such as tremor. These motor fluctuations can be prevented with higher and more frequent doses of levodopa that, in turn, lead to more side effects [10]. Other adjunctive therapies are deep brain stimulation (DBS)

or lesioning procedures, which are used when patients are unresponsive to levodopa therapy [1]. Although DBS or lesioning procedures can be effective in several cases, they also present drawbacks such as haemorrhage, infection, or postoperative confusion. In addition to this, it is worth adding the high cost such procedures entail, which is a deciding factor for many patients [14].

Due to the increased incidence of PD, the impact tremor has on the quality of life and social interactions, and the unreliability of the previously mentioned therapies, innocuous, affordable, and effective alternatives to reduce tremor are needed. Therefore, this Bachelor Thesis deepens on some of the neurophysiological effects of peripheral neuromuscular electrical stimulation[1] (PES) via afferent pathways[2]. Elucidating some of these neurophysiological mechanisms may set the basis for new strategies to reduce tremor.

1.2. RESEARCH IN CONTEXT

1.2.1. PREVIOUS PROJECTS COORDINATED BY THE NEURO-REHABILITATION GROUP

This Bachelor Thesis has been carried out at the Neuro-Rehabilitation Group (NRG) from the Cajal Institute of the Spanish National Research Council (*Consejo Superior de Investigaciones Científicas* (CSIC)),

In the past, the NRG coordinated different projects revolving around tremor reduction strategies to be applied in PD and essential tremor (ET) patients.

The first of those projects was Dynamically Responsive Intervention for Tremor Suppression (DRIFTS, QKL6-CT-2002-00536) that aimed at suppressing upper-limb tremor by creating wearable active orthoses. A prototyping platform was developed for evaluating and analysing the efficacy of the sensing, actuating and control technologies for tremor reduction [15]. However, these systems are deemed too bulky to be practical because they interfere with ADLs.

The next project called TREMOR (ICT-2007- 224051) was focused on mechanical tremor suppression through functional electrical stimulation[3] (FES) based on a (Brain Computer Interface) BCI-driven detection of involuntary tremor activity [16]. Notwithstanding, FES is a strategy that can lead to both muscle fatigue and discomfort.

Posteriorly, the project NeuroTREMOR (ICT-2011-287739) developed a platform for the study, assessment, and attenuation of upper-limb tremor by means of neuromodulation through afferent stimulation [17]. It consisted of recording windows, which used electromyography (EMG), and stimulation windows. In this way, the system estimated tremor from the recorded EMG signals and used these data for predicting the presence of tremor and localising the tremorgenic bursts. Based on these bursts, stimulation to the antagonist muscle was delivered during the stimulation window [18]. Nevertheless, this strategy is not completely efficient due to the unpredictable nature of tremor.

1.2.2. EU PROJECT EXTEND: BIDIRECTIONAL HYPER-CONNECTED NEURAL SYSTEMS (BHNS)

[1] Peripheral electrical stimulation activates motor and sensory pathways to produce muscle contractions.
[2] Afferent pathways are also referred as sensory pathways, so they need lower stimulation intensities (below motor threshold *i.e.*, the minimum current that elicits muscle contraction).
[3] Functional Electrical Stimulation uses high stimulation intensities (above motor threshold) to evoke muscle contractions that produce the desired forces opposing tremor.

This Bachelor Thesis and the results it attains are part of the currently ongoing project EXTEND, which is funded by the European Union's Horizon 2020 research and innovation program (grant agreement ID: 779982).

EXTEND aims at developing the novel concept of Bidirectional Hyper-Connected Neural Systems (BHNS) to extend the capabilities of neural interfaces with minimally invasive communication links between multiple nerves of the body and multiple external devices (see Figure 1.1.). This will be done through wireless injectable electrodes that will enable stimulation, processing, sensing, and analysis of neuromuscular activity [19, 20]. Therefore, this new technology will provide a feasible solution to manage tremor in ET and PD as well as to control exoskeletons for spinal cord injury [21].

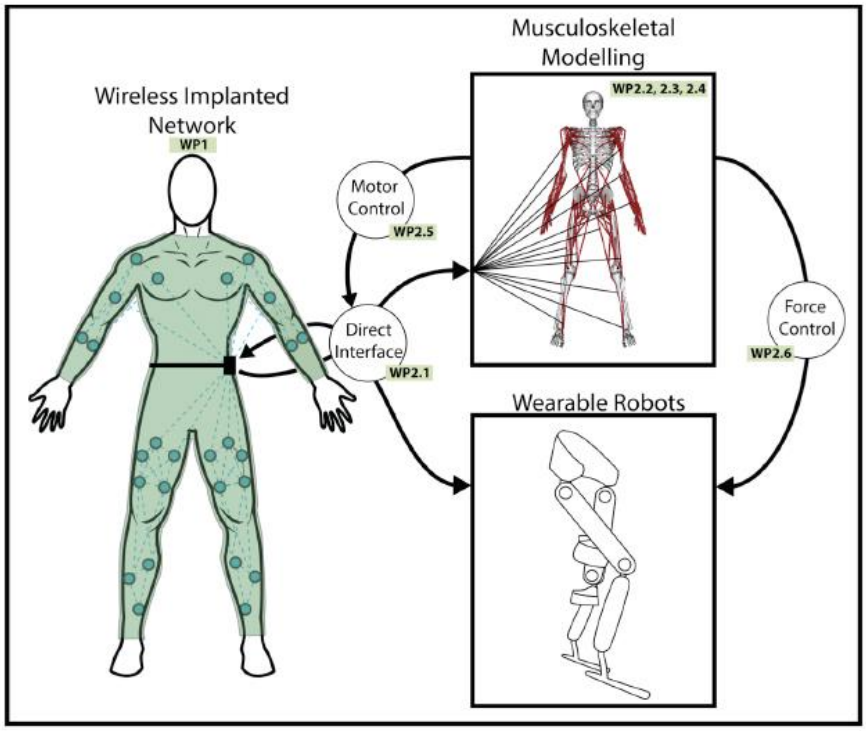

Figure. 1.1. Applications of BHNS on the EXTEND project. 'The objective of EXTEND is to develop all the necessary tools to achieve a minimally invasive bidirectional neural interface platform capable of distributed stimulation and sensing of neuromuscular activity, for attaining what we refer to as Bidirectional Hyper-connected Neural System, BHNS'. Extracted from [21].

Consequently, it will be possible to use BHNS as a closed loop to modulate muscle activity or sensory perception by neuromuscular activation and thus, change the sensorimotor behaviour to correct movement disorders (e.g., in pathological tremor suppression) [21].

1.3. OBJECTIVES AND HYPOTHESIS

The main objective of this Bachelor Thesis is to elucidate some of the physiological mechanisms responsible for the therapeutic effect of electrical stimulation of afferent fibres and use this knowledge to test novel strategies towards tremor reduction in PD patients. To achieve this main goal, two studies were designed, with each targeting a specific sub-goal.

As stated in Section 1.1., there is a palpable need to overcome the invasiveness and unreliability current therapies have for reduction of pathological tremor. Consequently, peripheral electrical stimulation will be addressed in this thesis. On the one hand, it is hypothesised that tremor reduction due to electrical stimulation of afferent pathways is related to plasticity changes at the spinal level [22]. Therefore, the first study consists of deepening the knowledge of neurophysiological processes that take place in upper limbs when afferent stimulation is applied. On the other hand, Pascual-Valdunciel et al. (2020) already demonstrated that the combination of time-selective stimulation[4] and the modulation of afferent pathways with intramuscular electrodes led to an acute and prolonged (24h after stimulation) tremor reduction in ET patients [22]. Therefore, the second study of this thesis aim at using the previous understanding of physiological mechanisms to optimize and test the present strategy of afferent electrical stimulation. This last study has the purpose of providing more knowledge about the effects SATS has on reciprocal inhibition[5] and how it can hypothetically be applied to reduce tremor in PD patients.

Hereafter, following the diagram in Figure 1.2. a brief introduction to each study is given:

1. During the first study, the role of Ia afferents[6] will be evaluated in healthy subjects by assessing reciprocal inhibition before and after vibration is applied at the wrist tendons. Prolonged vibration has been proved to acutely decrease spinal excitability after its cessation, resulting in a motor inhibition reduction [23]. We expect long-period vibration to increase the recruitment threshold for Ia afferents, which in turn will produce a decrease in reciprocal inhibition.
2. In the second study, afferent stimulation in healthy subjects will be applied in in-phase[7] and out-of-phase[8] with wrist muscles activation. We expect reciprocal inhibition to change in opposite directions in result of each of these stimulation techniques.

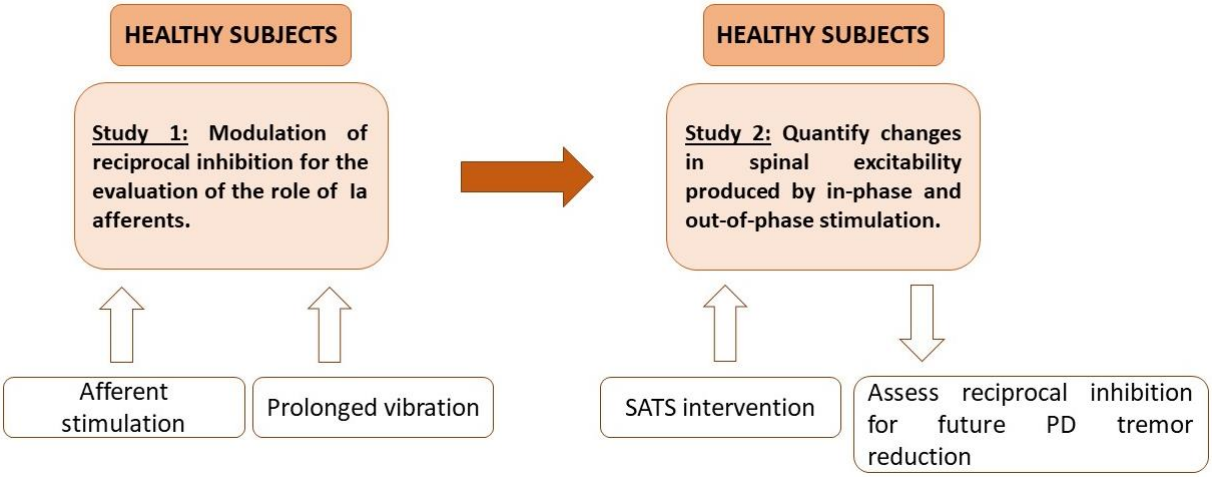

Figure 1.2. Diagram of the objectives and hypothesis of this Bachelor Thesis.

[4] Also called Selective and Adaptive Timely Stimulation (SATS). See Section 3.2. for more information.
[5] Reciprocal inhibition consists of the inhibition of the activity of the antagonist muscle when the agonist is activated. It's carried out by Ia afferents (see Section 2.3.1. for more information) [65].
[6] Ia afferents are a type of sensory/afferent fibres that provide information about both muscle velocity and length [64].
[7] In phase is a stimulation strategy that produces muscle contraction in the agonist when it has been previously activated [22].
[8] Out-of-phase is a stimulation strategy that produces muscle contraction in the antagonist when the agonist is activated [22].

2. STATE OF THE ART

2.1. PARKINSON'S DISEASE: PATHOPHYSIOLOGY AND ETIOLOGY

PD is the most frequent neurodegenerative movement disorder [24]. The diagnosis of PD is based on the presence of four cardinal motor signs: tremor-at-rest, rigidity[9], bradykinesia[10], and postural instability [24]. Other motor features can include freezing of gait[11] and abnormal posture. Moreover, it is also necessary to bear in mind non-motor symptoms, such as sleep disorders, cognitive impairment, constipation, or mood disturbances (depression, anxiety, or apathy), which can appear at all stages of the disease [24, 25].

Tremor is the most common and noticeable motor symptom of PD, and it is defined both as a rhythmic and oscillatory involuntary movement [26]. The classification of tremor is based on different parameters, including frequency, affected parts of the body, and activation state. Therefore, the Parkinsonian tremor that manifests when the body part is at rest, is referred to as resting tremor [26]. This type of tremor is more frequent and present in PD, and it oscillates with a frequency between 3 to 6 Hz [24, 26]. A typical involuntary movement at rest is 'pill rolling' hand tremor, whose manifestation consists of 'simultaneous rubbing of thumb and index fingers against each other' [24]. Rest tremor disappears when sleeping and moving voluntarily and it is re-emergent *i.e.*, its reappearance is delayed after the patient holds an outstretched horizontal position [8]. Thus, in addition to rest tremor, other coexisting tremors have been observed in PD patients: postural tremor[12], and kinetic tremor[13] [26].

The pathological findings of PD are characterised by the gradual death of dopaminergic neurons in the substantia nigra pars compacta and the presence of Lewy bodies (LBs). LBs are intra-cytoplasmic inclusions composed of more than 90 proteins, but whose main constituent is α-synuclein. This protein has the tendency to misfold, become insoluble, and form aggregates that result int the formation of these intraneuronal inclusions LBs are made of [27]. Lewy bodies are not specific for PD and are also found in other neurodegenerative disorders (e.g., dementia), yet pathological changes associated with their formation have reported that they are a presymptomatic stage of PD and, thus a marker for neurodegeneration. Nevertheless, many other hypotheses support the implication of other factors in neuronal degenerations, such as mitochondrial dysfunction, oxidative stress, or excitotoxins [28].

Different signals that come from the cortex are processed through the basal ganglia, enabling a correct performance of voluntary movements. The problem in PD resides in the loss of dopaminergic neurons that in turn impairs the function of basal ganglia and finally sets off a cascade of functional changes that disrupt the whole basal ganglia circuitry [29]. This network consists of the direct and indirect pathways, which are modulated by the nigrostriatal projection between the substantia nigra pars compacta and the striatum [30]. In the direct pathway, activation of striatal neurons leads to excitation of the thalamus and a pause of neuronal firing, whereas in the indirect pathway, the opposite occurs (for the detailed network, see Figure 2.1.). Since the output of basal ganglia involves the execution of a movement when neuronal firing pauses, and discharges are related to stopping movement, these two pathways are considered as opposite projection systems that produce or inhibit movements [31]. Furthermore, striatal neurons from the direct pathway have D1 dopamine receptors, which depolarize the cell in response to dopamine, whereas these neurons in the indirect pathway contain D_2 dopamine receptors that hyperpolarize the cell. Thus, the nigrostriatal pathway can activate the direct pathway (activation of body movement) when simultaneously inhibiting the indirect pathway (inhibition of body movement) [30]. This way, the loss of striatal neurons can be translated into the imbalance of these two pathways, that finally involves the expression of PD motor symptoms [29, 30].

[9] Rigidity refers to an increased muscle tone that involves both flexor and extensor muscle groups [24].
[10] Rigidity refers to an increased muscle tone that involves both flexor and extensor muscle groups [24].
[11] Freezing gait refers to 'freeze' of movement.
[12] Postural tremor manifests immediately after stretching out the arms [24].
[13] Type of tremor that appears when doing a voluntary movement [25].

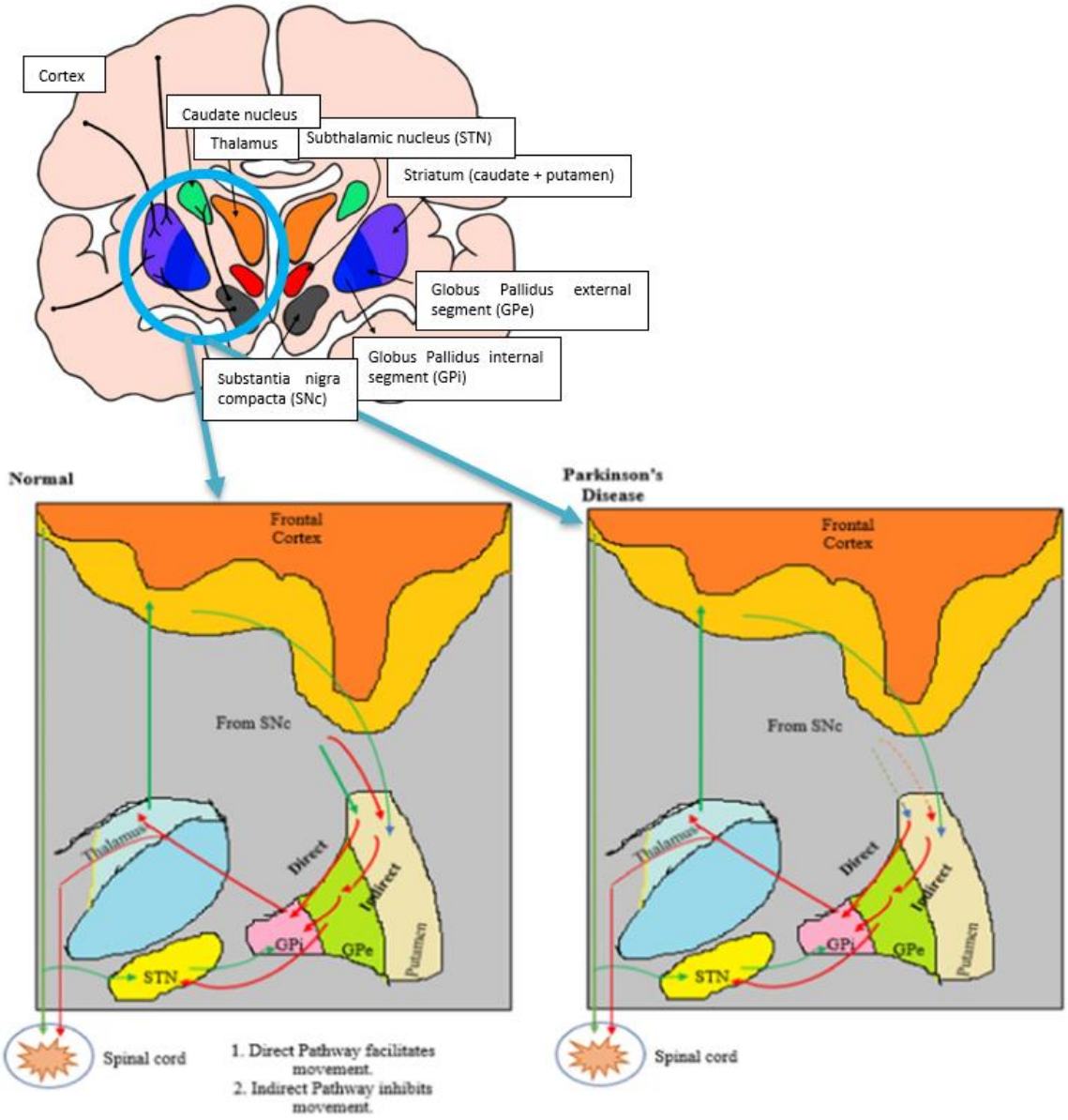

Figure 2.1. Basal ganglia structures (above) and their excitatory and inhibitory signals in a normal brain and one with PD (below). Green lines represent excitatory connections and red lines represent inhibitory connections. Adapted from [32, 33].

The cause of PD is unknown, yet it its hypothesised that genetic and environmental factors affect disease risk and progression. Age, nutrition, hereditary forms, exposure to toxics, and oophorectomy[14] are considered as risk factors, whereas an inverse effect has been associated with smoking, coffee, and black tea. On this point, it has also been found that high uric acid levels seem to protect from PD and decrease the progression of the disease [24].

Positive family history entails 5%-15% of PD cases [27]. Studies on these families have not only allowed to identify the genes involved in the pathogenesis (e.g., the discovery of a genetic alteration in the SNCA gene in 1997) but have also given details of disease mechanisms [27, 34]. Of the many candidates, the major causative genes are:

- LRRK2 (PARK8) refers to *Leucine-rich repeat kinase 2* and has been accounted for being the most prevalent gene in familial PD [35]. It is believed that high activity of LRRK2 picks up the risk for PD, for increased kinase activity has been involved with nigrostriatal degeneration and Lewy body formation [36].

[14] Oophorectomy is a surgical procedure where one or both ovaries are removed.

- SNCA (PARK1/4) is the gene encoding for α-synuclein, which is the main component of LBs and, thus shows that protein aggregation plays a role in PD pathophysiology. Typical clinical features of this mutation are parkinsonism, dementia, and autonomic dysfunction[15] [35].
- Parkin (PARK2) mutations are especially prevalent in PD with onset before age 30, representing 10%-20% of cases. A study on familial juvenile parkinsonism patients characterized this mutation with a female predominance onset of 27.8 years old and slow progression [35, 36].

Among the environmental factors, besides age, which is the leading risk factor for PD, the role of microbiota in neurological disorders, and neuroinflammation has drawn attention in the field of neurology. Research is currently focalised on the microbiota-gut-brain pathway, which consists of the bidirectional communication between gut bacteria and the CNS (see Figure 2.2.). 17 years ago, it was hypothesised that PD could start in the gut, due to the findings of α-synuclein aggregates in the mucosal and submucosal nerve fibres and ganglia of PD patients. The combination of this evidence and digestive dysfunctions, which often take place years before motor symptoms appear, suggests that this protein is transported to the brain via the vagal nerve. During a study, mice developed motor deficits and inflammation, two hallmark PD symptoms, after being colonised with the microbiota of PD patients. Nevertheless, diet has a great effect on the composition of microbiota and many neurological diseases affect nutrition. Furthermore, it hasn't come to an agreement whether a specific microbiota exists for PD. Therefore, a closer understanding of the relationship between diet, microbiota, composition, and the brain is needed in order to have a good dietary data for all human studies [37].

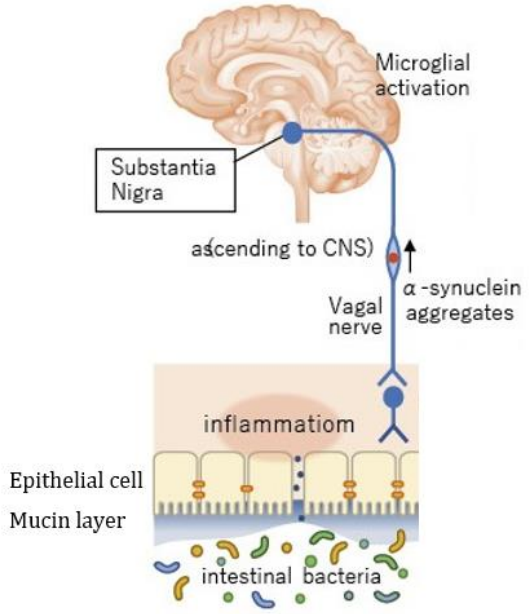

Figure 2.2. Microbiota-gut-brain pathway. Adapted from [38].

2.2. CURRENT THERAPEUTICAL APPROACHES FOR PARKINSON'S DISEASE

There is currently no known cure for PD, thus present therapeutical approaches are available to help manage motor and non-motor symptoms. Both the undetermined pathophysiology and the limited understanding of the mechanism(s) responsible for cell death in PD hinder the possibility of developing a neuroprotective strategy against PD progression — even at an asymptomatic stage. It is also worth bearing in mind that PD therapy must be individualised and tailored to the specific needs of each patient [25].

[15] Autonomic dysfunction develops when nerves of the autonomous nervous system are damaged

First-line treatments are medication approaches, which are often used when symptoms are mild. Once they become troublesome and begin interfering with the activities of the daily life despite medication, surgical and experimental therapies are considered [25].

2.2.1 PHARMACOLOGICAL THERAPIES

Pharmacological approaches of PD primarily focus on dopamine deficits or inappropriate dopamine imbalances [1]. Therefore, the main objective of present pharmacological approaches is the dopamine replacement therapy to alleviate motor symptoms [39]. To accomplish this, there are different approaches centred upon the expression and secretion of dopamine in addition to the activation of dopamine receptors (see Figure 2.3. for more information about dopamine metabolism) [32].

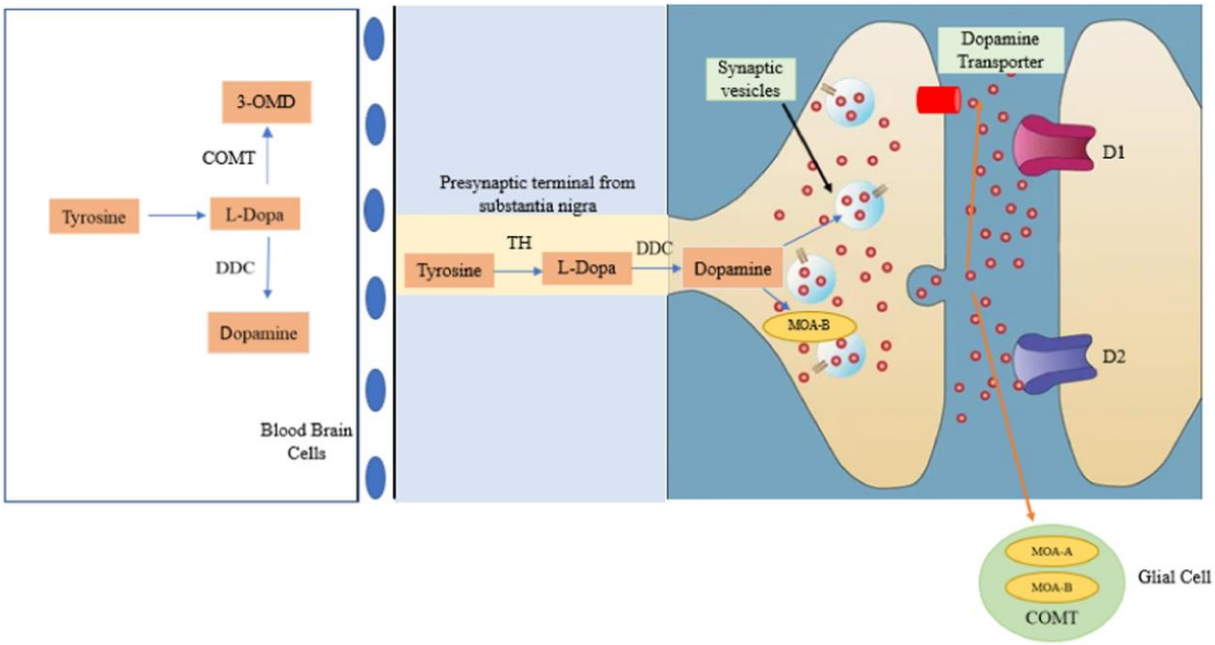

Figure 2.3. Pathways of dopamine metabolism. Extracted from [32].

Levodopa is the golden standard medication in PD, for it is the immediate precursor to dopamine and can cross the blood-brain barrier. It enables the depleted dopaminergic neurons to produce more dopamine, mitigating motor symptoms [40]. Levodopa is usually combined with carbidopa in order to prevent nausea/vomiting as well as the premature conversion of levodopa into dopamine in the bloodstream, letting more of it reach the brain [41]. However, long-term levodopa therapy leads to the emerge of motor-related complications due to progressive neurodegeneration of striatal cells, which in turn declines the capacity of storing levodopa [42]. These levodopa-induced complications can become disabling and deeply affect patient's quality of life, thus they are often the main reason why other medications or surgical interventions start being considered [43].

Other medications are, for instance, dopamine agonists that stimulate endogenous dopamine receptors [42]. They are suitable for managing mild to moderate PD and are often regarded as the first choice of medication in younger patients with the purpose of delaying levodopa therapy and the risks it attains. Typical side effects of dopamine agonists involve hallucinations, confusion, somnolence, leg edema and impulse control disorders. Therefore, they should be used with caution in older or cognitively impaired patients [25].

2.2.2. SURGICAL THERAPIES

Surgical therapies have become second-line treatments for advanced PD patients, when medication is no longer effective, or the side effects it attains become unbearable, specially continued motor fluctuations and dyskinesias [44].

The first surgical treatments for movement disorders were lesioning procedures, which consisted of removing a specific target brain area believed to be involved in the pathophysiology of motor symptoms. Nevertheless, such procedures are irreversible and destructive and, therefore, other non-destructive and adjustable alternatives such as deep brain stimulation (DBS) have overtaken them [44].

2.2.2.1. DEEP BRAIN STIMULATION (DBS)

Deep brain stimulation (DBS) is considered as the gold standard surgical treatment for many movement disorders when medication is no longer effective, cannot be tolerated due to the side effects, or results in motor fluctuations [44]. This therapeutical approach involves the implantation of an electronic device that can modulate or disrupt neuronal activity in a certain area or circuit of the brain by providing continuous high frequency electrical stimulation [1, 44]. This way, the procedure is non-destructive and adjustable, being possible to fine-tune different stimulation parameters. The stimulation is provided by the electrodes at the tip of the leads that reach the specific targeted brain area. They are connected to a small internal pulse generator placed under the skin in the chest that contains all the necessary electronics to generate stimulation pulses as well as to power the system (see Figure 2.4.) [44]. Although the mechanism of action of DBS is not known, this strategy has been reported to stop the propagation of tremor signals by disrupting pathological thalamic oscillatory activity, thus improving motor symptoms [45].

Figure 2.4. Current deep brain stimulation devices from Medtronic. Indicated by * is the internal pulse generator and with a black arrow are intracranial electrodes. Extracted from [46].

In the case of PD, the leads are implanted in the subthalamic nucleus (STN) and the globus pallidus internal (GPi), as these two regions of the brain are considered part of the cortico-basal ganglia-thalamo-cortical circuitry (see Figure 2.5. for DBS-targeted locations) [47]. On the one hand, STN-DBS studies have been associated with a reduction of levodopa medication after surgery, which in turn resulted in the decrease of dyskinesia and other side effects induced by dopaminergic medication. [46, 14]. On the other hand, GPi-DBS studies have been proved to be better suited to PD patients with postural instability and gait difficulty, even though in the long term these improvements lessen. [14]. Furthermore, GPi-DBS has been linked to fewer negative effects on mood and cognition, especially among older people [46].

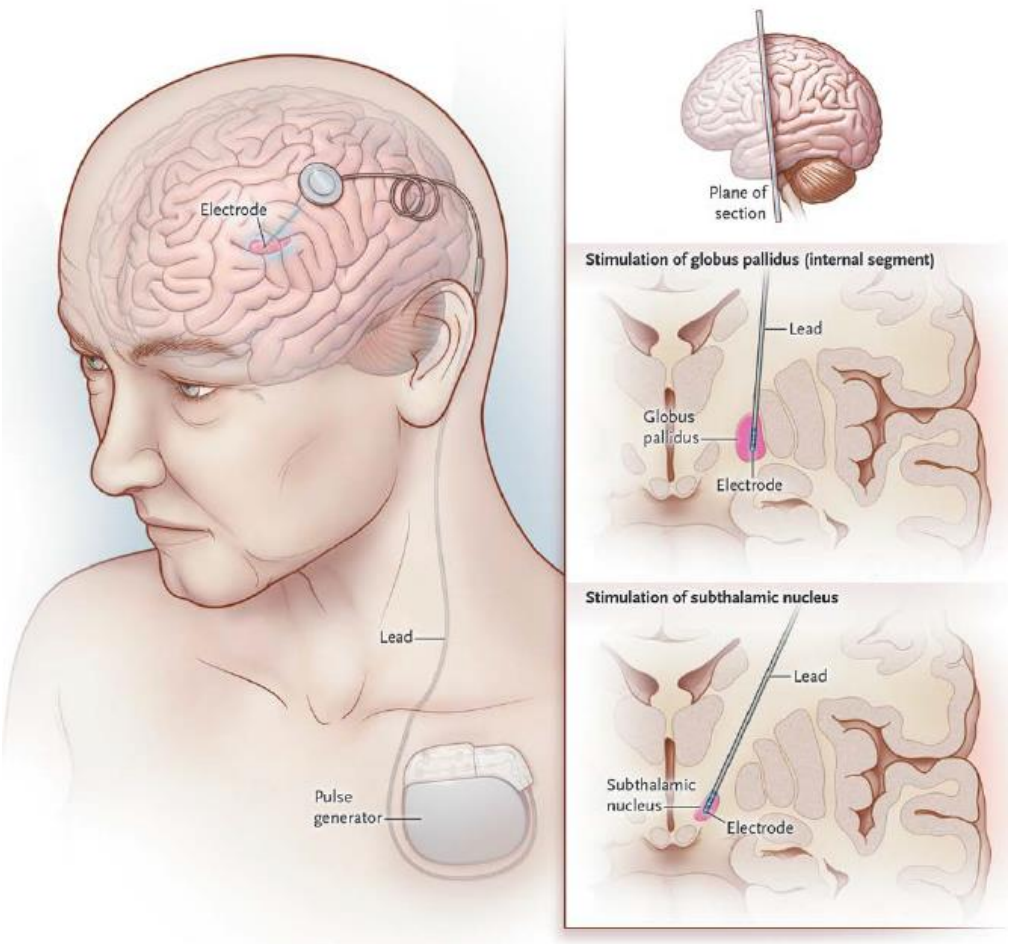

Figure 2.5. Electrode implantation for DBS. Extracted from [48].

Accurate clinical outcomes of DBS surgery are determined by a correct patient selection and electrode placement in the targeted area. Thus, optimising patient selection is crucial for coming by worthwhile results [14]. Worsening of PD symptoms and unresponsiveness to medical therapy, which typically happens after 5-15 years of treatment as the disease progresses, makes PD patients the ideal candidates for DBS therapy [14, 48]. Therefore, it is recommended for PD patients to have a disease period of at least 5 years, so atypical forms of parkinsonism fully manifest, but before they become disabling [46]. Age limit for DBS is not well established yet, however most studies have excluded patients older than 75 years, even though their clinical outcomes reported similar improvements in motor symptoms and dyskinesia than in patients with less than 70 years [14].

Despite its use and being an attractive solution among clinicians and patients, DBS also has its drawbacks. The placement of leads can entail infection and intracranial haemorrhages, which often result in both the removal of the device and antibiotic treatment [14]. Moreover, lead fractures and other issues related to hardware, such as mechanical malfunction or disconnection, are usual [14, 44]. There is also a wide range of neurologic and neuropsychological negative effects that have been associated with DBS surgery, including cognitive impairment, difficulties with speech, depression, and anxiety. Lastly, it is an expensive procedure that in 2010 could exceed $14.4/€13.5 thousand million [44].

2.2.2.2. LESIONING PROCEDURES

Before DBS appeared and took the lead in current surgical therapies, the treatment for movement disorders depended upon lesioning procedures (LP) [44]. Ablative surgeries involve selective and permanent destruction in a targeted brain area deemed to be associated with the pathogenesis of motor symptoms. Although clinically effective, lesioning surgeries are destructive and irreversible procedures

and, in 30%-60% of the cases, lead to speech and cognition deficits when performed bilaterally [14, 44].

Current available strategies are shown in Figure 2.6. and these consist of radiofrequency (RF) thermoablation, stereotactic radiosurgery (SRS) lesioning, and magnetic resonance-guided high-intensity focused ultrasound (MRgFUS) thermal ablation [49]:

- RF techniques involve the penetration of a probe into the brain connected to an RF generator [14]. Thus, once the intracranial target area is localised, sufficient heat by means of high-frequency electric currents is applied through the tip of the electrode, which finally results in thermocoagulation [49]. Risks related to RF interventions are infection and haemorrhage, for they require a burr hole or incision [14, 49].
- SRS strategies, also previously known as gamma knife neurosurgeries, deliver a single large dose of ionising radiation to a precise intracranial target location, leaving other tissues unharmed [14, 49]. Contrary to RF, SRS is non-invasive, but it also has its drawbacks: variable lesion size, exposure to ionizing radiation, and delayed effect (the benefit onset can take approximately 2 months) [14].
- MRgFUS is a modern and non-invasive intracranial technology that, with the guidance of MRI systems, applies ultrasonic waves through the skull into the targeted area in the brain to perform thermal ablation [14]. This is achieved by the conversion of wave energy into heat, which finally causes thermal lesions in deep brain structures [49].

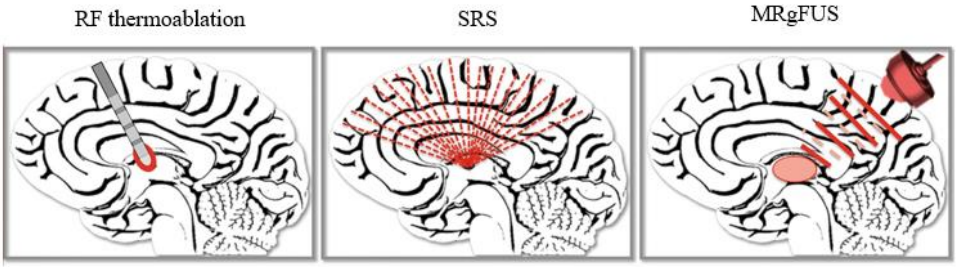

Figure 2.6. Different available lesioning procedures for motor- related complications. Surgical interventions from left to right: radiofrequency (RF) thermoablation, stereotactic radiosurgery (SRS) lesioning and magnetic resonance-guided focused ultrasound (MRgFUS) thermal ablation. Adapted from [50].

2.2.3. PERIPHERAL ELECTRICAL STIMULATION (PES)

As previously stated, there are important limitations in current therapeutical approaches for reducing motor symptoms, for they are unreliable and entail serious adverse side effects. Therefore, due to its affordability and innocuousness, electrical stimulation of muscles and peripheral nerves has been explored as an attractive alternative for tremor attenuation during the past few years.

Two main electrical stimulation approaches have been used for tremor reduction: functional electrical stimulation (FES) and stimulation of afferent pathways [51].

2.2.3.1. FUNCTIONAL ELECTRICAL STIMULATION (FES)

Functional electrical stimulation (FES) was first proposed 20 years ago in order to elicit muscle contraction by means of the activation of efferent pathways in tremorgenic muscles and, thus attenuate tremor [52, 53]. Therefore, this strategy uses electrical stimulation to recruit muscles fibres and thereby activate muscle contraction. In this regard, higher stimulation currents recruit more fibres, which in turn achieve stronger forces opposing tremor [51].

There are two stimulation techniques that can be distinguished in FES: out-of-phase and co-contraction. The most frequent approach is out-of-phase, which aims at applying electrical stimulation to the antagonist muscle when its agonist shows tremorgenic activity [54]. This way, opposite forces are

generated by the stimulation of the antagonist, which in turn will counteract the tremorgenic bursts coming from the agonist muscle. The other strategy is co-contraction that applies continuous stimulation to both muscles (agonist and antagonist) at the affected joint to increase its stiffness and thereby prevent tremorgenic movements. Both strategies have in common that they use stimulation intensities above motor threshold (MT) in order to achieve muscle contraction, which results in muscle fatigue, patient discomfort due to strong stimulation, and possible alteration of voluntary movements [54].

2.2.3.2. STIMULATION OF AFFERENT PATHWAYS

Alternatively, recent studies have reported that afferent stimulation below MT may also modulate tremorgenic activity without the difficulties stimulation above MT represents [22]. Stimulation below MT intends to modulate interneurons and reflex circuitries at the spinal level by means of stimulation of afferent pathways, resulting in tremor reduction at the muscular output [52]. As it happened with FES, afferent stimulation can also be applied in an out-of-phase or continuous manner [22]. The operating principles are the same: out-of-phase requires a precise stimulation pattern and stimulates the antagonist muscle when the agonist is activated by tremorgenic bursts, while continuous stimulation applies electrical stimulation to the antagonist muscle without any pattern or synchronization (see Figure 2.7. for a comparison between these two strategies) [54].

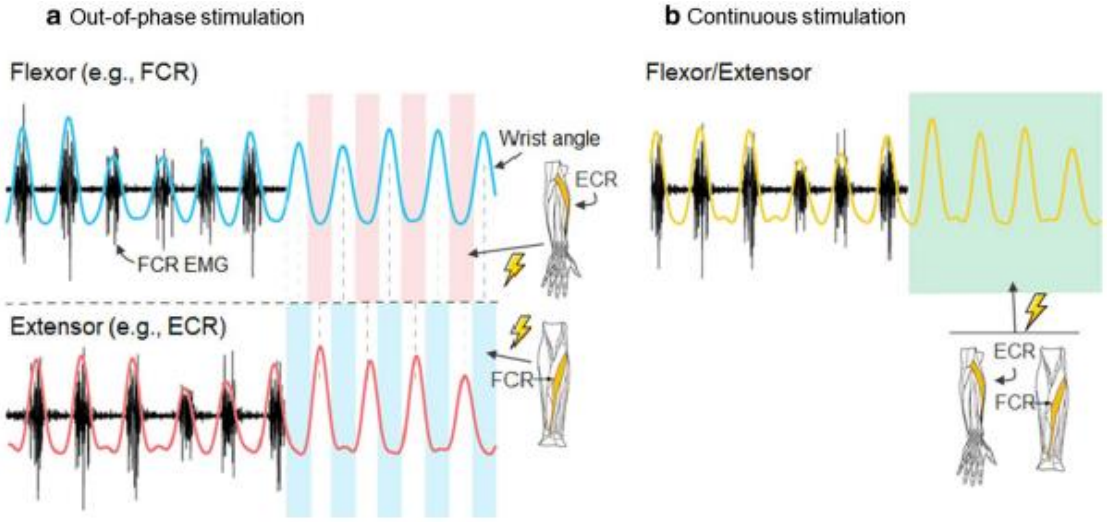

Figure 2.7. Stimulation strategies. (a) Out-of-phase stimulation. EMG recording window detects tremorgenic bursts and predicts them during the stimulation window (red and blue lines). When the flexor experiences a tremor burst, the extensor is stimulated (blue-coloured rectangles), whereas if it is the extensor, the flexor is stimulated (red-coloured rectangles). (b) Continuous stimulation. Stimulation is applied without a pattern during the entire stimulation window (green-coloured rectangle). Extracted from [51].

2.2.3.3. TREMOR SUPPRESSION STUDIES ON PD

The results obtained by peripheral electrical stimulation (PES) studies on PD patients are summarized in the following table:

TABLE 2.1. SUMMARY OF METHODOLOGY AND RESULTS WITH PERIPHERAL ELECTRICAL STIMULATION

Reference	Population	Strategy	Physiological mechanism	Results

Dosen et al. [18]	4 PD, 2 ET	FES: out-of-phase	Generation of opposite forces to tremor oscillations	60 ± 14%
Gallego et al. [55]	2 PD, 4 ET	FES: co-contraction	Increasing joint stiffness	52.33 ± 25.48%
Gillard et al. [56]	3 PD, 3 HV	FES: out-of-phase	Out-of-phase forces	84.50 ± 2.20%
Grimaldi et al. [57]	1 PD, 1 ET, 1 cerebellar syndrome	FES: co-contraction	Increasing joint stiffness	9 ± 35%
Javidan et al. [58]	4 PD, 3 ET, 6 cerebellar tremor	FES: out-of-phase	Generation of opposite forces to tremor oscillations	62 ± 5%
Popovic et al. [53]	4 PD, 3 ET, 5 HV	FES: out-of-phase	Generation of opposite forces to tremor oscillations	67 ± 13%
Dideriksen et al. [54]	5 PD, 4 ET	Afferent stimulation: out-of-phase	Ia afferent fibres, reciprocal inhibition	50 ± 14% (SF) 54 ± 20% (IM)
Dosen et al. [18]	4 PD, 2 ET	Afferent stimulation: out-of-phase	Generation of opposite forces to tremor oscillations	42 ± 5%
Hao et al. [59]	8 PD	Afferent stimulation: continuous	Cutaneous afferents and propriospinal interneurons	62%
Heo et al. [60]	14 PD	Afferent stimulation: continuous	Afferents might modulate supraspinal tremor oscillations	Finger: 68% Hand: 62% Forearm: 53%
Muceli et al. [61]	1 PD	Afferent stimulation: out-of-phase	Ia afferent fibres, reciprocal inhibition	58%
Xu et al. [62]	2 PD	Afferent stimulation: continuous	Cutaneous afferents and propriospinal interneurons	Significant acute reduction. No values provided

ET: essential tremor, PD: Parkinson's disease, HV: healthy volunteer, SF: surface stimulation, IM: intramuscular stimulation. Adapted from [51].

As shown in Table 2.1., results obtained from electrical stimulation studies on PD patients are highly variable, which therefore shows the great need for consensus and standardised procedures. In this way, the role of small sample sizes (< 10 patients), different stimulation parameters (frequency, amplitude, pulse width, among others) across studies, and the combination of ET and PD patients in the same groups, limit the conclusions about the effectiveness of PES as well as the identification of the mechanism(s) underlying plastic changes —primarily in the case of afferent stimulation [51]. Therefore, more research is needed to fully understand and customise the neuromodulation produced by afferent stimulation and exploit its potential [63].

Despite the inconclusive results between FES and afferent stimulation, it is worth mentioning that the results of tremor reduction by means of afferent stimulation are comparable to other strategies and has the advantage of not possessing the limitations stimulation above MT has [51].

2.3. NEUROMODULATION OF PHYSIOLOGICAL PATHWAYS

Abnormal spinal reflexes impair motor control and thereby limit mobility, disrupt sleep, and can cause pain and fatigue [64]. Therefore, discovering possible supraspinal mechanisms and identifying possible lasting plastic effects due to afferent stimulation is of great importance in order to better understand the role neuromuscular stimulation plays in modulating motor inhibition [51].

2.3.1. STRETCH REFLEX AND RECIPROCAL IA INHIBITION

The stretch reflex is a spinal reflex produced by the lengthening contraction of a muscle [65]. This change of muscle length is sensed by the muscle spindle, whose Ia afferent fibres conduct such information to the spinal cord and form excitatory synapses with motoneurons as well as inhibitory connections with interneurons (see Figure 2.8. for the spinal cord circuit). In order to achieve these excitatory pathways, Ia fibres from a muscle activate both α-motoneurons, which in turn innervate the muscle from which they arise (homonymous muscle), and other motor neurons that stimulate synergist muscles[16]. Alternatively, Ia inhibitory interneurons inhibit the activity of α-motoneurons, resulting in the relaxation of antagonist muscles[17]. This inhibitory pathway is called reciprocal Ia inhibition and prevents antagonist muscles from contracting when the antagonist is stretched [65]. This mechanism is very important for joints, for they are controlled by the synchronization of two opposing muscles: the flexors and the extensors [66]. Therefore, when a muscle is stretched, the Ia afferent fibres are activated, which leads to the contraction of the homonymous muscle and its synergists and the relaxation of the antagonist [65]. The full reflex pathway enables to coordinate voluntary movements by preventing agonist and antagonist muscles from working against each other [66].

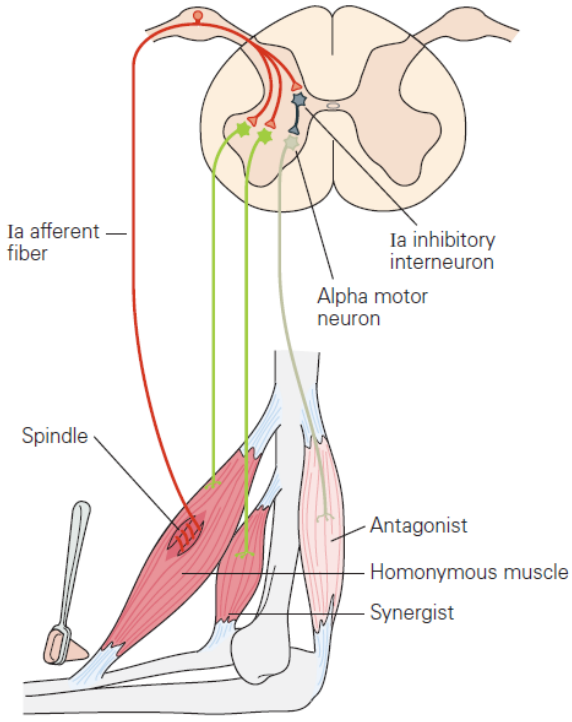

Figure 2.8. Stretch reflex and reciprocal Ia inhibition pathways. Muscle spindle senses the muscle being stretched when tapped with a reflex hammer. That way, the muscle spindle via Ia afferents makes excitatory synapses with α-motoneurons, contracting homonymous and synergist muscles, while making inhibitory connections through Ia inhibitory interneurons, thus preventing the antagonist muscle from contracting. Extracted from [65].

It is hypothesised that supraspinal input and spinal afferents are related to tremor generation because when the agonist muscle receives supraspinal tremor input tremor oscillations occur, entailing a passive

[16] Synergist muscles are muscles which work together in order to create movement.
[17] Antagonist muscles are the ones producing opposing forces to other muscles (e.g., wrist flexors and extensors).

stretch of the antagonist. This results in the activation of Ia afferent fibres and the generation of tremor-like behaviour [63, 67]. Therefore, stimulation below MT may be a way of modulating stretch reflex and reciprocal inhibition by means of Ia afferents and, thereby attenuate tremor on antagonist muscles due to the inhibitory connections to motor neurons in the spinal cord (see Figure 2.8.) [63]. Thus, following the scheme from Figure 2.9., the agonist muscle (extensor carpi radialis (ECR) in this case) is activated by stimulation below MT. This impulse travels to the spinal cord via Ia afferent fibres where it synapses with both the α-motor neuron, activating the agonist muscle, and the Ia inhibitory interneuron, which consequently inhibits the activity of the α-motor neuron of the antagonist muscle (tremorgenic muscle, flexor carpi radialis (FCR) in this case) [51]. This neuronal circuit has been studied in an out-of-phase strategy (see Table 2.1.) in order to reduce tremor between a pair of antagonistic tremorgenic muscles [51].

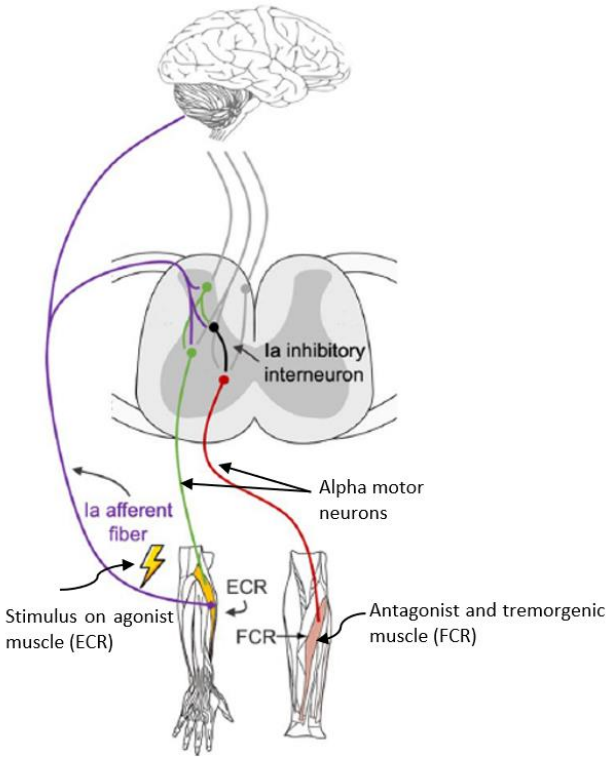

Figure 2.9. Out-of-phase afferent stimulation based on reciprocal Ia inhibition that will alter the transmission of tremorgenic input. Adapted from [51].

2.3.2. H-REFLEX AS A PROBE TO STUDY SPINAL NEURONAL PATHWAYS AND MECHANISMS

The Hoffman reflex (H-reflex) is one of the most studied reflexes in humans, which entails the existence of reciprocal inhibition [51, 68]. Analogous to the spinal stretch reflex from Section 2.3.1., H-reflex pathway consists of Ia afferents from muscle spindles that have excitatory connections to spinal α-motoneurons, which activate the muscle. H-reflex is also elicited by electrical stimulation below MT, but bypasses muscle spindles by directly stimulating the nerve that innervates a muscle [64, 69]. Therefore, H-reflex follows a 'simpler' neuronal circuit in contrast to the spinal stretch reflex, which is of great use for evaluating and investigating the mechanisms and therapeutical approaches of neuromotor control diseases [64, 68, 69]. To that end, H-reflex is recorded using an EMG [64].

Electrical stimulation produces two responses in the homonymous muscle: an M-wave and an H-reflex [68]. The M-wave occurs faster because of the direct stimulation of the motor axon innervating the muscle, whereas H-reflex must go first to the spinal cord and then travel across the synapse before reaching the muscle [65]. Therefore, the M-wave is the first response to be detected in an electromyogram (EMG), followed by the H-reflex (see (a) in Figure 2.10.).

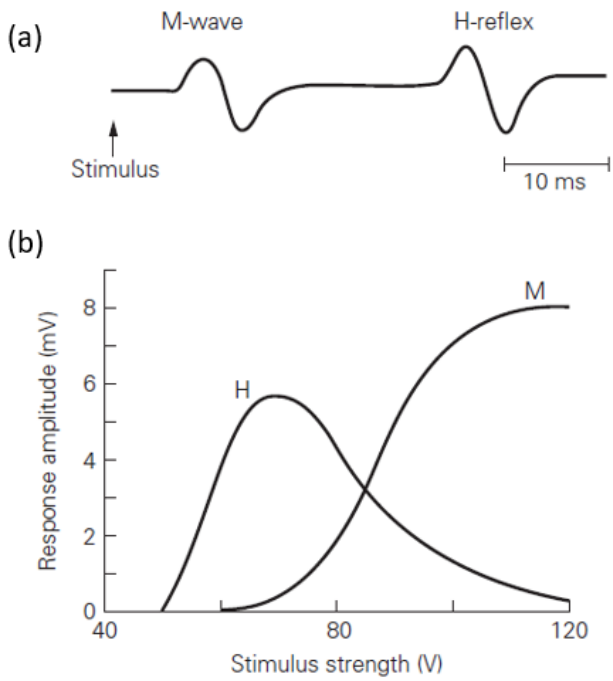

Figure 2.10. Recruitment curves for H-reflex and M-wave. (a) Activation of motor neurons produces an M-wave followed by the H-reflex in an EMG. (b) H and M-wave amplitude depending on stimulus intensity. Extracted from [65].

At low intensities, only the large-diameter and slow Ia afferents are activated [68]. This impulse travels to the spinal cord via Ia afferent fibres, where it connects to the homonymous α-motor neuron and travels to the muscle (red arrows in Figure 2.11.) [64]. As the stimulus intensifies and the afferents start reaching their threshold, more Ia afferents are recruited, increasing the amplitude of the H-reflex to a maximum (see H-wave in (b) from Figure 2.10.) [69]. On the other hand, as the stimulus strength continues to increase beyond the threshold of Ia afferents, the α-motoneurons are also stimulated. These have a higher threshold than Ia afferents due to their smaller motor axons and their excitation results in the presence of the M-wave (green-coloured arrow in figure 2.11.) and the progressive decline of the H-reflex [65, 69]. Analogous to the H-reflex, when the threshold of the motor axons is reached, the M-wave is maximum (see M-wave in (b) from Figure 2.10.).

The reason for the decline and further disappearance of the H-reflex is due to the antidromic collision effect [69]. The stimulation of motor axons produces antidromic activity (green dashed arrow in Figure 2.11.) that travels to the wrong direction in the motor axons up to the spinal cord, colliding with the afferent orthodromic (going in the correct direction) signal. Therefore, when the antidromic volley is equal or larger than the reflexive volley, H-reflex disappears [69].

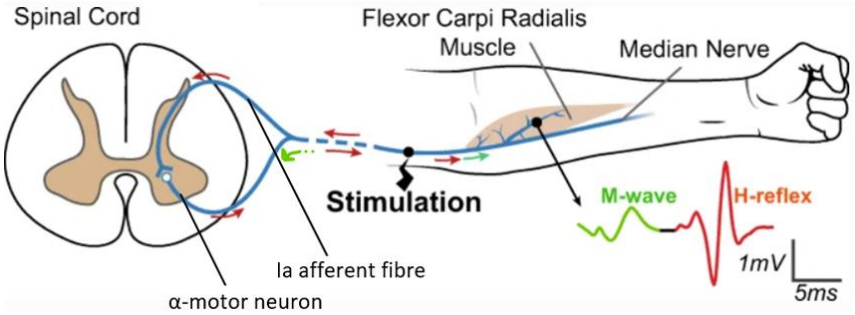

Figure 2.11. H-reflex and M-wave pathways. Ia afferent pathways are represented by the red arrows, whereas the green-coloured arrows represent the excitation of the spinal α-motoneurons going to the muscle and antidromically (dashed green arrow). Adapted from [64].

2.3.3. MUSCLE INHIBITION BY NERVE STIMULATION

It has been demonstrated that stimulation of Ia afferent fibres results in the inhibition of antagonist α-motoneurons, which are responsible for innervating the skeletal muscle and, therefore, for contracting muscle [66, 69]. This mechanism is known as reciprocal inhibition and ensures the reduction of antagonist muscle activity when the agonist is activated [70].

Pascual Valdunciel et al. (2019) already studied the conditioning of reciprocal inhibition of antagonist activity during voluntary wrist flexion/extension task by means of electrical stimulation above and below MT. In the case of the wrist, the radial nerve is responsible for innervating the extensor carpi radialis (ECR), whereas the median nerve innervates the flexor carpi radialis (FCR). Therefore, depending on which muscle was being activated during the flexor/extension task, surface stimulation was delivered to one nerve or the other with the final purpose of inhibiting the activity of the antagonist muscle *i.e.,* if ECR was contracted via an extension task, the stimulation was applied to the median nerve in order to measure ECR inhibition (see Figure 2.12. for ECR inhibition). Stimulation below MT achieved a statistically significant mean inhibition in both ECR (12 ± 4%) and FCR (16 ± 6%) in 7 out of 8 subjects, while stimulation above MT accomplished reciprocal inhibition in all the 8 subjects with an ECR mean inhibition of 22 ± 6% and a mean inhibition of 25 ± 10% in the FCR. Thus, this study demonstrated that afferent stimulation makes it possible to achieve muscle inhibition and that stimulation below MT is an effective neurophysiological strategy to activate Ia afferent pathways without recruiting motor nerves, thereby avoiding stimulation above MT limitations [70].

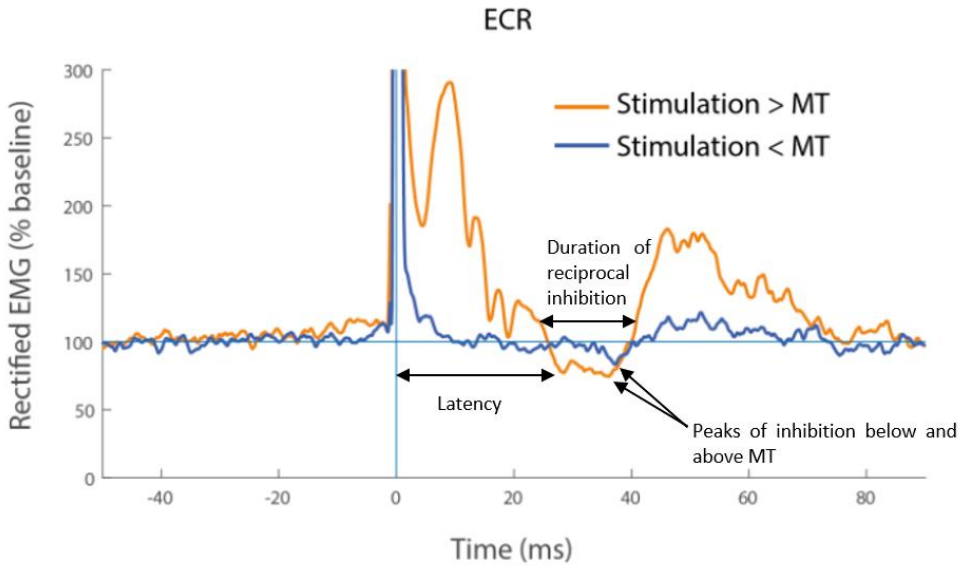

Figure 2.12. Average rectified EMG from ECR in response to stimulation below MT (blue-coloured line) and above MT (orange-coloured line) of the median nerve. Adapted from [70].

3. EXPERIMENTS

3.1. STUDY 1: EFFECT OF AFFERENT STIMULATION AND PROLONGED VIBRATION IN HEALTHY SUBJECTS

3.1.1. BRIEF INTRODUCTION AND GOALS OF THE STUDY

This study aimed at characterising neurophysiological mechanisms when performed on healthy subjects, which is the first step to be considered to develop novel strategies to reduce tremor. Therefore, the main objective of this study was to elucidate the effect low-current stimulation and long-duration vibration have on Ia afferent fibres. In order to achieve this, reciprocal inhibition of wrist flexors was addressed and studied as specified in Section 2.3.3. by means of delivering surface electrical stimulation below MT to the radial nerve, before and after prolonged vibration was applied to the wrist tendons.

The first hypothesis is that, when conducting a flexion task, the stimulation of the radial nerve below MT produces the activation of the ECR (the agonist muscle) and the inhibition of the FCR (the antagonist). This would demonstrate that stimulation below MT activates Ia afferent pathways and that the modulation of reciprocal inhibition at the wrist is a similar mechanism to reciprocal Ia inhibition [70]. The second hypothesis is that long-lasting vibration locally applied to a muscle, or its tendons acutely diminishes spinal excitability, resulting in the attenuation of Ia afferent input onto spinal motoneurons. [23]. In fact, Robin Souron et al. (2019) [23] proved that long-lasting tendon vibration at the soleus raised the recruitment threshold for Ia afferents. Therefore, during this experiment, prolonged vibration is addressed in the tendons of the wrist in order to prove this hypothesis at the wrist tendons and further demonstrate the implication of Ia afferents in reciprocal inhibition.

The findings of this study will provide more knowledge on the role Ia afferent pathways have in the modulation of voluntary movement as well as the effect long-period vibration has on muscle inhibition through surface stimulation.

3.1.2. MATERIALS AND METHODOLOGY

3.1.2.1. PARTICIPANTS

Fourteen healthy subjects volunteered to participate in this study. Only one was lefthanded and none of them had neurological injuries. Nine of them were females and five males, with a mean age and standard deviation (SD) of 23.28 ± 3.71 years. Even though the sample number was N=14, the full procedure could only be carried out in nine subjects. All the participants were given verbally and written all the necessary information about the procedure and possible discomfort. After that, doubts were cleared, and they signed a n informed consent form. All experiments were conducted according to the Declaration of Helsinki and approved by a local Ethical Committee.

3.1.2.2. EQUIPMENT

For the full procedure, different surface electrodes (see Figure 3.1.), were used for either nerve stimulation or EMG recording. On the one hand, four bipolar electrodes (Ag/AgCl 2.2x2.2 cm, Vermed Neuroplus, USA) were used in order to measure EMG activity: one pair was placed on the flexor carpi radialis (FCR) and the other on the extensor carpi radialis (ECR). Two pairs of self-adhesive bipolar electrodes (Ag/AgCl, Ambu, Denmark) were also used for recording EMG activity. On the other hand, in order to deliver surface electrical stimulation, two bipolar round electrodes (4 cm diameter, EN-Trode, Enraf Nonius, The Netherlands) were placed on the radial nerve.

Figure 3.1. Surface electrodes used for the full study. (a) Vermed Neuroplus bipolar electrode for EMG recording. (b) Ambu® Neuroline 720 bipolar electrode for EMG recording. (c) Enraf Nonius bipolar electrodes for nerve stimulation. Extracted from [71, 72, 73].

Surface electromyography (sEMG) signals were obtained by the bio-signal amplifier Quattrocento (OT Bioelettronica, Italy) and its respective accessories, as shown in Figure 3.2. and Figure 3.3. Although this device allows the acquisition of signals from up to 400 different channels (384 standard channels and 16 auxiliary input channels), for these experiments only two standard channels for the two muscles (in addition to one auxiliary input channel for force signals) were used. By means of a 16-channel bipolar adapter jack connector (OT Bioelettronica, Italy) and male banana or snap-on adapters (OT Bioelettronica, Italy), the previously mentioned surface electrodes were connected to the EMG amplifier in order to record muscle activity. Furthermore, a wet wristband around the elbow of the dominant arm with male clip connector (OT Bioelettronica, Italy) was clipped to a ground patient cable (OT Bioelettronica, Italy) to work as a reference. Therefore, this setup enabled the amplification, digital conversion, and transfer of these biosignals to a computer with a USB2/Ethernet cable. The software OT Biolab+ (version 1.5.6.0) was used in order to display, record and check the quality of the measured EMG signals.

Figure 3.2. Quattrocento EMG amplifier. Extracted from [74].

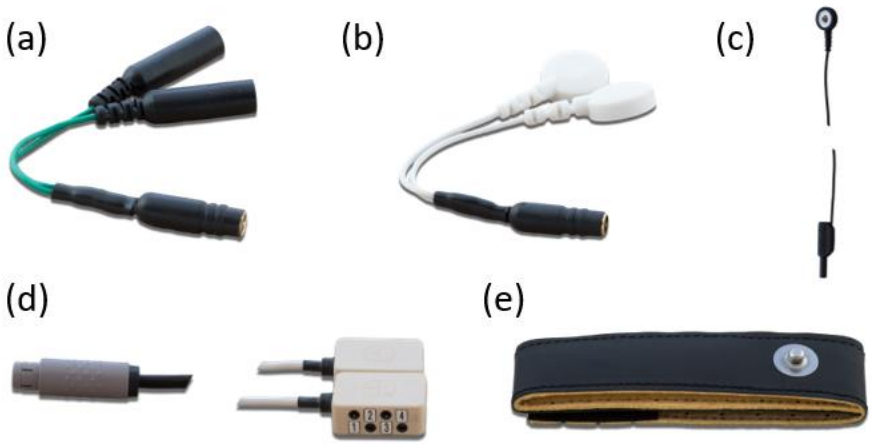

Figure 3.3. Quattrocento's accessories used during experiments. (a) Male banana electrode adapter. (b) Snap-on electrode adapter. (c) Ground patient cable. (d) 16-channel bipolar adapter jack connector. (e) Wristband with male clip connector. Extracted from [75-79].

The constant current stimulator DS8R (Digitimer Ltd, UK) shown in Figure 3.4. (a) was used to deliver electrical pulses to the radial nerve. In order to localise the nerve and afterwards place the stimulation electrodes from Figure 3.1. (c) on the correct area of the arm, the bar electrode (Digitimer Ltd, UK) in Figure 3.4. (b) was employed. Because this bar electrode applies more pressure to the nerve than when only having sEMG electrodes on the skin, pre-existing hand-sewn straps with 3D printed structures of different sizes were used to better reach the nerve by placing them on the stimulation electrodes. The length of these straps could be adapted to each participant due to the added Velcro in the strap and its webbing adjusters' buckles (see Figure 3.4. (c)). These adjustable straps were specially designed for volunteers with more muscle mass or a deep nerve.

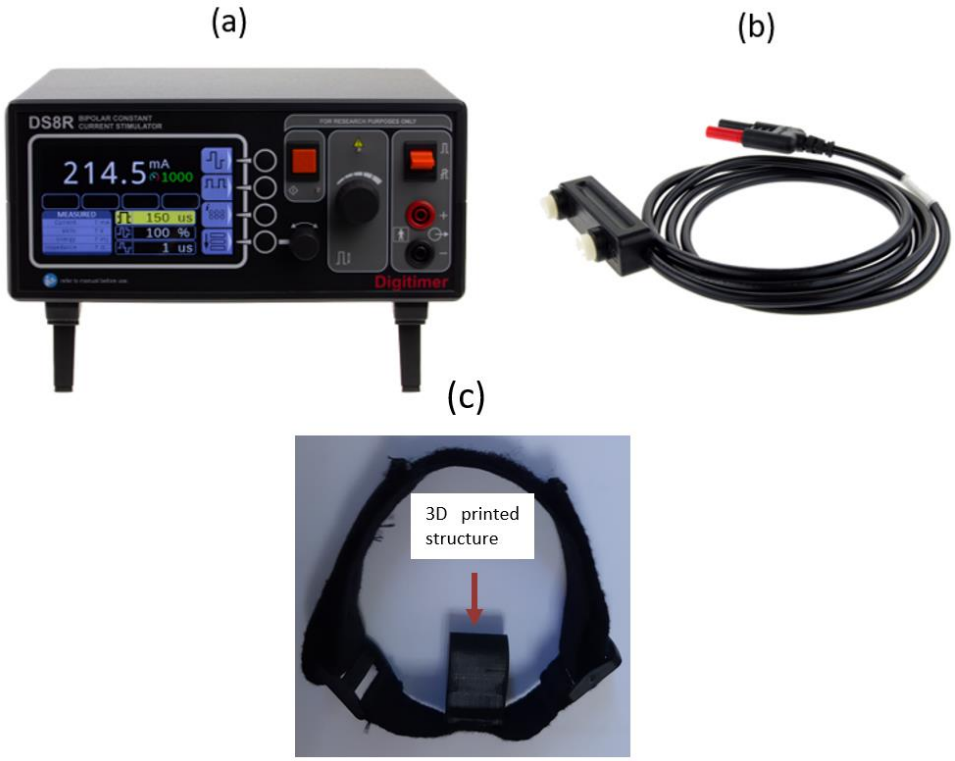

Figure 3.4. Equipment for electrical stimulation and electrode localisation. (a) Digitimer DSR8 for delivery of electrical pulses. (b) Bar electrode. (c) Strap for applying pressure to the stimulation electrodes. (a) and (b) extracted from [80, 81].

During a part of the procedure, volunteers were asked to maintain 20% of their maximum voluntary contraction (MVC) in order to employ this value during the flexion task. For this purpose, a load cell

(Model: FC2231-0000-0100-L, TE Connectivity, USA) was used to measure the compression force by means of a 3D printed structure drilled into the wall. This arrangement (see Figure 3.5.) was printed at the NRG from the Cajal Institute and consisted of two sub-structures that enclosed the load cell and that applied pressure on the sensor when the subject pulled a strap (Decathlon, France) set on his wrist. Velcro was sewn to this band so it could be adjusted to the wrist depending on the participants' arm length. The strap was connected to the whole structure enclosing the sensor by a hole in one of the sub-structures. The software OT Biolab+ was also used in order to visualise and measure the exerted flexion wrist force.

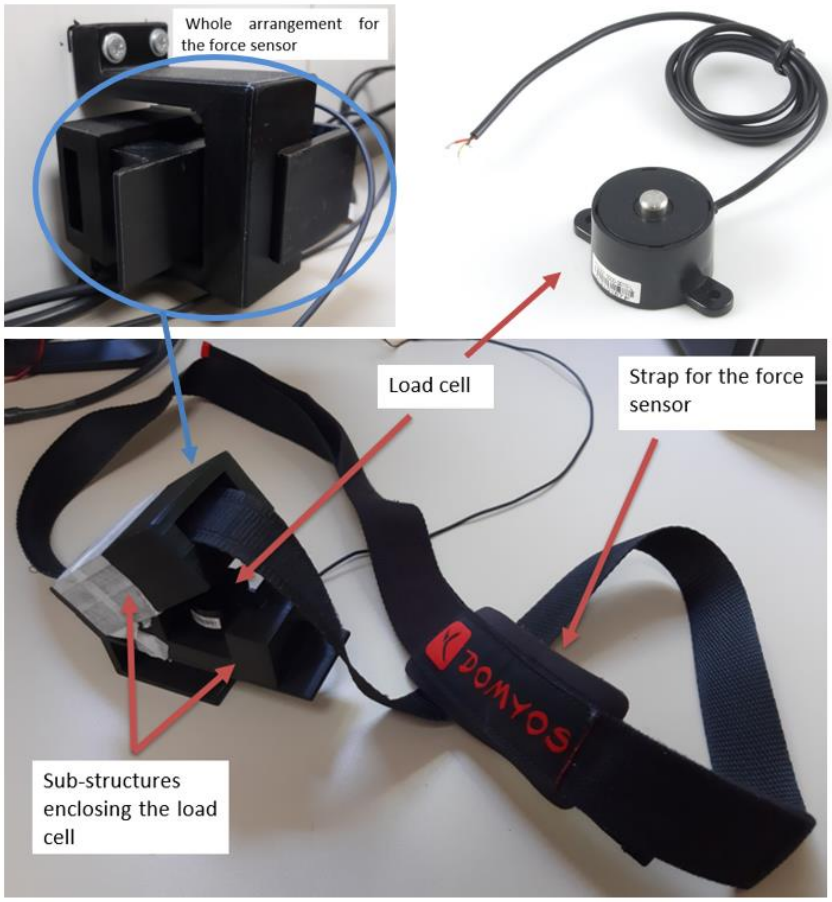

Figure 3.5. Arrangement for the compression sensor. Load cell picture extracted from [82].

In order to control the stimulation pulses from the constant current stimulator DSR8 and receive the information from the compression sensor, the Arduino M0 Pro board shown in Figure 3.6. was used. Pre-existing Arduino codes were employed for this purpose.

Figure 3.6. Arduino M0 Pro board. Extracted from [83].

During the last part of the procedure, the effect of long-period vibration on Ia afferents was studied. For this purpose, the muscle massager MANFLY (Amazon, USA) (see Figure 3.7.) was employed. In order to place and fix this device on the tendons of the wrist, elastic straps with added Velcro were used.

Figure 3.7. Muscle massager for evaluation of the role of long-vibration. Extracted from [84].

Lastly, a pre-existing guided-user interface (GUI) – developed by the Neuro-Rehabilitation Group– was used to visualise and save the EMG activity, especially the H-reflexes and M-waves, when delivering electrical pulses. The user could select different settings: the sampling frequency, which was always set to 2,042.48 Hz for these experiments; the EMG and auxiliary channels; the plotting mode that in the case of these studies was always the stimuli mode; the plotting scale (normally RMT scale); and different file settings, such as the name of the file and subject, or the intensity. After recording EMG activity, the MATLAB file was used for data analysis. In Figure 3.8. all these GUI settings are shown.

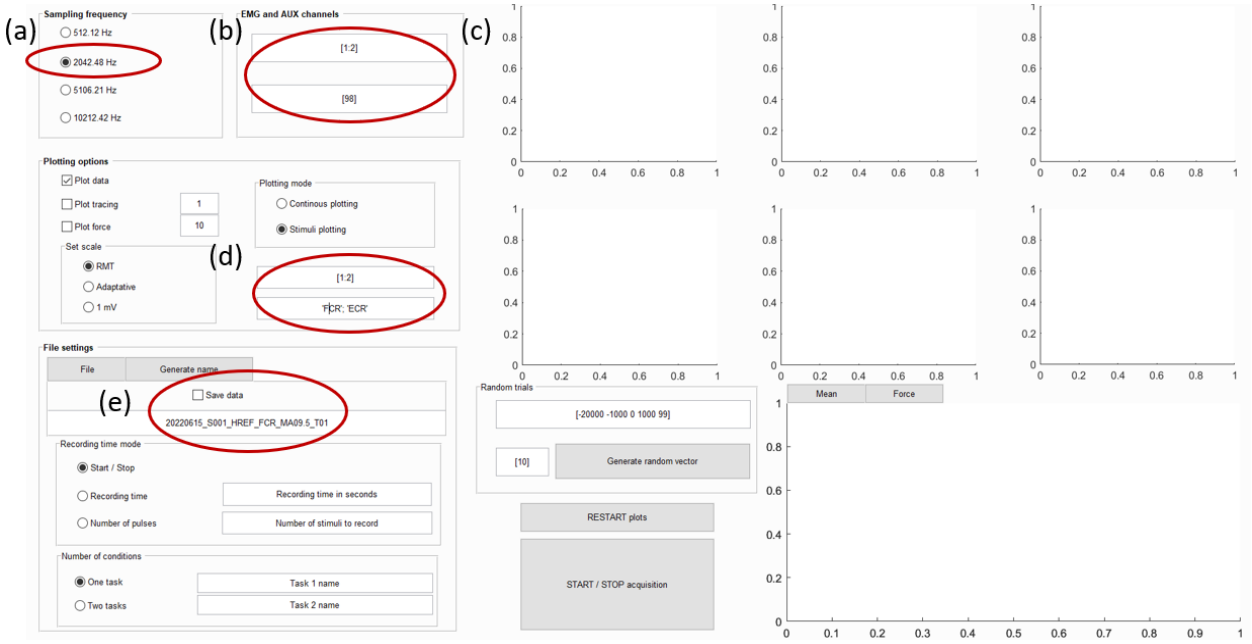

Figure 3.8. EMG amplifier GUI. (a) Sampling frequency set to 2042.48 Hz. (b) EMG channels indicated by [1, 2] and force channel indicated by [98]. (c) Stimulation plots. (d) Plotting mode of ECR and FCR set to stimuli plotting. (e) File settings.

3.1.2.3. EXPERIMENTAL PROTOCOL

The experimental procedure took approximately 3-3.5 hours and was divided into three parts:

- PRE: it consisted of two sub-experiments. The first one was H-reflex and M-wave recruitment curves, followed by reciprocal inhibition during flexion task (voluntary contraction).

- POST30': 30 minutes after PRE stage, the sub-experiments of PRE were repeated. We expect to obtain the same results than in the PRE stage and thus, demonstrate the reproducibility of reciprocal inhibition.
- POSTVIB: immediately after finishing POST30', 20 minutes of vibration were applied to the wrist tendons. Posteriorly, the sub-experiments of PRE were repeated right away.

It is worth mentioning that during the three parts of this study (PRE, POST30', and POSTVIB), different parameters (range of intensities, stimulation intensity, and 20% MVC) were measured at the beginning of the experiment and remained the same throughout the three sub-experiments in order to maintain the same conditions during the entire procedure. Furthermore, all participants were submitted to the same conditions (20% of their MVC, stimulation below MT to the radial nerve, among others) to standardise the procedure and obtain more reliable results.

Before starting the actual experiment and meeting the participant, all the instrumentation needed to be set up. Therefore, the following connections between the different devices were made:

- The 16-channel bipolar adapter jack connector (Figure 3.3. (d)) was connected to the signal amplifier Quattrocento (Figure 3.2.) to register ECR and FCR muscle activity.
- The reference wristband (Figure 3.3. (e)) positioned on the participant's elbow was also connected to the Quattrocento via a patient ground cable (Figure 3.3. (c)).
- The signal amplifier and the current stimulator DSR8 (Figure 3.4. (a)) were also connected to each other.
- The Arduino M0 Pro board (Figure 3.6.) was adequately connected to the current stimulator and the force sensor.
- Lastly, the Quattrocento and the Arduino board were connected to the computer via a USB/Ethernet cable in order to receive the EMG and force signals.

After setting up all the instrumentation, the volunteer was prepared following the scheme in Figure 3.9.

Firstly, the skin of the dominant arm was cleaned with alcohol to later place different sEMG electrodes. The cleaned areas were the FCR and ECR muscles, the elbow, and the spinal groove. Afterwards, the participant was asked to flex or extend the wrist to identify by palpation both muscles. Once localised, a pair of recording sEMG electrodes (Figure 3.1. (a) or (b)) were placed on the muscle bellies with a 2cm inter-electrode distance to measure FCR and ECR activity. Posteriorly, a wet wristband was placed on the elbow to work as a reference. The bio-signal amplifier Quattrocento and its respective software (OT Biolab+) were then used in order to check the proper position of the sEMG electrodes and the quality of the EMG signals.

Secondly, to locate the radial nerve, the bar electrode (Figure 3.4. (b)) connected to the current stimulator DSR8 was employed. Electric pulses were manually delivered at the same time the bar electrode was moved around the spinal groove in order to find the best spot that gave the highest wrist response. Once these two points were identified and marked by a pen, a pair of round stimulation electrodes (Figure 3.1. (c)) were placed on the skin. Posteriorly, the adjustable straps with 3D printed shapes from Figure 3.4. (c) were fastened in the volunteer's arm to apply pressure to the stimulation electrodes. This step was essential, as additional pressure was needed to obtain an adequate response of the wrist. Lastly, the radial stimulation electrodes were connected to the constant current stimulator.

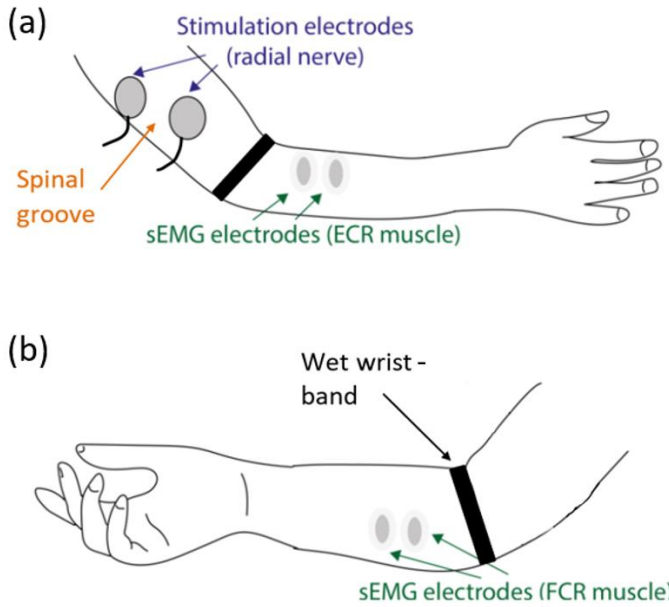

Figure 3.9. Complete electrode arrangement for study 1. (a) Surface electrodes were placed over the ECR muscle belly to record its activity and over the radial nerve for ECR activation in the spinal groove. (b) Another pair of electrodes were placed over the FCR muscle belly to record its activity. In addition, a wet wristband on the elbow was used as a reference. Adapted from [70].

After finishing the complete electrode arrangement, the three stages of this experiment (PRE, POST30' and POSTVIB) were conducted as shown in the diagram from Figure 3.10. These three parts of the study 1 could be summarised into three sub-experiments: H-reflex and M-wave recruitment curves, reciprocal inhibition during flexion task and reciprocal inhibition during flexion task after long-period tendon vibration.

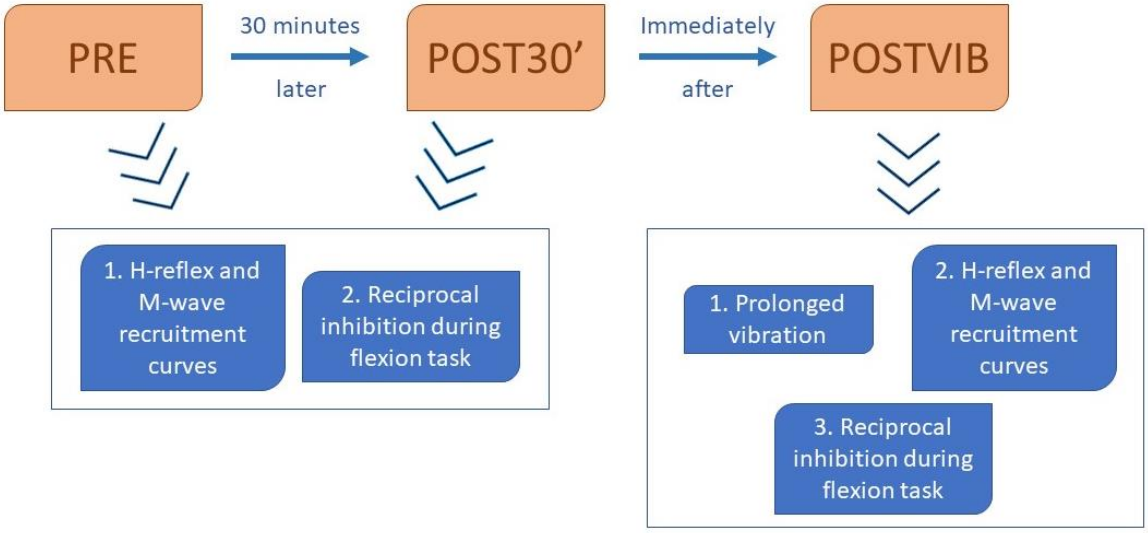

Figure 3.10. Experimental protocol of study 1.

H-reflex and M-wave recruitment curves

The objective of this sub-experiment was to obtain ECR H and M-waves by delivering a range of stimulation intensities in order to guarantee proper nerve stimulation. This enabled the assessment and modulation of reciprocal inhibition from FCR.

As described in the protocol from Pascual-Valdunciel et al. (2019) [70], volunteers were asked to comfortably sit on an armchair with their dominant arm stretched on a table in front of them. The shoulder joint was kept at 45º whereas the elbow joint was kept at 120º. In addition, participants were

also asked to slightly extend the wrist in order to further facilitate the appearance of ECR reflexes during electrical stimulation.

For each participant, 5 to 6 different stimulation intensities were delivered via the constant current stimulator DSR8 to the radial nerve ranging from 4 to 22 mA, with the purpose of measuring an illustrative excitation of Ia afferent pathways. For each stimulation intensity, 5 electrical pulses of 1ms duration were manually delivered to the radial nerve with an inter-stimulus interval of 5 ± 0.2 ms in order to avoid post-activation depression[18]. Additionally, the order of each stimulation intensity value was random. Each elicited EMG response was recorded using the EMG amplifier Quattrocento and further saved and plotted by means of MATLAB (version 2020) EMG GUI (Figure 3.8.).

Lastly, the motor threshold (MT) was defined. To this end, the minimum stimulation intensity that produced a visible muscle response was selected as the MT. This was visually confirmed with the H-reflexes and M-waves recruitment curves that resulted from the stimulation below MT of the radial nerve (see Section 2.3.2.). For this part, participants were asked to slightly flex their wrist to facilitate the definition of the MT.

This sub-experiment was conducted in the PRE stage, 30 minutes after the PRE stage (POST30') and immediately after 20 minutes of tendon vibration (POSTVIB).

Reciprocal inhibition during flexion task

The aim of the second sub-experiment was to assess the amount of reciprocal inhibition resulting from a voluntary wrist flexion task and compare the obtained reciprocal inhibition from each stage. The inhibition of the FCR muscle is presumably due to the stimulation of Ia afferent fibres of the antagonist muscle – the ECR – that produces reciprocal inhibition mechanisms. Therefore, the main objective of this sub-experiment was to demonstrate the implication of Ia afferents in reciprocal inhibition when stimulation below MT was applied.

Volunteers were positioned as previously described, but in this case, they were asked to perform their maximum voluntary contraction (MVC) by means of a wrist flexion task. To that end, an adjustable strap connected to a structure enclosing a compression sensor (see Figure 3.5.) was set on the participants' wrist in order to measure their MVC. In this way, subjects were asked to perform their maximum wrist flexion for 5 seconds by pulling the strap and avoiding using other muscles such as the biceps. The procedure was repeated 3 times with a rest period of 30 seconds between each trial and was recorded using OT Biolab+. Afterwards, the MVC was calculated as the mean of the three trials.

Once the MVC was determined, electrical stimulation below MT during flexion task was addressed. Surface stimulation was delivered to the radial nerve, activating the ECR and inhibiting the antagonist muscle – the FCR. During this experiment 4 trials were performed, each one of 30 electrical pulses with an interstimulus interval of 2 ± 0.2 ms. Each try-out consisted of a ramp corresponding to 20% of the MVC that the participants had to follow while stimulation below MT was applied to the radial nerve. The stimulation intensity used was the calculated MT from the previous sub-experiment.

The software OT Biolab+ was used to help participants maintain a constant muscle activity during the procedure and record EMG activity of both muscles. Furthermore, the Arduino board was employed to control the constant current stimulator and receive the information from the force sensor and transmit it to the Quattrocento for visualisation.

This sub-experiment was conducted in the PRE stage, 30 minutes after the PRE stage (POST30') and immediately after 20 minutes of tendon vibration (POSTVIB).

Long-period tendon vibration

The goal of the third sub-experiment was to assess the effect long-period vibration had on reciprocal inhibition when applied to the wrist tendons.

[18] Post-activation depression is due to the activation of some motoneurons aside from Ia afferents and, therefore, limits the amplitude of H-reflexes. Thus, low stimulation rates are needed [85].

The muscle massager from Figure 3.8. was employed to apply 20 minutes of constant vibration to the tendons of the wrist. In order to prevent the device from falling off the forearm, two adjustable and elastic straps were used. After 20 minutes of tendon vibration, afferent stimulation to the radial nerve during flexion task was immediately conducted with the same stimulation and force parameters.

This sub-experiment was conducted immediately after POST30' stage finished.

See Annex F for the complete experimental protocol used in study 1.

3.1.3. DATA ANALYSIS

Signals were processed using MATLAB R2020b (MathWorks, USA) with a similar approach as the one described in Pascual Valdunciel et al. (2019) [70].

H-reflex and M-wave recruitment curves

Raw EMG signals were bandpass filtered by means of a Butterworth filter (2nd order, 50-500 Hz) and then segmented in trials with respect to each stimulus. Lastly, the segmented data was averaged. Afterwards, peak-to-peak amplitudes of the averaged signals were determined for both waves (see Figure 3.11. (a)). The H-reflex of each participant and of each stimulation intensity was calculated and expressed as a proportion of maximum M-wave (see Figure 3.11. (b)). These values allowed us to know if the stimulation and recording electrodes were properly positioned.

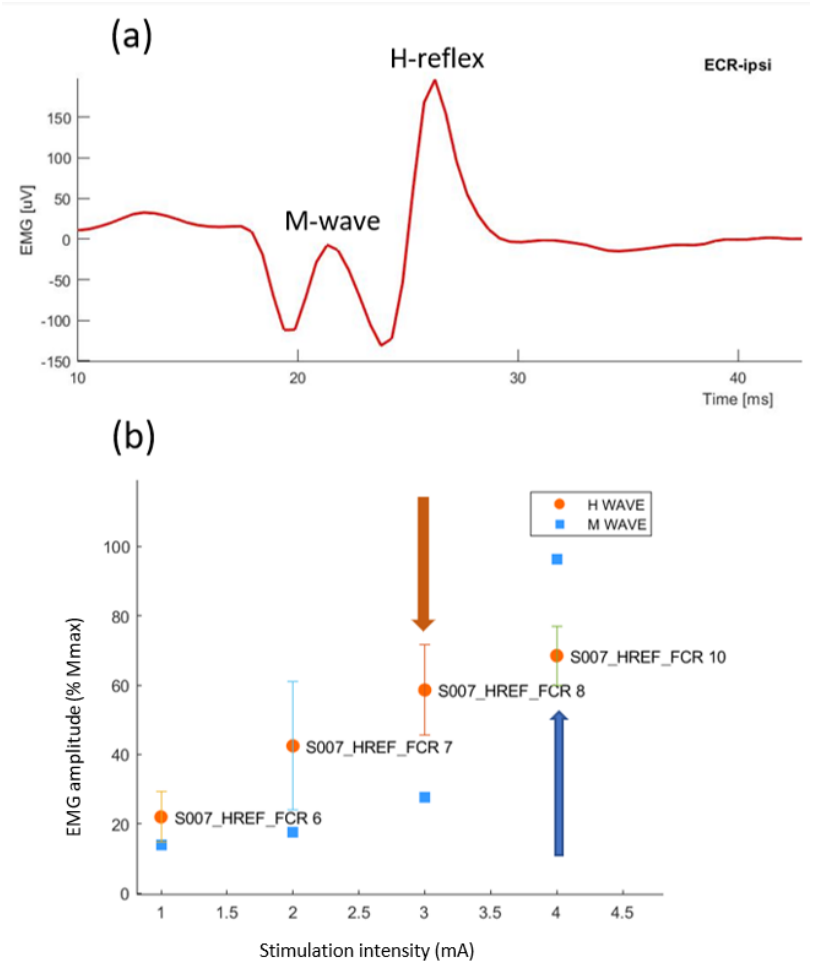

Figure 3.11. H-reflex and M-wave recruitment curves from ECR muscle. (a) Individual EMG sample from S007 after stimulating the radial nerve with 8mA (below MT). (b) Recruitment curves from H-reflex (orange) and M-wave (blue) from S007. Maximum H-reflex is indicated by an orange arrow, whereas maximum M-wave is indicated by a blue arrow.

Reciprocal inhibition during flexion task and after long-period vibration

Raw EMG signals were also bandpass filtered with the previous Butterworth filter and then rectified. Posteriorly, data corresponding to the total 120 electrical pulses from the 4 trials were segmented and then averaged to determine the degree of FCR inhibition for each volunteer. The baseline activity (see Figure 3.12. (a)) was computed by means of the averaged and rectified EMG activity ranging from -140 to -40 ms (before stimulation). The start of the inhibition window was characterised by the time where the signal was lower than the baseline during at least 5 ms. Conversely, the end of the inhibition window was defined as the time when the signal stayed above the baseline for 5 ms. The inhibition window was manually calculated for each subject and for each stage. Lastly, the amount of inhibition was determined as the mean activity in the previously defined inhibition window and maximum inhibition was calculated as the minimum value of the inhibition window (see Figure 3.12. (b) and (c)).

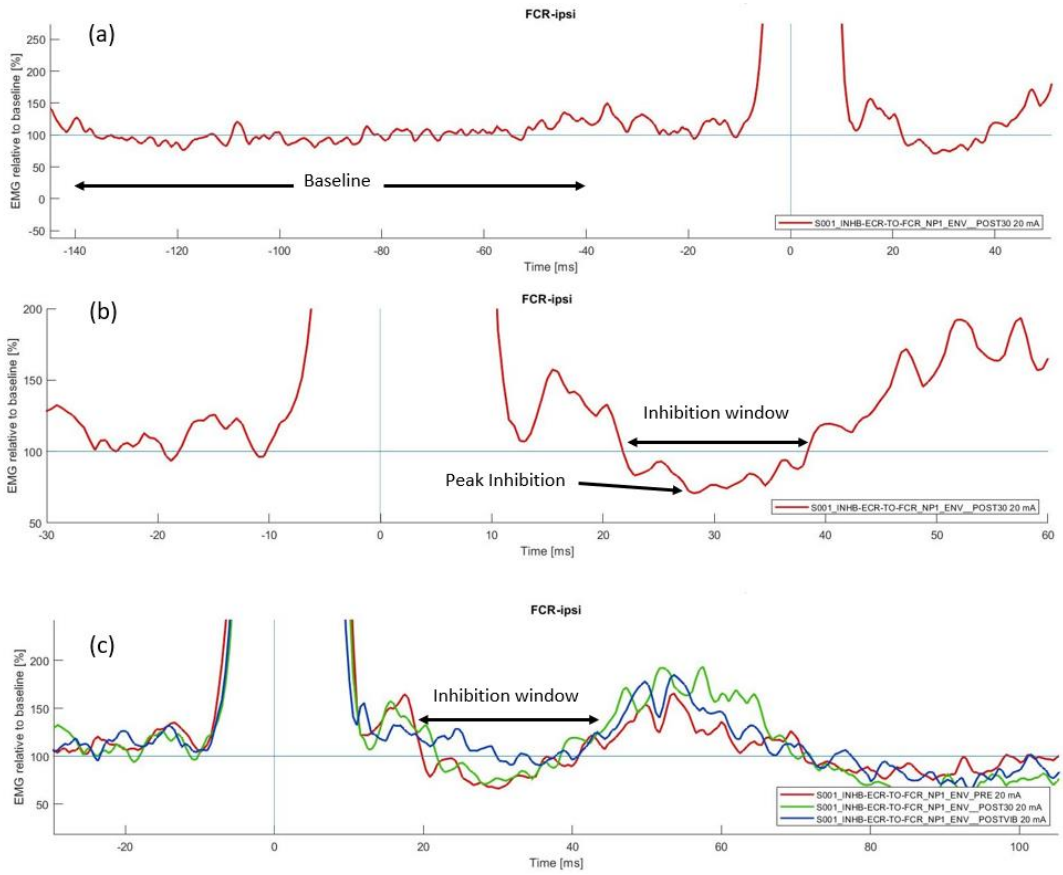

Figure 3.12. Individual example of FCR inhibition by radial stimulation. (a) Baseline activity. (b) FCR inhibition window and its maximum peak inhibition. (c) Example of FCR inhibition in the three stages (PRE (red line), POST30' (green line) and POSTVIB (blue line)) from S001.

3.1.4. STATISTICAL ANALYSIS

In order to check the normal distribution of the samples, Shapiro-Wilk's test was employed.

Two sample t-tests were performed in order to evaluate the differences between the baseline and the inhibition time window for each of the stages (PRE, POST30' and POSTVIB) across subjects. Therefore, if the mean of the 120 delivered electrical was less than the mean baseline, electrical stimulation below MT was validated as a strategy that reduces muscle activity and entails reciprocal inhibition.

One-way ANOVA was used in order to test muscle inhibition differences between PRE, POST30', and POSTVIB. Then, Post-hoc Tukey´s test was employed to find statistically significant differences

between those three groups. We expect less muscle inhibition after long-period tendon vibration (POSTVIB) in comparison to the first two stages.

Statistical significance was set by a *p*-value of 0.05.

3.1.5. RESULTS

H-reflex and M-wave recruitment curves

During this experiment, H and M-waves were elicited by stimulating the radial nerve in 9 out of 14 participants.

Figure 3.11. shows an individual EMG sample from S007 after stimulation of the radial nerve with 8mA. For this specific participant, maximum H-reflex was elicited at 8mA (orange-coloured arrow in Figure 3.11. (b)), whereas maximum M-wave was elicited at 10 mA (blue-coloured arrow in Figure 3.11. (b)). Therefore, maximum H-reflex of ECR for this subject was 39% of his maximum M-wave.

H-reflexes obtained during these experiments were visually confirmed using the EMG GUI in Figure 3.8., with the final purpose of ensuring proper positioning of the stimulation and recording electrodes. MT intensity values were based on the stimulation amplitudes that elicited a bigger H-reflex response than M-wave response and were used in order to conduct the next sub-experiment.

Reciprocal inhibition during flexion task and after long-period tendon vibration

On average, a stimulation intensity of 11.09 ± 4.48 mA was delivered to the radial nerve in 9 out of 14 volunteers in order to modulate reciprocal inhibition during the three stages this experiment consisted of, with the purpose of demonstrating the implication of Ia afferents in this mechanism when electrical stimulation below MT was applied.

Regarding the inhibition windows, for the PRE stage the inhibition started at 23.13 ± 3.48 ms and finished at 42.50 ± 4.57 ms, with a total duration of 19.37 ± 4.49 ms. In the case of the POST30' stage, the onset of the inhibition was at 22.91 ± 5.26 ms and ended at 42.20 ± 3.84 ms, with a total duration of 19.28 ± 6.64. Lastly, for the POSTVIB stage, the inhibition window lasted a total of 15.43 ± 5.05 ms, started at 25.42 ± 3.06 ms, and finished at 40.84 ± 4.01 ms (see Table 3.1.).

TABLE 3.1. RESULTS OF RECIPROCAL INHIBITION DURING FLEXION TASK IN STUDY 1

Subject	Stage	Inhibition window start (ms)	Inhibition window end (ms)	Duration of the inhibition window (ms)	Mean inhibition (%)	Maximum inhibition (%)
	PRE	19.87	40.92	21.05	17.60	17.14
S001	POST30'	21.83	38.47	16.64	16.16	13.56
	POSTVIB	0	0	0	0	0
	PRE	19.38	35.04	15.66	20.72	21.08
S002	POST30'	15.46	38.72	23.25	23.32	17.01
	POSTVIB	20.85	38.47	17.62	20.16	26.09
	PRE	23.78	40.92	17.14	15.87	16.29
S004	POST30'	23.29	41.41	18.12	10.66	31.13
	POSTVIB	22.80	41.90	19.10	9.80	31.22
	PRE	25.25	38.47	13.22	23.59	13.04
S005	POST30'	25.25	40.43	15.18	20.25	17.56
	POSTVIB	26.23	41.90	15.67	19.49	23.08
	PRE	20.36	39.45	19.09	23.15	15.34
S007	POST30'	19.87	40.92	21.05	24.07	17.78
	POSTVIB	0	0	0	0	0

	PRE	30.15	45.82	15.67	14.96	13.82
S008	POST30'	18.89	46.3	27.41	16.26	32.07
	POSTVIB	28.68	43.37	14.69	15.07	28.38
	PRE	25.74	46.31	20.57	10.16	24.98
S009	POST30'	25.74	49.24	23.50	13.62	18.49
	POSTVIB	25.74	45.33	19.59	6.33	19.87
	PRE	21.83	47.77	25.94	18.31	9.41
S010	POST30'	21.83	45.33	23.50	25	15.66
	POSTVIB	28.19	34.07	5.88	10.06	15.18
	PRE	21.82	47.77	25.95	15.27	27.48
S014	POST30'	34.06	38.96	4.9	8.63	9.02
	POSTVIB	0	0	0	0	0
	PRE	23.13	42.50	19.37	17.74	17.62
Mean	POST30'	22.91	42.20	19.28	17.55	19.14
	POSTVIB	25.42	40.84	15.43	8.99	15.98

FCR mean inhibition of each volunteer can be found in Figure 3.13.

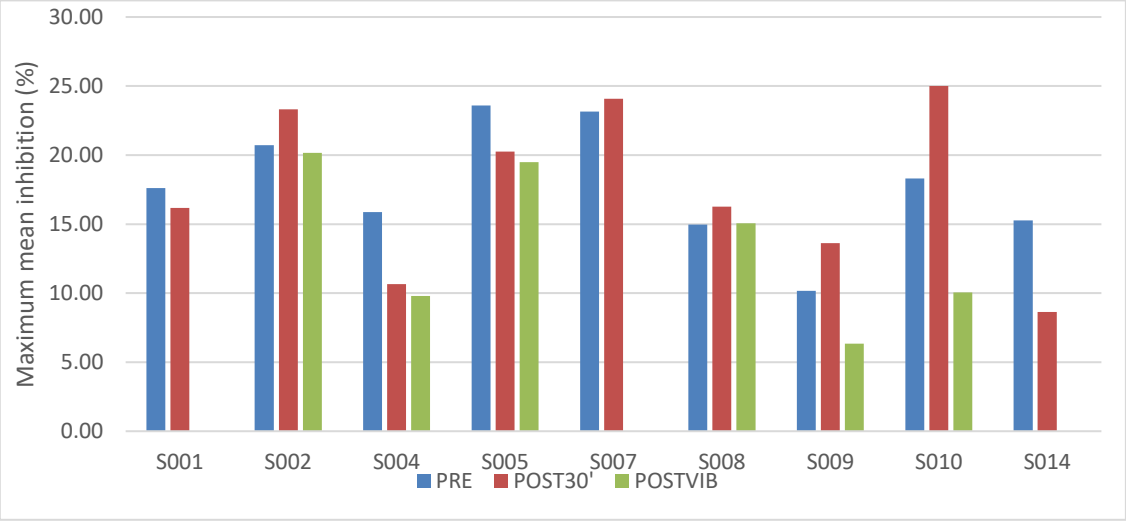

Figure 3.13. Results of reciprocal inhibition during wrist flexion task for each participant. Blue-coloured bars indicate the inhibition obtained during PRE stage, red-coloured ones during the POST30' stage and green-coloured bars represent FCR inhibition after POSTVIB was conducted.

Even though reciprocal inhibition after 20-minute vibration decreased in all volunteers, only in S001, S007 and S014 were the mean inhibition values from PRE and POST30' statistically different from POSTVIB, demonstrating the effect tendon vibration had on the modulation of reciprocal inhibition. Moreover, no statistical significance was found between the mean inhibitions of PRE and POST30' from each subject, which proved the reproducibility of the strategy when no tendon vibration had been applied (see Table 3.2.).

TABLE 3.2. STATISTICAL DIFFERENCES FOR EACH SUBJECT AFTER STIMULATION BELOW MT

Subject	p- value PRE, POST30', POSTVIB	Statistical significance
S001	$p<0.001$	POSTVIB has means significantly different from PRE and POST30'

S002	p=0.68	No stages have means significantly different
S004	p=0.44	No stages have means significantly different
S005	p=0.80	No stages have means significantly different
S007	p<0.001	POSTVIB has means significantly different from PRE and POST30'
S008	p=0.95	No stages have means significantly different
S009	p=0.36	No stages have means significantly different
S010	p=0.097	No stages have means significantly different
S014	p<0.001	POSTVIB has means significantly different from PRE and POST30'

In green: expected values.

For all subjects, afferent stimulation to the radial nerve achieved a mean FCR inhibition of 17.74 ± 4.29% in the PRE stage, of 17.55 ± 5.97 % in the POST30' stage, and of 8.99 ± 8.09% in the POSTVIB stage. By means of a Bonferroni Post-Hoc test, a Bonferroni p-value of 1.00 was obtained between PRE and POST30', which demonstrated the reproducibility of the study because no statistically significant difference was found between these two stages. Conversely, the Bonferroni p-values between PRE-POSTVIB, and POST30'-POSTVIB were 0.036 and 0.043, respectively. Both p-values proved that POSTVIB mean inhibition was different from both PRE and POST30' mean inhibition, which meant that long-period vibration influenced the excitatory threshold of Ia afferent pathways and in turn also affected the degree of reciprocal inhibition. Figure 3.14. shows the total mean inhibition of this study in the three stages.

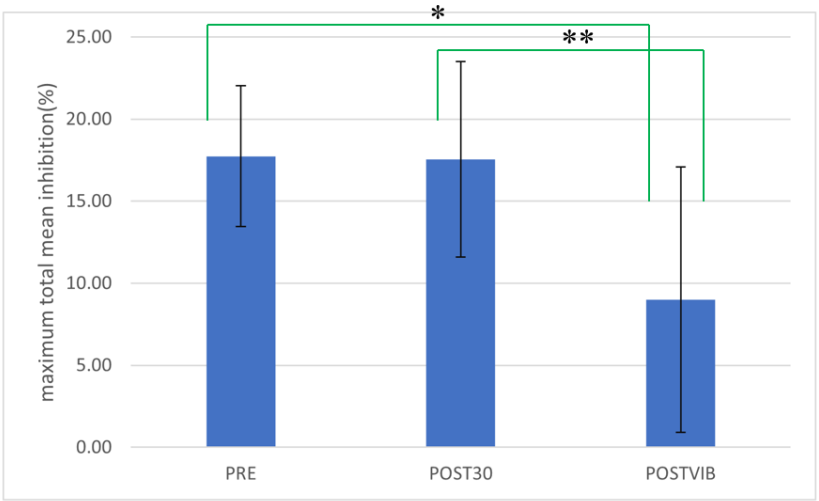

Figure 3.14. Total mean inhibition in PRE, POST30' and POSTVIB. * $p_{Bonferroni}$= 0.036; ** $p_{Bonferroni}$= 0.043.

3.1.6. DISCUSSION

The first study of this Bachelor Thesis aimed at modulating reciprocal inhibition during FCR voluntary contraction by means of peripheral electrical stimulation below MT before and after long-period vibration was applied. Thus, the main goal of this study was to demonstrate the involvement of Ia afferents in the modulation of reciprocal inhibition by means of a standardised procedure with low-current intensity.

An important obstacle encountered during the conduction of this study was the inability to find a response of the radial nerve in 5 out of 14 participants once the adjustable straps (see Figure 3.4. (c))

were fastened in the arm. As explained in Section 3.1.2.3. these straps were employed in order to apply more pressure to the stimulation electrodes with the final purpose of better reaching the radial nerve. Since the participant had first to extend the arm in order to facilitate the appearance of ECR reflexes and then flex the arm to measure the MVC, the inner conformation of the arm slightly changed and hid even more the radial nerve within muscle and skin. This movement of the arm prevented the 3D printed structures from pressuring the surface stimulation electrodes enough to get to the radial nerve, which made impossible to carry on with the study in those 5 volunteers. In addition, this movement also contributed to the instability of the 3D printed structures pressuring the stimulation electrodes, which end up falling sideways. Therefore, the same pressuring conditions were difficult to maintain throughout the experiments and in turn the wrist response to afferent stimulation.

Regarding the outcomes of this experiment, on the one hand, the results from H-reflex recruitment curves positively confirmed a proper electrode arrangement for each subject and muscle, which had the final purpose of modulating reciprocal inhibition by means of spinal reflexes. Moreover, M-wave recruitment curves were employed to set the stimulation below MT for each volunteer. This was determined by comparing H and M-wave recruitment curves and selecting the stimulation intensity that produced a bigger amplitude in H-reflex than in M-wave.

On the other hand, it was demonstrated that the stimulation below MT to the radial nerve was a viable and effective procedure to produce muscle inhibition during FCR voluntary contraction. This was evaluated via Paired T-tests that resulted in significantly different means between baseline and inhibition windows of each subject and stage. Furthermore, the modulation of reciprocal inhibition provided evidence for the presence of Ia afferents between a pair of antagonist wrist muscles (FCR-ECR). This was achieved by delivering low-current stimulation pulses to the radial nerve while FCR was voluntarily contracted. In this way, modulation of Ia afferent pathways from ECR connected with inhibitory interneurons in the spinal cord, and then inhibited the activity of FCR. In this respect, PRE and POST30' inhibition results showed that peripheral stimulation strategy below MT produced the same amount of reciprocal inhibition in ten subjects ($p_{Bonferroni}$=1.00) undergoing the same conditions and, thereby demonstrating that the same group of I fibres was involved in the performance of FCR muscle inhibition: Ia afferents.

The implication of Ia afferent fibres in reciprocal inhibition was further assessed during this experiment by applying long-duration tendon vibration before stimulation below MT. According to Robin Souron et al. (2019) [23], long-lasting vibration locally applied to a muscle, or its tendons produces a raise in the electrical threshold of Ia afferent fibres. This hypothesis was investigated during POSTVIB stage by means of applying 20 minutes of long-period vibration on the wrist tendons. After that, afferent stimulation was delivered to the radial nerve to later assess possible spinal changes that could be reflected in the amount of reciprocal inhibition. FCR mean inhibition from POSTVIB stage was proved to be statistically different (see Figure 3.14.) than the reciprocal inhibition obtained in PRE and POST30', being FCR muscle inhibition after POSTVIB 50.67% smaller than in PRE and 51.25% smaller than in POST30' stages. Therefore, following the hypothesis by Robin Souron et al. (2019), such decrease in reciprocal inhibition could only have meant that less Ia afferents were recruited due to the increased recruitment threshold produced by prolonged vibration, which in turn demonstrated again the involvement of Ia afferents in FCR muscle inhibition.

Therefore, by means of stimulation below MT to the radial nerve, this study successfully demonstrated the implication of Ia afferent fibres in the modulation of reciprocal inhibition during wrist flexion task.

The results of this study matched the conclusions from previous experiments assessing low-intensity stimulation. B. Day et al. (1984) [86], P. Fuhr and M. Hallett (1993) [87] and N. Petersen et al. (1999) [88], already demonstrated that electrical pulses below MT presumably involved the implication of Ia afferent pathways in peripheral reciprocal inhibition and that such intensities didn't saturate this reciprocal inhibitory pathway. Similarly, in more recent studies, Pascual-Valdunciel et al. (2019) [70], demonstrated that reciprocal inhibition was possible in both FCR (16 ± 6%). and ECR (12 ± 4%) muscles by means of stimulation of Ia afferent pathways. It is worth mentioning that this study used a similar approach as this experiment. Another example is Nito et al. (2018) [89], who evaluated the effects of afferent stimulation between the brachioradialis (BR) and FCR. The stimulation below MT

of both muscle nerves produced a significant muscle inhibition in both cases (6.8 ± 1.8% for BR and 8.8 ± 0.9% for FCR), which allowed them to conclude that such intensities could have only activated group Ia and Ib afferents. Since the delay of reciprocal inhibition was longer than in the homonymous facilitation, they deduced that reciprocal inhibition had to be mediated by Ia afferent fibres.

3.2. STUDY 2: EFFECT OF AFFERENT STIMULATION ON HEALTHY SUBJECTS SIMULATING PATHOLOGICAL TREMOR

3.2.1. BRIEF INTRODUCTION AND GOALS OF THE STUDY

The aim of the second and final study of this Bachelor Thesis was to test a novel strategy of electrical stimulation called selective and adaptive timely stimulation (SATS). In order to do so, SATS was applied for twenty minutes in seven healthy participants that mimicked wrist flexion-extension tremor.

Two different SATS strategies were tested: out-of-phase and in-phase stimulation.

Out-of-phase stimulation pattern is based on the stimulation of the antagonist muscle when the agonist contracts *i.e.*, when ECR is activated, stimulation is delivered to the FCR and vice versa. Conversely, in-phase stimulation consists of activating the agonist muscle when it contracts *i.e.*, when ECR is activated, stimulation pulses are delivered to the ECR and vice versa. Therefore, the main goal of this study was to assess the modulation of reciprocal inhibition between a pair of antagonistic muscles produced by afferent stimulation strategies with SATS.

Our other hypothesis was that FCR mean inhibition would be smaller when afferent stimulation with an out-of-phase pattern was delivered. Conversely, we also hypothesised that in-phase stimulation strategy would produce a higher reciprocal inhibition.

The findings of this study can provide additional knowledge on the role low-current stimulation of afferent fibres have in the modulation of voluntary movement and, thereby in the possible reduction of pathological tremor.

3.2.2. MATERIALS AND METHODOLOGY

3.2.2.1. PARTICIPANTS

Seven healthy subjects volunteered to participate in this study. Six of them were right-handed and none presented any neurological injuries. Five of them were females and two males, with a mean age and a standard deviation (SD) of 24.57 ± 4.20 years. All participants were verbally given all the necessary information about the procedure and possible discomfort they could perceive. After that, doubts were cleared, and they signed an informed consent form. All experiments were conducted according to the Declaration of Helsinki and approved by a local Ethical Committee.

3.2.2.2. EQUIPMENT

For the entire procedure, four bipolar electrodes (Ag/AgCl 2.2x2.2 cm, Vermed Neuroplus, USA) were used in order to measure EMG activity: one pair was placed on the FCR and the other on the ECR (see Figure 3.1(a)). For surface electrical stimulation, two bipolar round electrodes (4 cm diameter, EN-Trode, Enraf Nonius, The Netherlands) (see Figure 3.1. (c)) were placed on both the radial nerve and the median nerve. In this study, another surface electrode (5x5 cm, ValuTrode Cloth, Denmark) was placed on the elbow to work as ground for the stimulation (see Figure 3.15.).

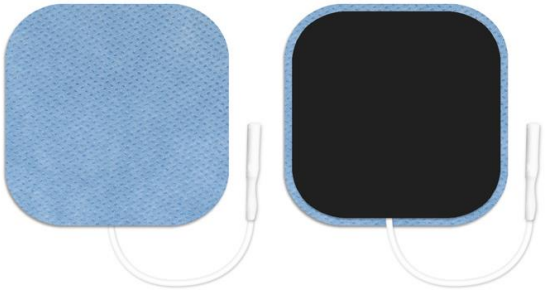

Figure 3.15. Ground electrodes from ValuTrode Cloth for afferent stimulation. Extracted from [90].

All the equipment in Figure 3.2. and Figure 3.3. (OT Bioelettronica, Italy) was again used to record and amplify sEMG signals. The Quattrocento and its accessories were arranged in the same way as in the first study. Similarly, the software OT Biolab+ was also employed in order to display and check the quality of FCR and ECR muscle activity.

As in the first experiment, one constant current stimulator DS8R (Digitimer Ltd, UK) shown in Figure 3.4. (a) was used to deliver electrical pulses to the radial nerve. In this case, in order to localise also the median nerve, the same procedure by means of the bar electrode in Figure 3.4. (b) was carried out. Because the stimulation pattern during SATS was based on ECR activity, the adjustable straps from Figure 3.4. (c) were not employed in order to apply pressure to the electrodes placed on the median nerve, but again only on the radial nerve.

In order to measure the maximum voluntary contraction (MVC) of each volunteer, the same arrangement as Figure 3.5. was used. As performed during the first study, the software OT Biolab+ was employed to guide and help the participant during the flexion task as well as to measure the exerted force.

Arduino M0 Pro board (Figure 3.6.) was yet again used to control de stimulation pulses from the constant current stimulator DSR8 and receive the information from the compression sensor.

Lastly, for afferent stimulation during SATS intervention, a pre-existing device called EAST (see Figure 3.16.) was employed. This one consisted of a customised processing unit with an EMG amplifier and a voltage-controlled stimulator. Different cables were used in order to register EMG activity and deliver afferent electrical stimulation. This device was connected to the computer via an USB cable.

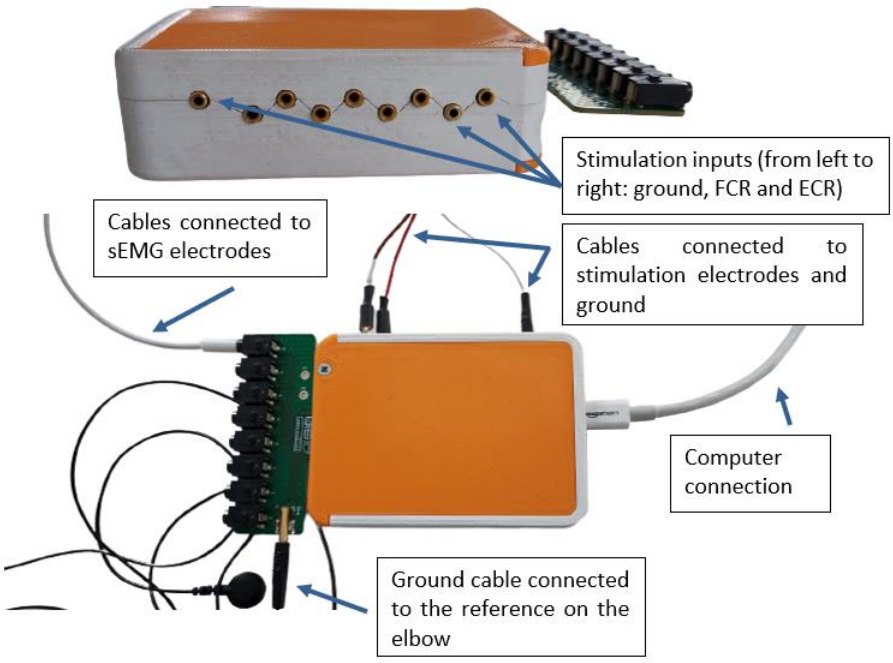

Figure 3.16. EAST stimulator and its input arrangement.

A pre-existing GUI developed by the NRG was used to switch on and off the EAST as well as the stimulation. Furthermore, this interface allowed to visualise and save EMG activity while the participant was simulating tremorgenic activity. The user could select different stimulation parameters, such as the time length of stimulation, tremor threshold, or the stimulation intensity (see Figure 3.17.). The entire stimulation consisted of 5 trials of 120 seconds each with a total recording time of 600 seconds. The tremor threshold was defined as the minimum ECR contraction (due to simulated tremor) in order to deliver electrical pulses either to the radial nerve (in-phase strategy) or median nerve (out-of-phase strategy). It was normally set to 20.

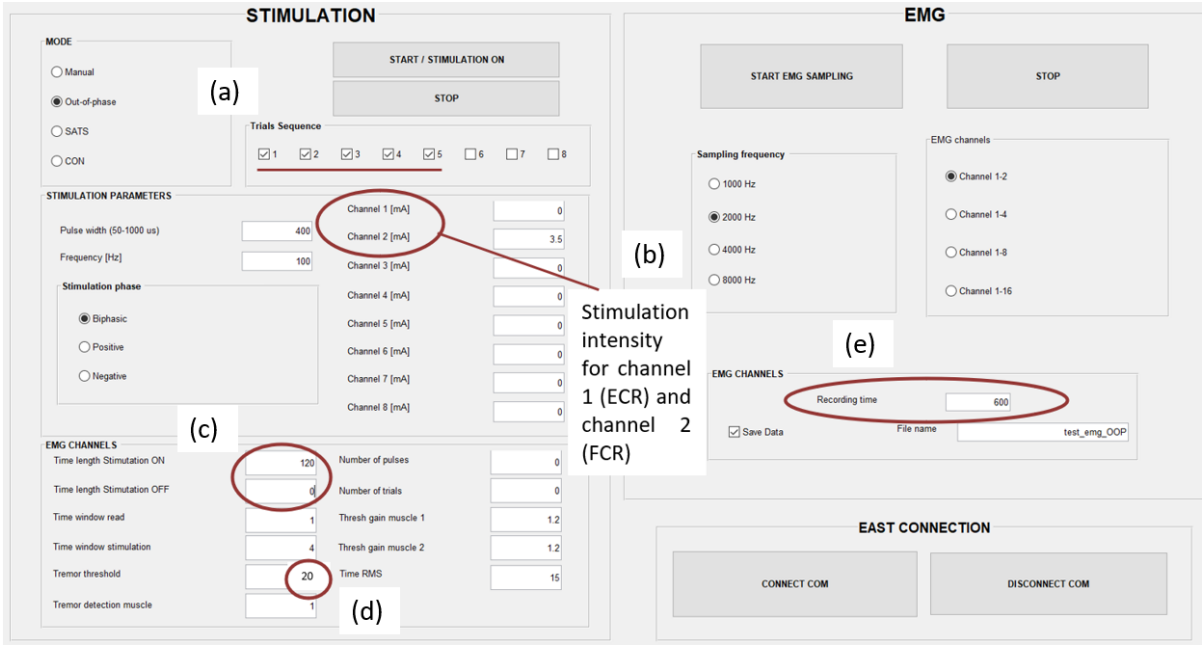

Figure 3.17. Example of the parameters used for S004 for out-of-phase stimulation. (a) Number of trials set to 5. (b) Stimulation intensity for channel 2 (stimulation delivered to FCR) set to 3.5 mA. (c) Stimulation time length of each trial set to 120 seconds. (d) Tremor threshold set to 20. (e) Recording time of the entire stimulation set to 600 seconds (120*5).

3.2.2.3. EXPERIMENTAL PROTOCOL

The experimental procedure took approximately 2-2.5 hours and was divided into four parts:

- PRE: it consisted of applying stimulation below MT during voluntary contraction (flexion task) in order to assess reciprocal inhibition mechanisms.
- SATS intervention: it consisted of a 20-minute afferent stimulation session to the radial (in-phase) or to the median nerve (out-of-phase). The stimulation pattern was based on ECR muscle activity.
- POST: immediately after SATS intervention was completed, the sub-experiment of the PRE stage was repeated.
- POST30': 30 minutes after SATS intervention, sub-experiment of the PRE stage was repeated.

It is worth mentioning that the stimulation intensity and the maximum voluntary contraction for each volunteer remained the same throughout the procedure.

As during the first study, before starting the actual experiment and meeting the participant, all the instrumentation needed to be set up. Therefore, the following connections were made between the different devices:

- The 16-channel bipolar adapter jack connector was connected to the signal amplifier Quattrocento to register ECR and FCR muscle activity.
- The reference wristband positioned on the participant's elbow was also connected to the Quattrocento via a patient ground cable.

- The signal amplifier and the constant current stimulator DSR8 were also connected to each other.
- The Arduino M0 Pro board was adequately connected to the current stimulator and the force sensor.
- The EAST stimulator was prepared by connecting the stimulation and EMG cables (see Figure 3.16.).
- Lastly, the Quattrocento, the Arduino board, and the EAST stimulator were connected to the computer via an Ethernet/USB cable to receive and record the EMG and force signals.

After setting up all the instrumentation, the volunteer was prepared following the scheme in Figure 3.18.

First, the skin of the dominant arm was cleaned with alcohol (FCR and ECR muscles, the elbow, olecranon, the cubital fossa, and the spinal groove). Afterwards, FCR and ECR muscles were localised by palpation under resisted flexion and extension, respectively. Once identified, a pair of recording sEMG electrodes were placed on both muscle bellies to measure FCR and ECR activity. Posteriorly, a wet wristband was set on the elbow to work as a reference and a square electrode was set on the olecranon to work as ground for the stimulation. The bio-signal amplifier Quattrocento and the software OT Biolab+ were then used to check the position of the sEMG electrodes and the quality of the EMG signals.

Secondly, the bar electrode connected to the constant current stimulator was employed in order to place the stimulation electrodes. To do so, electric pulses were manually delivered at the same time the bar electrode was moved around the spinal groove in order to locate the radial nerve. Once these two points were identified and marked by a pen, a pair of round stimulation electrodes were placed on the skin. The same procedure was repeated for the median nerve but in the cubital fossa. Posteriorly, the adjustable straps with 3D printed shapes from Figure 3.4. (c) were fastened in the volunteer's arm to apply pressure to the radial stimulation electrodes. Finally, the radial stimulation electrodes were connected to the constant current stimulator.

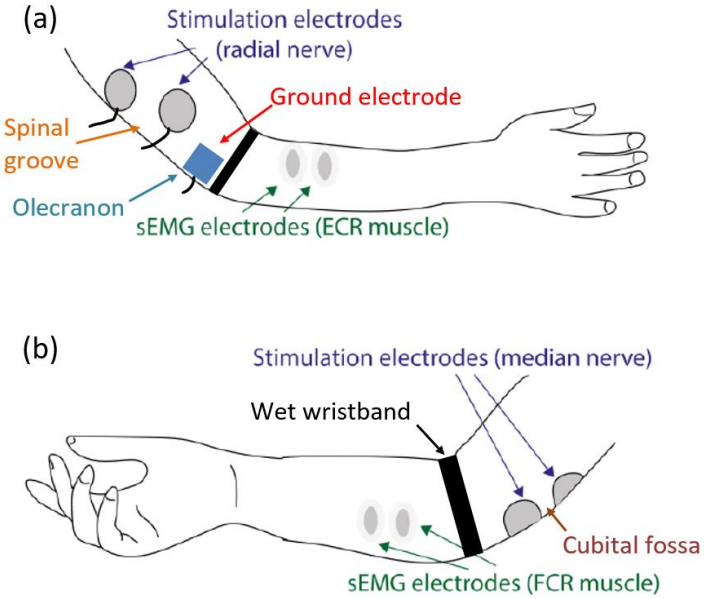

Figure 3.18. Complete electrode arrangement for study 2. (a) Surface electrodes were placed over the ECR muscle belly to record its activity and over the radial nerve for ECR activation in the spinal groove. Furthermore, the ground electrode was placed on the olecranon (b) Another pair of electrodes were placed over the FCR muscle belly to record its activity and on the median nerve in the cubital fossa. In addition, a wet wristband on the elbow was used as a reference. Adapted from [70].

After finishing the complete electrode arrangement, the four stages of this experiment (PRE, SATS intervention, POST, and POST30') were conducted as shown in the diagram from Figure 3.19. The sub-experiments of these stages can be summarised into reciprocal inhibition during flexion task and afferent stimulation during SATS intervention.

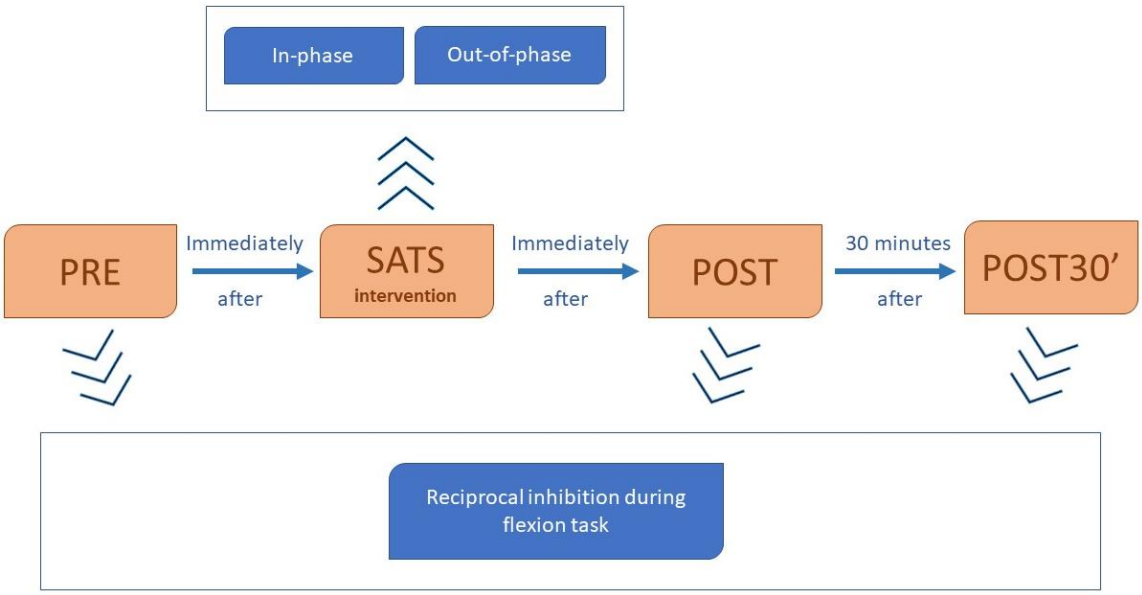

Figure 3.19. Experimental protocol study 2.

Reciprocal inhibition during flexion task

As in the previous study, the aim of the second sub-experiment was to assess the amount of reciprocal inhibition resulting from a voluntary wrist flexion task and compare it to the obtained reciprocal inhibition from each stage.

As described in the protocol from Pascual-Valdunciel et al. (2019) [70], volunteers were asked to comfortably sit on an armchair with their dominant arm stretched on a table in front of them. The shoulder joint was kept at 45º whereas the elbow joint was kept at 120º. In addition, participants were also asked to slightly flex the wrist in order to facilitate the determination of the MT. The MT was defined as the minimum stimulation intensity that produced a visible muscle response. This was visually confirmed with H-reflex and M-wave recruitment curves that resulted from the stimulation below MT of the radial nerve.

Then, volunteers were asked to perform their maximum voluntary contraction (MVC) with a wrist flexion task. To achieve this, an adjustable strap connected to a structure enclosing a compression sensor (Figure 3.5.) was set on the participants' wrist. They were asked to perform their maximum wrist flexion for 5 seconds by pulling the strap and avoiding using other muscles such as the biceps. The procedure was repeated 3 times with a rest period of 30 seconds between each trial and was recorded using OT Biolab+. Afterwards, the MVC was calculated as the mean of the three trials.

Once the MVC was determined, electrical stimulation below MT during flexion task was addressed. Surface stimulation was delivered to the radial nerve, activating the ECR and inhibiting the antagonist muscle – the FCR. During this experiment 4 trials were performed, each one of 30 electrical pulses with an interstimulus interval of 2 ± 0.2 ms. Each try-out consisted of a ramp corresponding to 20% of the MVC that the participants had to follow while stimulation below MT was applied to the radial nerve. The stimulation intensity used was the MT.

The software OT Biolab+ was used to help the participants maintain a constant muscle activity during the procedure and record EMG activity of both muscles. Furthermore, the Arduino board was employed to control the constant current stimulator and receive the information from the force sensor and transmit it to the Quattrocento for visualisation.

This sub-experiment was conducted in the PRE stage, immediately after the 20-minute SATS intervention (POST) and after 30 minutes SATS intervention finished (POST30').

Afferent stimulation during SATS intervention

The goal of this sub-experiment was to assess reciprocal inhibition after a 20-minute interval (with one-minute break between the 10-minute trials) of afferent stimulation by means of sequential and adaptive timely stimulation (SATS), while imitating wrist extensor-flexor tremor.

SATS is a stimulation strategy designed by Pascual-Valdunciel et al. (2020) [22] that consists of detecting tremorgenic bursts in each wrist muscle (ECR and FCR) and delivering electrical pulses to the antagonist, when these tremorgenic bursts become larger than an adaptive threshold. As depicted in Figure 3.20., this strategy employs both sequential recording and stimulation in order to avoid EMG contamination by stimulation artifacts. Therefore, SATS begins analysing one-second recording windows of EMG data from FCR and ECR activity, which afterwards are demodulated. Tremor is detected in these signals if their main frequency falls in the range 3-12 Hz thus, enabling a two-second stimulation window for the antagonist. But if tremor is not detected, the recording window restarts. Within the stimulation window, the root mean square (RMS) of two ten-millisecond sub-windows, one for each muscle, is calculated. If any of the two RMS surpasses an adaptive threshold calculated from the RMS of the previous recording window, a short stimulation pulse is sent to the antagonist (see green- and red-coloured rectangles in Figure 3.20. (b)) Conversely, if the two RMS don't exceed the threshold value, another ten-millisecond sub-window starts. The cycle repeats until the two-second stimulation window finishes, and then the next one-second recording window begins again [22].

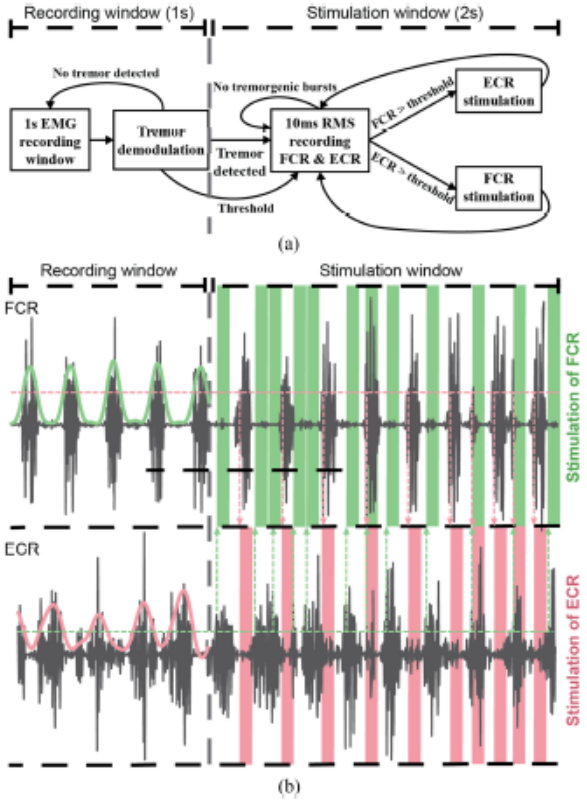

Figure 3.20. SATS stimulation strategy. (a) Control flow diagram of SATS strategy. (b) Illustration of SATS strategy for a pair of tremorgenic muscles (FCR and ECR). Extracted from [22].

During this experiment, the stimulation pattern was based on ECR activity and, therefore, out-of-phase stimulation consisted of delivering stimulation pulses to the median nerve (innervating FCR– the antagonist muscle) when ECR was activated due to simulated wrist flexor-extensor tremor. On the other side, in-phase strategy stimulated the radial nerve when ECR was contracted. Therefore, before starting this strategy, the cables from the Quattrocento and the constant current stimulator were disconnected, and conversely, EAST recording, stimulation, and reference cables (see Figure 3.16.) were connected. Posteriorly, different stimulation parameters (tremor threshold and stimulation intensity) as well as the time length of the stimulation were defined in the EAST GUI. After completing the EAST setup, two ten-minute-duration trials of afferent stimulation with SATS, with a rest period of one minute, were conducted. During SATS strategy, healthy volunteers were asked to simulate flexion-extension tremor.

Immediately after the 20-minute SATS intervention, reciprocal inhibition during flexor contraction was addressed (POST). Similarly, after 30 minutes since SATS strategy ended, POST30' was conducted.

See Annex G for the complete experimental protocol of study 2.

3.2.3. DATA ANALYSIS

As in the previous study, EMG signals were processed using MATLAB R2020b (MathWorks, USA) with a similar approach as Pascual Valdunciel et al. (2019) [70].

Raw EMG signals were bandpass filtered with the previous Butterworth filter and then rectified. Posteriorly, data corresponding to the total 120 electrical pulses from the 4 trials were segmented and then averaged to determine the degree of FCR inhibition for each volunteer. The baseline activity (see Figure 3.21. (a)) was computed by means of the averaged and rectified EMG activity ranging from -140 to -40 ms (before stimulation). The start of the inhibition window was characterised by time where the signal was lower than the baseline during at least 5 ms. Conversely, the end of the inhibition window was defined as the time when the signal stayed above the baseline for 5 ms (see Figure 3.21. (b)). The inhibition window was manually calculated for each subject. Lastly, the amount of inhibition was determined as the mean activity in the previously defined inhibition window and maximum inhibition was calculated as the minimum value of the inhibition window (see Figure 3.21. (c)).

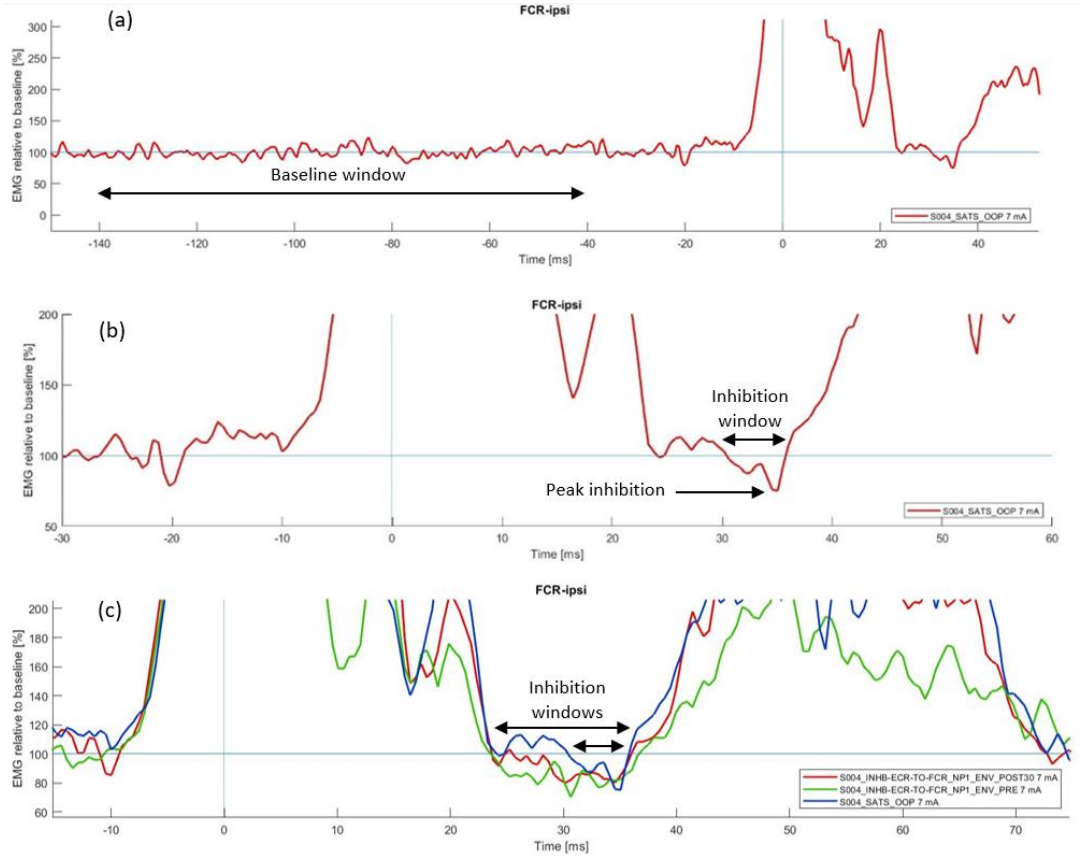

Figure 3.21. Individual example of FCR inhibition by radial stimulation after out-of-phase intervention. (a) Baseline activity. (b) FCR inhibition window and its maximum peak inhibition. (c) Example of FCR inhibition in the three stages (PRE (green), POST(blue) and POST30'(red)) from S004.

3.2.4. STATISTICAL ANALYSIS

As in the first study, the normal distribution of the samples was checked by using Shapiro-Wilk's test.

Two sample t-tests were performed to evaluate the differences of rectified EMG activity between baseline and the inhibition time window for three of the stages (PRE, POST, and POST30'). Therefore, if the mean of the 120 delivered electrical pulses was less than the mean baseline, electrical stimulation below MT was validated as a strategy that reduced muscle activity and entailed reciprocal inhibition.

One-way ANOVA was used in order to test muscle inhibition differences between PRE, POST and POST30'. Post-hoc Tukey´s test was then employed to find statistically significant differences in the previous three groups. We expect to have a bigger mean muscle inhibition in POST stage in comparison to PRE and POST30' when in-phase strategy has been conducted. Conversely, out-of-phase stimulation is speculated to result in a smaller mean muscle inhibition compared to PRE and POST30'.

Statistical significance was set by a *p*-value of 0.05.

3.2.5. RESULTS

A stimulation intensity of 8.38 ± 1.80 mA was delivered to the radial nerve in 4 volunteers that went through out-of-phase intervention, whereas, for in-phase intervention, a stimulation intensity of 8.24 ± 2.85 was used in 5 participants – 2 participants volunteered to take part in both in-phase and out-of-phase interventions. Reciprocal inhibition was evaluated before (PRE), after (POST) and 30 minutes after (POST30') out-of-phase and in-phase afferent stimulation was delivered to the radial nerve.

Regarding the inhibition windows for out-of-phase stimulation, the PRE stage started at 23.05 ± 1.81 ms and finished at 38.35 ± 2.41 ms, with a mean duration of 15.30 ± 1.76 ms. The POST stage inhibition window lasted 13.71 ± 5.86 ms, began at 23.30 ± 4.68 ms, and finished at 37.00 ± 2.89 ms. Lastly, the POST30' stage started at 22.16 ± 3.15 and finished at 37.82 ± 2.70, with a mean duration of 16.32 ± 5.28 ms.

Conversely, the PRE inhibition windows for in-phase stimulation began at 23.10 ± 1.75 ms, finished at 40.24 ± 2.41ms and took 17.14 ± 3.64 ms. The inhibition window of the POST stage had a duration of 15.42 ± 4.40 ms, started at 22.81 ± 3.15 ms, and finished at 38.23 ± 1.97 ms. Lastly, the POST30' stage began at 22.71 ± 2.91ms and finished at 40.24 ± 2.34 ms, with a mean duration of 17.52 ± 4.83 ms.

Table 3.3. summarises the different results obtained in the three stages of this study.

TABLE 3.3. RESULTS OF RECIPROCAL INHIBITION DURING FLEXION TASK IN STUDY 2

Subject	Stage	Inhibition window start (ms)	Inhibition window end (ms)	Duration of the inhibition window (ms)	Mean inhibition (%)	Maximum inhibition (%)
	PRE	23.78	40.43	16.65	16.37	16.32
S001 (OOP)	POST	22.31	37.01	14.69	19.13	15.65
	POST30'	0	0	0	0	0
	PRE	21.34	42.39	21.05	27.68	21.24
S001 (IP)	POST	23.30	36.02	12.72	23.75	11.72
	POST30'	23.30	41.41	18.11	25.08	11.26
	PRE	20.36	36.02	15.66	21.34	12.08
S002 (OOP)	POST	19.87	34.06	14.19	16.9	14.57
	POST30'	17.91	36.52	18.61	18.93	17.16
	PRE	22.80	37.01	14.21	26.89	13.38
S002	POST	22.80	36.52	13.72	12.99	16.97

(IP)						
	POST30'	23.79	37.50	13.71	29.15	15.98
	PRE	24.28	40.43	16.15	24.51	13.668
S003 (OOP)	POST	20.85	40.92	20.07	18.93	4.46
	POST30'	20.85	40.92	20.07	21.57	35.01
	PRE	23.79	36.51	12.72	14.84	18.21
S004 (OOP)	POST	30.15	36.02	5.87	7.88	24.23
	POST30'	25.75	36.02	10.27	10.89	23.86
	PRE	25.75	39.45	13.71	24.44	23.96
S005 (IP)	POST	26.72	38.23	11.50	17	19.2
	POST30'	26.72	38.47	11.74	32.79	22.41
	PRE	23.79	39.45	15.66	18.54	11.1
S006 (IP)	POST	23.30	39.94	16.64	19.33	15.64
	POST30'	19.87	43.37	23.5	16.88	8.4
	PRE	21.83	42.88	21.05	30.85	14.47
S007 (IP)	POST	17.91	40.43	22.52	28.84	12.79
	POST30'	19.87	40.43	20.56	39.26	14.38
	PRE	23.05	38.35	15.30	19.27	15.07
Mean OOP	POST	23.30	37	13.71	15.71	14.73
	POST30'	22.16	37.82	16.32	12.85	19.01
	PRE	23.10	40.24	17.14	25.68	16.83
Mean IP	POST	22.81	38.23	15.42	20.38	15.26
	POST30'	22.71	40.24	17.52	28.63	14.19

OOP: Out-of-phase stimulation strategy, IP: In-phase stimulation strategy.

The comparison of the different mean inhibitions after out-of-phase afferent stimulation across stages and volunteers is shown in Figure 3.22.

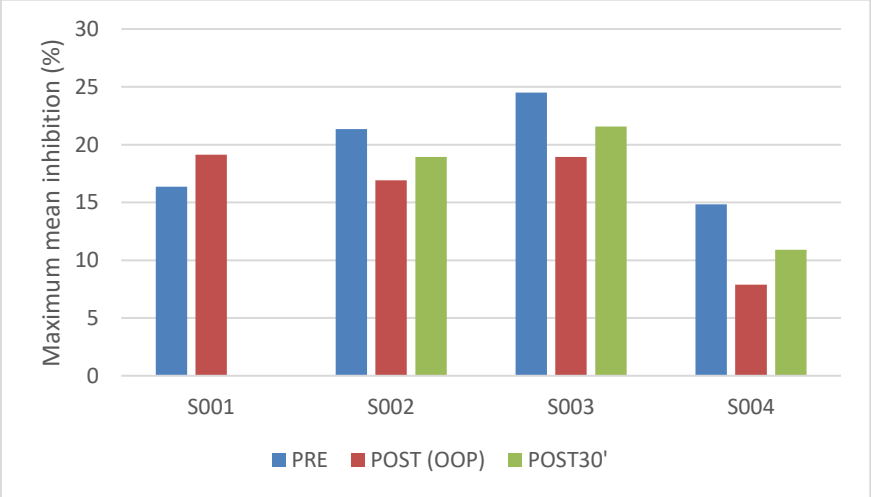

Figure 3.22. Results of reciprocal inhibition during flexor voluntary contraction after out-of-phase intervention.

Even though out-of-phase stimulation strategy was expected to decrease reciprocal inhibition, only a slight reduction was shown in S002, S003 and S004, as shown in Figure 3.22. In this regard, no statistically significant differences were found across the stages of any subject, except in S001 between POST30' and PRE and POST due to setup problems (see Table 3.4.).

TABLE 3.4. STATISTICAL DIFFERENCES AFTER OUT-OF-PHASE INTERVENTION

Subject	P-value	Statistical significance
S001	p<0.001	POST30' has means significantly different from PRE and POST
S002	p=0.76	No stages have means significantly different
S003	p=0.57	No stages have means significantly different
S004	p=0.48	No stages have means significantly different

In red: not expected values. In red and underlined: incorrect values.

Afferent stimulation with an out-of-phase stimulation pattern produced a mean reciprocal inhibition of 19.27 ± 4.46% in the PRE stage, of 15.71 ± 5.32% in the POST stage (after out-of-phase stimulation), and of 12.85 ± 9.69 % in the POST30' stage. The total mean inhibition of each stage is represented in Figure 3.23. Although out-of-phase afferent stimulation produced a slight decrease in the total mean reciprocal inhibition (POST), no significantly mean difference was observed among the three stages.

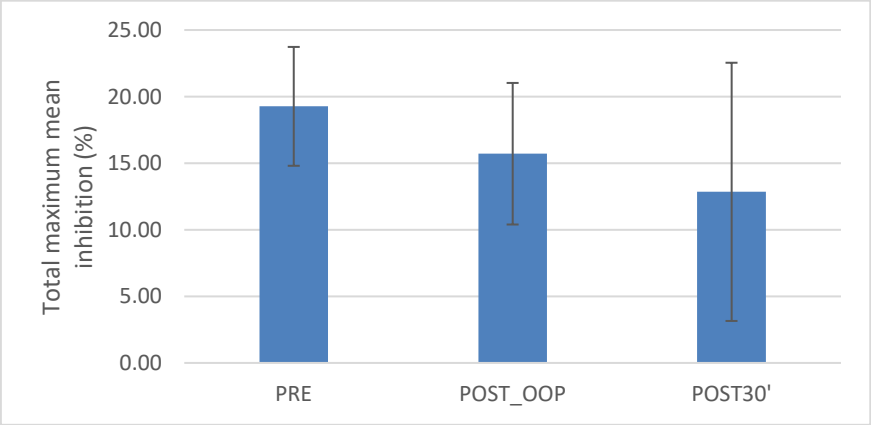

Figure 3.23. Total mean inhibition after out-of-phase intervention.

On the other hand, the comparison across stages and subjects after in-phase stimulation is shown in Figure 3.24.

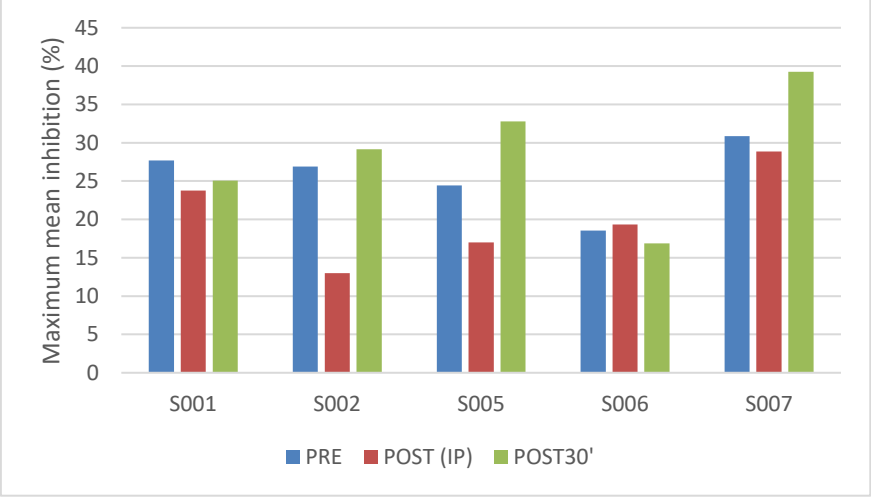

Figure 3.24. Results of reciprocal inhibition during flexor voluntary contraction after in-phase intervention.

After in-phase intervention, only S006 obtained a very slight increase in reciprocal inhibition (see Figure 3.24.). Furthermore, S002 obtained a smaller amount of FCR inhibition after the same strategy, which is the opposite of what was expected after this strategy. Therefore, none of the subjects had

statistically differences in reciprocal inhibition after in-phase stimulation except S002. These results are summarised in Table 3.5.

TABLE 3.5. STATISTICAL DIFFERENCES AFTER IN-PHASE INTERVENTION

Subject	P-value	Statistical significance
S001	p=0.82	No stages have means significantly different
S002	p=0.02	POST has means significantly different from PRE and POST30'
S005	p=0.07	No stages have means significantly different
S006	p=0.99	No stages have means significantly different
S007	p=0.10	No stages have means significantly different

In red: not expected values. In red and underlined: incorrect value.

After in-phase, FCR mean inhibition of 25.68 ± 4.60%, 20.38 ± 6.13%, and 28.63 ± 8.38% was obtained in PRE, POST and POST30', respectively. Furthermore, no statistical difference was found between the total mean inhibition of each stage. It is also worth mentioning that the total mean inhibition from POST after in-phase intervention was smaller than in the other two cases, when the opposite was expected after in-phase stimulation strategy. The total mean inhibition after in-phase intervention of each stage is shown in Figure 3.25.

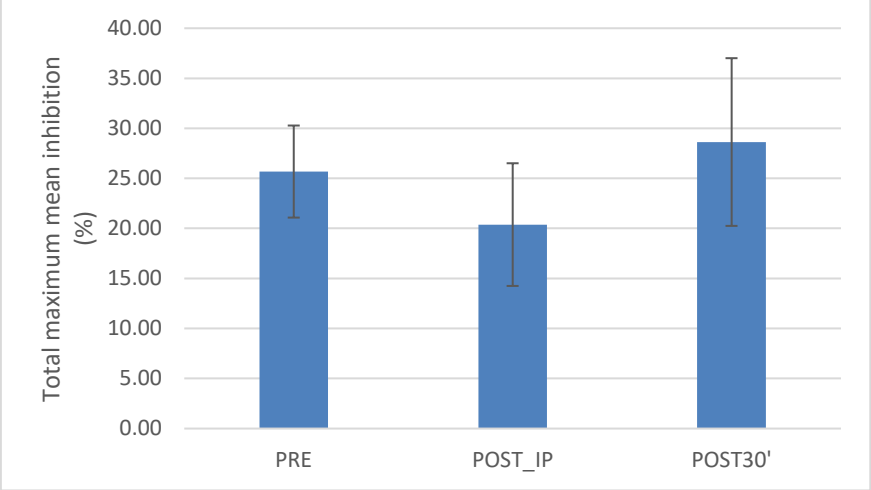

Figure 3.25. Total mean inhibition after in-phase intervention.

Therefore, the results of this experiment were not significantly different in either in-phase intervention or in out-of-phase intervention.

3.2.6. DISCUSSION

The objective of this study was to assess the modulation of reciprocal inhibition between a pair of antagonistic muscles (FCR, ECR), when afferent stimulation with SATS was delivered. To that end, SATS was applied for 20 minutes in either an out-of-phase or in-phase manner, while healthy participants mimicked wrist flexion-extension tremor. We expected to obtain more reciprocal inhibition after in-phase intervention and less reciprocal inhibition after out-of-phase intervention.

On the one hand, out-of-phase stimulation pattern during this study consisted of stimulating the median nerve whenever ECR was activated due to simulated tremor. Although not statistically significant, out-of-phase stimulation was correctly delivered as shown in Figure 3.26. Despite this, low intensity values in addition to the constant movement of the electrodes attached to the skin may have lessened the presumed effects of out-of-phase stimulation. In this way, electrode movement may have led to

stimulation variations over time. Therefore, it could have been worth considering increasing the stimulation intensity for each participant in order to achieve more representative results.

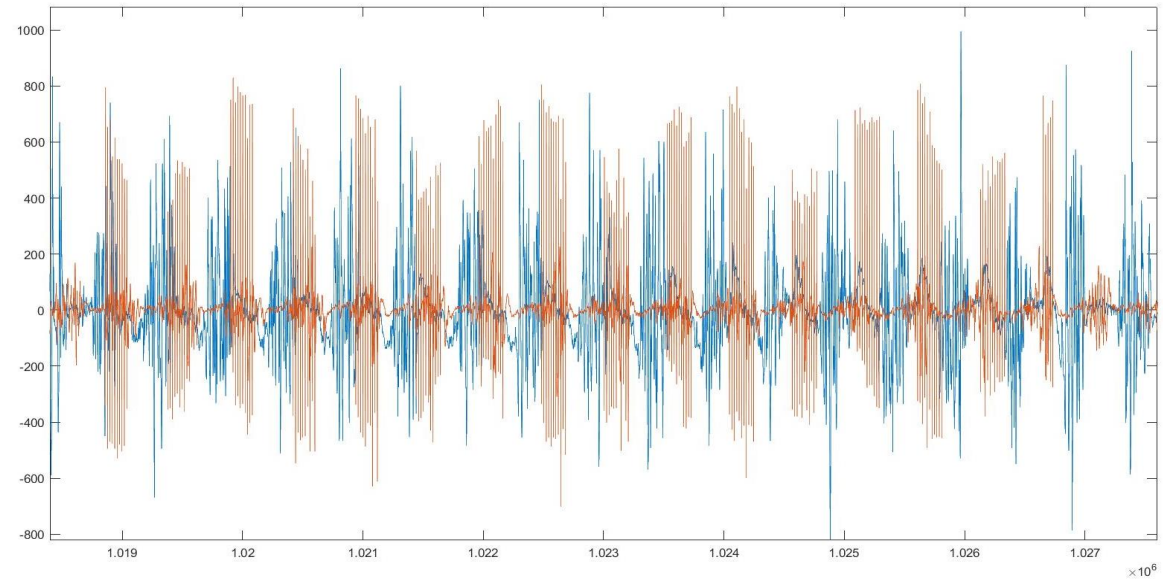

Figure 3.26. Example of a stimulation window during in-phase stimulation strategy from S001.

The only result that turned out to be statistically significant was the mean inhibition difference between PRE and POST30' stages in S001 (see Figure 3.22 and Table 3.4.). This big difference –16.37% in PRE compared to 0% in POST30'– might have been because of the adjustable straps set on the stimulation electrodes (see Figure 3.4. (c)). Since the participant had to move the orientation of the arm in order to first exert his maximum voluntary contraction (MVC) and then simulate tremor, the 3D printed structures sewn to the straps that were applying pressure to the nerve also moved along with the arm. The slightest arm movement may have entailed a little variation of the point to which those structures were pressuring, which eventually could have resulted in a different response to afferent stimulation *i.e.*, instead of stimulating the nerve, part of the surrounding muscle was receiving those stimulation pulses. This obstacle was encountered during the entire procedure in both in-phase and out-of-phase interventions, but only in few volunteers it produced such a big difference between PRE and POST30' stages, like in the case of S001. Nevertheless, if POST30' total mean inhibition was discarded– for that 0% was the main representation of such decrease in that stage– a slight reduction in the total mean inhibition from 19.27 ± 4.46% in the PRE to 15.71 ± 5.32% in the POST stage after out-of-phase intervention was obtained and, thereby modulation of reciprocal inhibition by spinal reflexes was produced.

On the other hand, none of the participants obtained a significantly higher mean inhibition after in-phase intervention (see POST in Figure 3.24.). This general decrease in the POST mean inhibitions in each subject may have been due to an incorrect synchronous stimulation. It is possible that this could have happened because of a delay between the recording EMG signals and the stimulation trigger, or a lack of sensitivity of EMG measuring. The best example of such a case was the results obtained in S002 after in-phase intervention, which led to a statistically significant decrease in reciprocal inhibition due to this false stimulation pattern, *i.e.*, instead of delivering afferent stimulation to the radial nerve when ECR was activated, electrical pulses were delivered when the FCR was activated, which corresponds to an out-of-phase strategy. Therefore, taking as an example two different stimulation windows from S002 during in-phase SATS intervention (see Figure 3.27.), it was observed that only 4 out of 15 ECR tremorgenic bursts were correctly perceived and only then stimulation was accordingly delivered (horizontal lines in Figure 3.27. that should only appear when ECR was contracted). Notwithstanding, these results confirmed the presumed effect afferent stimulation by means of out-of-phase intervention should have had on reciprocal inhibition.

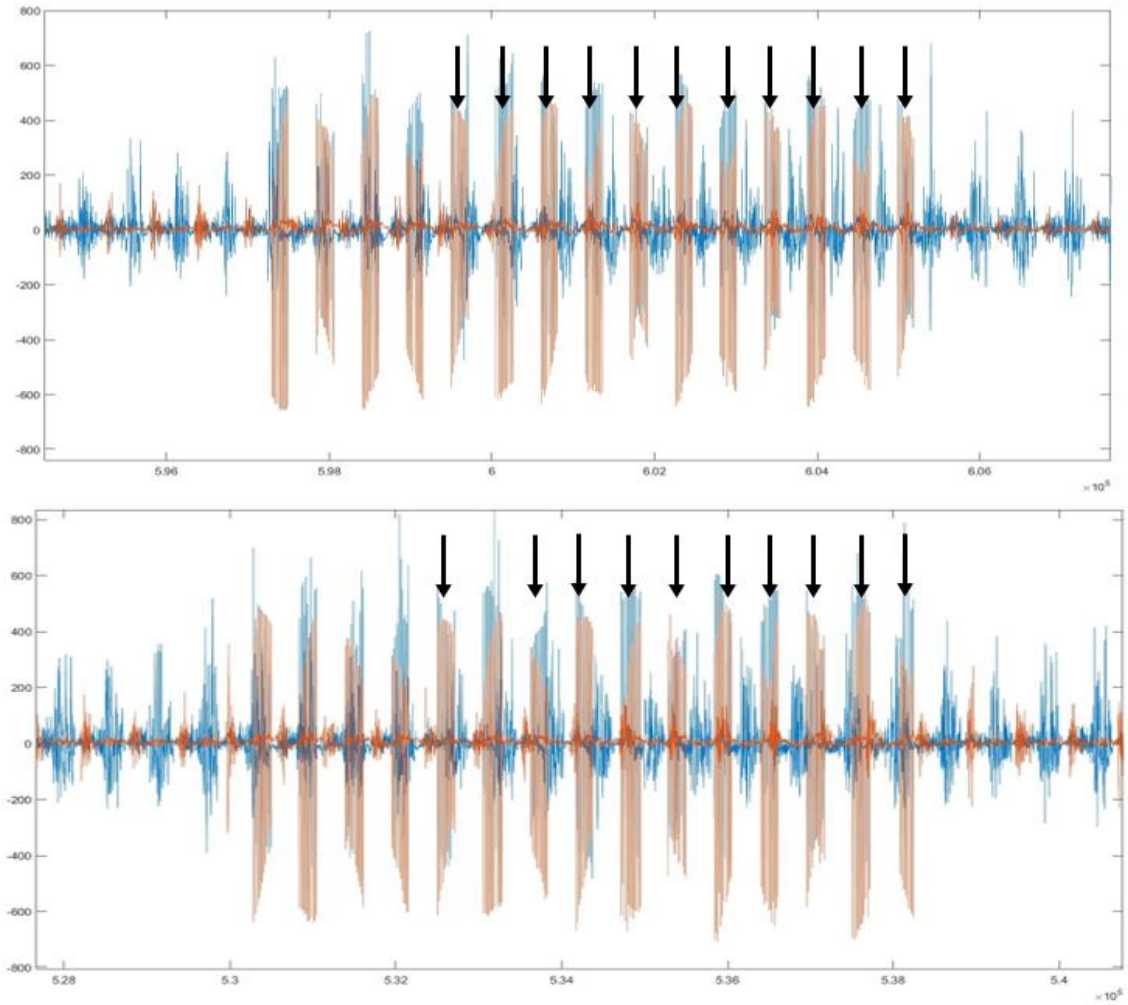

Figure 3.27. Stimulation windows during in-phase intervention in S002. Blue-coloured EMG activity corresponds to the ECR muscle, whereas FCR activity is represented in red. Stimulation is represented by horizontal lines that should only appear in ECR activity. Black arrows show the incorrect stimulation pattern.

To a greater or lesser extent, this false synchronous stimulation repeated itself in all the participants during in-phase intervention, thus preventing us from having real and representative in-phase stimulation results. This issue was observed in the total mean inhibition (see Figure 3.25.) after in-phase intervention (20.38 ± 6.13%), which was less in comparison to the total mean reciprocal inhibition obtained in PRE and POST30' stages (25.68 ± 4.60% and 28.63 ± 8.38%, respectively).

4. CONCLUSIONS AND FUTURE DIRECTIONS

4.1. CONCLUSIONS OF BOTH STUDIES

Both studies supported the hypothesis that the modulation of reciprocal inhibition is possible by afferent stimulation to the radial nerve and that it is mediated by Ia afferent fibres.

During the first experiment with healthy subjects, it was demonstrated that stimulation below MT to the radial nerve was a reliable and feasible procedure to produce reciprocal inhibition during voluntary contraction. Furthermore, by means of two sessions of low-intensity stimulation with a rest period of 30 minutes between each, it was proven that the same physiological actuators were involved in this physiological mechanism, for in these two stages the same degree of reciprocal inhibition was obtained. Results suggested that the mediators of this physiological mechanism seemed to be – at least to a great extent– Ia afferent fibres due to the decrease in the total mean inhibition after a 20-minute session of long-period vibration. It has been shown that Ia afferents take part in the modulation of reciprocal inhibition and that when prolonged vibration is applied to the tendons or muscles, Ia afferents' electrical excitatory threshold raises. This means that Ia afferents need to be stimulated by a higher stimulation intensity in order to mediate reciprocal inhibition. Therefore, if fewer afferent fibres are recruited, the degree of antagonist inhibition decreases after afferent stimulation has been delivered to the agonist muscle. Since the same conditions were used throughout the experiment, the involvement of Ia afferents in reciprocal inhibition was demonstrated due to the decrease of muscle inhibition. Thus, the first study of this Bachelor Thesis succeeded in demonstrating the implication of Ia afferent pathways in the modulation of reciprocal inhibition.

In addition to the previous results, this study validated a platform developed to assess the neuromodulation at the spinal level of reciprocal inhibition, in which the same conditions were used for each participant and throughout the entire procedure (20% of MVC, stimulation below MT, among others). Standardised conditions across subjects are important in order to obtain more reliable measurements.

Based on the previous results, SATS strategy was tested with the objective of demonstrating the modulation of reciprocal inhibition on healthy subjects simulating wrist flexion-extension tremor. Nevertheless, the results from this second experiment could not provide evidence for this as discussed in Section 3.2.6. On the one hand, the observed changes in out-of-phase could have been consistent with an increase in the stimulation intensity. Since surface electrodes move on the skin to which they are attached, stimulation pulses may not have been always delivered in the same place nor with the same strength. Therefore, the future use of intramuscular electrodes may sort out this issue, for they provide a more repeatable outcome. On the other hand, in-phase intervention produced a decrease in the total mean inhibition, which was the opposite of what was expected. This occurred because of a false synchronous stimulation pattern due to a delay between EMG recording and the stimulation trigger, or low sensitivity in EMG recording. Therefore, from this second study it can be concluded that both in-phase and out-of-phase stimulation patterns require accurate synchronous stimulation with muscle activation. Furthermore, a proper configuration is also crucial in order to sensitively record muscle activity and thereby deliver stimulation accordingly. In addition, since during the second study results weren't conclusive regarding the effects of both strategies in terms of reciprocal inhibition, bigger sample sizes are needed on this regard.

Even though this study could not provide additional evidence on the key role of reciprocal inhibition after SATS towards its therapeutic application to be applied in patients with pathological tremor, previous studies suggested its possible role in tremor reduction after SATS (see Table 2.1.). The recent study of Pascual-Valdunciel et al. (2020) [22] used a similar experimental approach as in this Bachelor Thesis and demonstrated that applying SATS out-of-phase, either with surface or intramuscular electrodes, led to a tremor reduction in ET patients. Mean tremor reduction after intramuscular stimulation was 64%, whereas with superficial stimulation was 14%. Furthermore, both strategies also showed tremor reduction for the stimulated wrist 24 hours after the intervention, accomplishing a mean

tremor reduction of 70% for intramuscular and 26% for surface afferent stimulation. In this regard, this Bachelor Thesis aimed at getting more insights on the physiological mechanisms after applying SATS, having in mind the improvement of the strategy and its use in PD patients as well.

4.2. FUTURE DIRECTIONS OF BOTH STUDIES

This Bachelor Thesis provided evidence of the modulation of FCR muscle inhibition by means of low-intensity stimulation to the radial nerve. In addition, the role of Ia afferent fibres in this physiological mechanism was demonstrated by assessing reciprocal inhibition after a 20-minute intervention of prolonged vibration. This provides novel insights on the physiological mechanisms resulting from applying SATS, which will help improving this strategy having in mind its use in PD patients as well. In this regard, this Bachelor Thesis represents an important step in that direction.

As previously discussed, the arm was in constant motion during the experiments, producing slight changes in the pressuring points of the 3D printed structures and consequently, giving rise to different wrist responses– or none at all– when afferent stimulation was applied. Therefore, the next step in surface electrical stimulation below MT would be to design new structures with different shapes. A flat structure would provide more stability against movement due to its bigger contact surface with the electrode. Thus, two flat square structures –one for each electrode– with a smaller one protruding from it to work as a pressure point, could help to both maintain the stability on the arm and apply pressure to the nerve. The little structures would need to have similar dimensions than the stimulation electrodes to increase the chances of finding the optimal stimulation points. Another option would be to substitute superficial electrodes for intramuscular ones. This could be the best method to better activate Ia afferents located at the muscle spindles. Therefore, intramuscular electrodes should still be taken into account when designing novel strategies based on electrical stimulation. Furthermore, the use of intramuscular stimulation would require lower stimulation intensities since the electrodes would be placed inside the muscle.

Another aspect to be considered is the characterisation of stimulation parameters in order to conduct standardised procedures that in turn would facilitate the identification of the mechanism(s) underlying plastic changes. To that end, bigger sample sizes would be needed in order to analyse and confirm these stimulation parameters so that more reliable results were obtained.

Lastly, once the stimulation parameters were characterised, the next step would be to study the effects SATS strategies could have on the modulation of reciprocal inhibition. This would allow to exploit the potential use of afferent stimulation in tremor reduction. To achieve this, a bigger sample size would also be needed. Once this were achieved, the next step would be to test the strategy with the objective of reducing pathological tremor. To achieve this, it would be crucial to discern between the pathological tremors from different diseases, and adapt this strategy only to PD.

5. BIBLIOGRAPHY

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ANNEX A: ETHICAL, ECONOMIC, SOCIAL AND ENVIRONMENTAL ASPECTS

A.1. INTRODUCTION

PD is a progressive neurodegenerative disease of the central nervous system (CNS) that gradually leads to a wide range of motor and non-motor symptoms. Tremor is a crucial issue for both early and advance-stage PD patients, which gravely impacts their quality of life and social interactions. In order to treat and reduce tremor in PD patients, pharmacological and surgical approaches are often employed. Nevertheless, there can be significant side effects arising from these therapies, such as the decline effectiveness in the case of medication, or infection and intracranial haemorrhages in the case of surgical interventions. Therefore, current treatment options do not provide a reliable solution to manage tremor, which leads to the development of innocuous, affordable, and effective alternatives to reduce it.

Electrical stimulation below motor threshold has been proved to be a reliable and feasible option to reduce tremor in essential tremor patients, thereby the objective of this Bachelor Thesis is to give more insight of the neuromuscular mechanisms accounted for the possible therapeutic effects of electrical stimulation below MT and use this knowledge to improve the present strategy for PD.

A.2. ETHICAL ASPECTS

All the experiments carried out during this Bachelor thesis were conducted according to the Declaration of Helsinki, thus ensuring the ethical principles for medical research.

Before conducting the experiment, all the volunteers were informed both verbally and with an information sheet about the purpose, material, protocol, and possible discomfort (see Annex C and D for the information sheets of both studies). Afterwards, all queries from the volunteers were clarified. Finally, an informed consent form was signed by the participant in order to be accepted for the experiment (see Annex E for the informed consent form for both studies).

All the equipment used in these experiments were previously certified and validated according to the current national legislation (Spanish Royal Decree 1591/2009).

The participation of the healthy subjects was completely voluntary and, therefore, at any time of the experimental process their consent could be revoked without further explanation.

Videos and personal data were kept and archived under strict confidentiality based on the current Organic Law 15/1999 of December 13 (in Spanish: *Ley Orgánica de Protección de Datos de Carácter Personal 15/1999, de 13 de Diciembre).* Therefore, the right to access, rectification, cancellation and opposition were guaranteed.

A.3. SOCIAL ASPECTS

As stated in Section 1.1., PD involves a wide variety of motor and non-motor symptoms that gravely affects patients' quality of life and social interactions.

During a survey, half of the life stories from PD patients revolved around the shame they felt for having the disease, which finally resulted in social withdrawal: 'I stopped walking in the streets during the day, I found it very awkward to be so visibly in a bad condition' [12]. These social consequences could be reflected in a survey, in which at least 58% of PD patients suffered from feeling ashamed of their condition, perceiving other persons were uncomfortable with them or even avoided them [13].

In the same way, more than one-third of patients with early-stage PD patients reported that tremor-related complications adversely impacted on their activities of the daily living (ADLs), such as writing,

dressing, eating, or holding material [11]. Even though PD is often associated with an older age population, it is also diagnosed in persons younger than 50 years (5% of the cases) or 65 years old (30% of the cases) [91]. Therefore, given that many patients are still working at the time PD begins, tremor was reported in a survey of 88 PD patients with less than 65 years old as one of the most challenging symptoms faced at work. Consequently, employment was lost in 50% of the PD participants 5-9 years after the first PD symptom [91].

A.4. ECONOMIC ASPECTS

PD is a significant economic burden for both the medical health system and the patients themselves. Due to the PD increasing incidence, it is estimated that the economic costs are going to increase at the same rate. TABLE A.1 shows the current economic burden PD has on several countries. It is worth mentioning that most of the total costs in these countries were defined as a sum of the following sub-costs:

- Direct medical costs: associated with primary care, treatments, medical equipment, or lab tests [5, 92, 93].
- Non- medical costs: refer to home renovations, motor vehicle modifications, and expenses on daily non-medical care [5].
- Indirect costs: involve the loss of productivity due to premature death or loss of employment, home care and caregivers [5, 92].

TABLE A.1. ECONOMIC BURDEN FOR PARKINSON'S DISEASE

Country	Year	Annual costs (in millions of €)
Australia [94]	2014	6,590.00 €[19]
China [95]	2015	7,977.00 €[20]
France [96, 97]	1999	663.15 €
Germany [98]	2008	2,300.00 €
Italy [99, 100]	2010	3,040.80 €
The Netherlands [101]	2011	240.96 €
Spain [93]	2016	5,100.00 €[21]
United Kindom [102]	1998	525.00 €[22]
United States of America [5]	2017	51,900.00 €[23]

Table A.1. represents an estimation of the PD economic burden in each respective country because some of the available studies are quite outdated and the PD incidence and the price of the disease has boosted up since then. What it is clearly shown in Table A.1., is the palpable need to reduce the expenses National Health Systems and public figures are taking from conventional therapeutical treatments, such as medication or DBS. Therefore, peripheral electrical stimulation is being investigated as an affordable, innocuous, and more effective solution to manage tremor in PD.

A.5. ENVIRONMENTAL ASPECTS

The only single-use materials employed during the two studies of this Bachelor Thesis were electrodes. Volunteers who came more than once–like in the case of S001 and S002 in the second study in order to

[19] The conversion of AUD to € was calculated on the 17th of June 2022.
[20] The conversion of $ to € was calculated on the 17th of June 2022.
[21] This value was calculated as the multiplication of the annual costs (17,000 €) and the number of national PD patients (300,000).
[22] The conversion of £ to € was calculated on the 17th of June 2022.
[23] The conversion of $ to € was calculated on the 17th of June 2022.

carry out both in-phase and out-of-phase afferent stimulation– reused the same electrodes so less waste was generated. Furthermore, the equipment to conduct afferent stimulation had a long deprecation period and, thereby no devices needed to be replaced.

ANNEX B: BUDGET

The experiments of this Bachelor Thesis were conducted in collaboration with the Neuro-Rehabilitation Group (NRG) from the Cajal Institute of the Spanish National Research Council (*Consejo Superior de Investigaciones Científicas* (CSIC)). The data obtained during both studies were given to the ongoing European project EXTEND- Bidirectional Hyper-Connected Neural system, which is a project from the NRG.

An approximate budge of the expenses the experiments of this Bachelor Thesis attained has been quoted and further divided into technical equipment (Table B.1.), software licenses (Table B.2.), and personnel (Table B.3.). The total estimated cost amounted to 14,992.83 €.

TABLE B.1. BUDGET FOR EQUIPMENT

Equipment	Deprecation period (months)	Number of units	Cost per unit(€)	Time of use (month)	Total cost (€)
Quattrocento EMG amplifier	72	1	27,950.00 €	6.00	2,329.17 €
Male banana adapter	72	1	12.00 €	6.00	1.00 €
Snap-on adapter	72	1	12.00 €	6.00	1.00 €
Ground cable patient	72	1	10.00 €	6.00	0.83 €
Jack Connector	72	1	300.00 €	6.00	25.00 €
Wristband	72	1	10.00 €	6.00	0.83 €
Digitimer DSR8	72	1	9,680.27 €	6.00	806.69 €
Bar electrode	72	1	371.75 €	6.00	30.98 €
Force sensor	72	1	100.00 €	6.00	8.33 €
Force strap	12	1	9.00 €	6.00	4.50 €
Arduino M0 Pro Board	48	1	52.00 €	4.00	4.33 €
Cordless Electrical Massager MANFLY	48	1	29.03 €	4.00	2.42 €
EAST	12	1	5,000.00 €	2.00	833.33 €
Programmer	48	1	300.00 €	2.00	12.50 €
Square sEMG	-	64	0.32 €	Single use	20.48 €
Oval sEMG	-	20	1.38 €	Single use	27.60 €
Surface stimulation electrode	-	56	1.00 €	Single use	56.00 €
Surface ground stimulation electrode	-	7	1.00 €	Single use	7.00 €
Laptop	72	1	550.00 €	6.00	45.83 €
Total estimated cost for equipment					**4,217.83 €**

TABLE B.2. BUDGET FOR SOFTWARE LINCENSES

Software	Cost per year (€)	Time of use (months)	Total cost (€)
MATLAB (annual license)	800 €[24]	6	400 €
Arduino	0 €	1	0 €

[24] With an academic license the price would be zero.

OT Biolab+	0 €	6	0 €
Total estimated cost for software licenses			**400 €**

TABLE B.3. BUDGET FOR PERSONNEL

Personnel	Salary (€/hour)	Time (hours)	Total cost (€)
Undergraduate student	10 €	700 €	7,000 €
Predoctoral researcher	20 €	75 €	1,500 €
Postdoctoral researcher	25 €	75 €	1,875 €
Total estimated cost for personnel			**10,375 €**

ANNEX C: INFORMATION SHEET STUDY 1

HOJA DE INFORMACIÓN PARA EL PACIENTE PARA PARTICIPAR EN LA FASE 1 DEL PROYECTODE INVESTIGACIÓN:

"SISTEMAS NEURONALES BIDIRECCIONALES HIPERCONECTADOS PARA MANEJO DEL TEMBLOR ESENCIAL Y DEL TEMBLOR DE LA ENFERMEDAD DE PARKINSON: PROYECTOEXTEND"

MEDIANTE EL USO DE ELECTRODOS DE SUPERFICIE

El proyecto EXTEND se trata de un estudio de investigación financiado por la Unión Europea que pretende desarrollar un nuevo método de control del temblor.

Los dos tipos de temblor más frecuentes son el Temblor Esencial y el temblor de la Enfermedadde Parkinson. Actualmente su tratamiento se basa en el uso de medicamentos, aunque en aquellos pacientes en los que no se consigue controlar con fármacos, se propone la Estimulación Cerebral Profunda. Este método consiste en la implantación en una zona profunda del cerebro unos electrodos que, del mismo modo que un marcapasos cardiaco, consigue "controlar" el temblor. Al tratarse de un proceso quirúrgico, hay muchos pacientes que no pueden beneficiarse de él. Por ello son muchos los esfuerzos por encontrar otros métodos de control del temblor menos agresivos y que actúen a nivel del músculo, y no del cerebro.

Es por esto por lo que le invitamos a participar en un estudio que tiene como finalidad el desarrollo tecnológico y clínico de un método de control del temblor mediante unos dispositivos que actúan a nivel muscular. Estos dispositivos se llaman electrodos, y lo que hacen es administrara los músculos que intervienen en el temblor una corriente eléctrica, completamente inocua e indolora, que inhibe el impulso que viene del cerebro y de la médula generando el temblor.

Hemos dividido el estudio en dos partes bien diferenciadas para lograr un desarrollo óptimo deestos dispositivos:

- Fase 1: se realizará en personas sin temblor. Pretendemos demostrar que los métodos de estimulación eléctrica por parte de los electrodos que estamos estudiando son capaces de modificar la activación de los músculos en gente sana.
- Fase 2: se realizará en personas con temblor. Veremos si las técnicas de estimulación que probamos con éxito en la fase 1 logran controlar el temblor en pacientes con Temblor Esencial y Enfermedad de Parkinson.

Puesto que usted no sufre temblor, le proponemos su participación en la **FASE 1** del estudio.

¿Qué pretendemos demostrar?

Creemos que la estimulación eléctrica del músculo mediante electrodos produce una inhibiciónde la actividad muscular, es decir, que es capaz de relajar un músculo que está contrayéndose gracias a que activa a un grupo de neuronas inhibitorias que existen en la médula espinal de todas las personas. Demostrar que esta inhibición muscular es posible es el primer paso para lograr controlar la actividad muscular "anormal", como sería el caso del temblor. Por eso es tanimportante primero conocer y probar cómo funciona en un músculo sano. Además, si los resultados de la inhibición fuesen satisfactorios, le aplicaremos una vibración al músculo para después realizar la estimulación eléctrica, pudiendo demostrar así si se activa otro grupo distinto de neuronas inhibitorias.

¿Qué son los electrodos de estimulación?

Los electrodos son unos pequeños dispositivos que se colocan en los músculos y suministran una corriente eléctrica de baja intensidad en un punto muy localizado del músculo. Hay varios tipos de electrodos y también queremos conocer cuáles es el tipo de electrodo que mejor funciona para inhibir la actividad del músculo. Los tipos de electrodos que vamos a probar son:

- Electrodos de superficie: se colocan sobre la piel. Son los que pretendemos probar en usted y por tanto, los que vamos a detallar a continuación.
- Electrodos de aguja: se colocan dentro del músculo.

Los electrodos de superficie se pegan en la superficie de la piel y son completamente inocuos. Utilizaremos un total de 3 pares de electrodos que colocaremos en los músculos que se encargan de flexionar y extender la muñeca del brazo que más usan, y en el nervio que se encarga de extender **l**a muñeca del otro brazo.

¿Me van a colocar algún otro aparato?

Por un lado, para poder demostrar cómo se modifica la actividad muscular mediante los electrodos, necesitamos un método fiable y objetivo que nos registre y traduzca la actividad de esos músculos. Para ello utilizaremos un Electromiograma, un aparato que se utiliza de forma rutinaria en la práctica clínica en los hospitales para poder registrar la actividad muscular y diagnosticar diferentes problemas médicos. Por ello le colocaremos otros electrodos correspondientes al Electromiograma junto a los descritos en el punto anterior.

Por otro lado, se utilizará un masajeador de varita inalámbrico. Éste se colocará en el interior del antebrazo y aplicará una vibración al músculo durante 15 minutos, para así poder ver posteriormente los resultados de dicha vibración tras la segunda estimulación.

¿En qué consistirá todo el procedimiento?

Tras la lectura de este documento, procederemos a responderle todas las dudas que le surjan. Si está de acuerdo con participar, firmará la hoja de consentimiento informado. Tras ello recogeremos una serie de datos personales (edad, enfermedades previas…) y le colocaremos loselectrodos de superficie y los electrodos del Electromiograma, junto con las cintas de fijación de los electrodos. Posteriormente, sentado cómodamente sobre una silla, apoyará el antebrazo sobre la superficie de una mesa, con la palma de la mano hacia arriba, y colocaremos una cincha en la muñeca que le fijarán el brazo a la mesa y le permitirá flexionar la muñeca para realizar una fuerza durante las pruebas.

Una vez esté todo colocado, iniciaremos estas mismas. En cada una le indicaremos cuál es el músculo que tiene que utilizar, por ejemplo, le podremos pedir que durante unos segundos esté intentando flexionar la muñeca con todas sus fuerzas. Cada prueba dura 60 segundos y realizaremos un total de cuatro, en las cuales usted tendrá que estar tirando de una cincha con la muñeca para hacer una fuerza con el músculo, mientras se le aplicará la estimulación eléctrica mediante los electrodos. Entre cada prueba dispondrá de 1 minuto de descanso. Posteriormente, se llevará a cabo la vibración: se le colocará en el antebrazo el dispositivo masajeador durante un periodo de 15 minutos y después se llevará a cabo nuevamente las 4 pruebas de estimulación.

La duración de todo el proceso tendrá una duración aproximada de 120 minutos.

¿Qué efectos indeseables puedo notar?

Durante las pruebas, cuando estamos estimulando, es posible que note una sensación de cosquilleo u hormigueo, no se espera que note sensación de calambre. Si notara cualquier tipo de sensación anormal, nos lo tiene que comunicar en el acto. Estos efectos sólo los podría notarmientras se esté estimulando. Además, la colocación de cinchas para fijar la posición de los electrodos puede resultar incómoda y producir alguna sensación de presión o irritación sobre la piel, siendo un efecto pasajero.

Durante la vibración, podría notar una sensación de cosquilleo y adormecimiento de los dedos, pero ésta solo los podría sentir durante la vibración. Si sintiese alguna incomodidad, comuníquenoslo.

¿Tengo que tener algún tipo de precaución especial antes o después de las pruebas?

No es necesario ningún tipo de preparación ni observación posterior. No obstante, si cree que tiene algún tipo de problema que pudiera estar relacionado con el procedimiento, se pone en contacto con nosotros, llamando al teléfono +34 915868339 (Secretaría de Neurología) ypreguntando por la Dra Muñoz o el Dr Grandas.

¿Qué va a pasar con mis datos personales y los resultados obtenidos en el estudio?

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 y publicados en diferentes conferencias y revistas médicas, sin aportar datos personales.

Su participación en este estudio es voluntaria por lo que puede negarse a participar o revocar su consentimiento en cualquier momento sin que precise dar ninguna razón para ello.

ANNEX D: INFORMATION SHEET STUDY 2

HOJA DE INFORMACIÓN PARA EL VOLUNTARIO SANO PARTICIPAR EN LA FASE 1 DEL PROYECTO DEINVESTIGACIÓN:

"SISTEMAS NEURONALES BIDIRECCIONALES HIPERCONECTADOS PARA MANEJO DEL TEMBLOR ESENCIAL Y DEL TEMBLOR DE LA ENFERMEDAD DE PARKINSON: PROYECTOEXTEND"

MEDIANTE EL USO DE ELECTRODOS DE SUPERFICIE

El proyecto EXTEND se trata de un estudio de investigación financiado por la Unión Europea que pretende desarrollar un nuevo método de control del temblor.

Los dos tipos de temblor más frecuentes son el Temblor Esencial y el temblor de la Enfermedadde Parkinson. Actualmente su tratamiento se basa en el uso de medicamentos, aunque en aquellos pacientes en los que no se consigue controlar con fármacos, se propone la Estimulación Cerebral Profunda. Este método consiste en la implantación en una zona profunda del cerebro unos electrodos que, del mismo modo que un marcapasos cardiaco, consigue "controlar" el temblor. Al tratarse de un proceso quirúrgico, hay muchos pacientes que no pueden beneficiarse de él. Por ello son muchos los esfuerzos por encontrar otros métodos de control del temblor menos agresivos y que actúen a nivel del músculo, y no del cerebro.

Es por esto por lo que le invitamos a participar en un estudio que tiene como finalidad el desarrollo tecnológico y clínico de un método de control del temblor mediante unos dispositivos que actúan a nivel muscular. Estos dispositivos se llaman electrodos, y lo que hacen es administrara los músculos que intervienen en el temblor una corriente eléctrica, completamente inocua e indolora, que inhibe el impulso que viene del cerebro y de la médula generando el temblor.

Hemos dividido el estudio en dos partes bien diferenciadas para lograr un desarrollo óptimo deestos dispositivos:

- Fase 1: se realizará en personas sin temblor (voluntarios sanos). Pretendemos demostrar que los métodos de estimulación eléctrica por parte de los electrodos que estamos estudiando son capaces de modificar la activación de los músculos en gente sana.
  Fase 2: se realizará en personas con temblor. Veremos si las técnicas de estimulación que probamos con éxito en la fase 1 logran controlar el temblor en pacientes con Temblor Esencial y Enfermedad de Parkinson.

Puesto que usted no sufre temblor, le proponemos su participación en la **FASE 1** del estudio.

¿Qué pretendemos demostrar?

Creemos que la estimulación eléctrica del músculo mediante electrodos produce una inhibiciónde la actividad muscular, es decir, que es capaz de relajar un músculo que está contrayéndose gracias a que activa a un grupo de neuronas inhibitorias que existen en la médula espinal de todas las personas. Demostrar que esta inhibición muscular es posible es el primer paso para lograr controlar la actividad muscular "anormal", como sería el caso del temblor. Por eso es tanimportante primero conocer y probar cómo funciona en un músculo sano.

¿Qué son los electrodos de estimulación?

Los electrodos son unos pequeños dispositivos que se colocan en los músculos y suministran una corriente eléctrica de baja intensidad en un punto muy localizado del músculo. Los tipos de electrodos que vamos a probar son electrodos de superficie. Estos se pegan en la superficie de la piel y son completamente inocuos. Utilizaremos un total de 3 pares de electrodos que colocaremos en los músculos que se encargan de flexionar y extender la muñeca del brazo que más usa.

¿Me van a colocar algún otro aparato?

Por un lado, para poder demostrar cómo se modifica la actividad muscular mediante los electrodos, necesitamos un método fiable y objetivo que nos registre y traduzca la actividad de esos músculos. Para ello utilizaremos un Electromiograma (EMG), un aparato que se utiliza de forma rutinaria en la práctica clínica en los hospitales para poder registrar la actividad muscular y diagnosticar diferentes problemas médicos. Por ello le colocaremos otros electrodos correspondientes al Electromiograma junto a los descritos en el punto anterior.

¿En qué consistirá todo el procedimiento?

Tras la lectura de este documento, procederemos a responderle todas las dudas que le surjan. Si está de acuerdo con participar, firmará la hoja de consentimiento informado. Tras ello recogeremos una serie de datos personales (edad, enfermedades previas…) y le colocaremos loselectrodos de superficie (para estimulación muscular) y los electrodos del EMG, junto con las cintas de fijación de los electrodos. Una vez esté todo colocado, iniciaremos las pruebas, que se realizarán mientras usted está sentado en una silla con respaldo con el antebrazo apoyado encima de una mesa. En cada prueba le indicaremos cuáles serán los pasos a seguir y qué movimientos tendrá que realizar. El estudio consistirá en dos partes:

- Inhibición de la actividad muscular voluntaria mediante la estimulación eléctrica superficial del nervio. En esta primera parte del estudio se le colocarán todos los electrodos superficiales en las zonas correspondientes. Posteriormente, se le pedirá que realice una flexión máxima de muñeca mediante una cincha colocada en su muñeca. Esto se realiza con el fin de obtener el porcentaje de flexión que tendrá que mantener durante la realización de las pruebas, pero sin fatigarse. Una vez obtenido este porcentaje, se procederá a la estimulación eléctrica superficial con un intervalo de 2 segundos sobre el nervio que controla al antagonista- el extensor de muñeca. El valor de dichos pulsos eléctricos habrá sido previamente obtenido con el fin de maximizar la inhibición y minimizar la contracción muscular. Esta prueba se realizará al comienzo del experimento y 30 minutos después de la segunda prueba.
- Estimulación de las vías aferentes sincronizada con la actividad simulada de temblor. En esta segunda parte se busca reproducir la estrategia SATS ya habiendo sido testada anteriormente en voluntarios. Para ello, se le pedirá que realice movimientos de flexión y extensión de muñeca que simulen el temblor de un paciente con temblor patológico. Mientras realice este movimiento, se le aplicará estimulación eléctrica sincronizada con la actividad en 2 tandas de 10 minutos cada una con una pausa de 1 minuto entre cada una. El objetivo de esta prueba es estudiar los cambios inducidos a nivel medular por la estimulación.

Tras finalizar ambas pruebas se le retirarán todos los electrodos de estimulación y EMG.

La duración de todo el estudio será aproximadamente 120 minutos.

¿Qué efectos indeseables puedo notar?

Durante las pruebas, cuando estamos estimulando, es posible que note una sensación de cosquilleo u hormigueo, no se espera que note sensación de calambre. Si notara cualquier tipo de sensación anormal, nos lo tiene que comunicar en el acto. Estos efectos sólo los podría notarmientras se esté estimulando. Además, la colocación de cinchas para fijar la posición de los electrodos puede resultar incómoda y producir alguna sensación de presión o irritación sobre la piel, siendo un efecto pasajero.

¿Tengo que tener algún tipo de precaución especial antes o después de las pruebas?

No es necesario ningún tipo de preparación ni observación posterior. No obstante, si cree que tiene algún tipo de problema que pudiera estar relacionado con el procedimiento, se pone en contacto con nosotros, llamando al teléfono +34 658391730 y preguntando por Doctor Filipe Oliveira Barroso, que es el Investigador Principal del Proyecto EXTEND y responsable de este estudio.

¿Qué va a pasar con mis datos personales y los resultados obtenidos en el estudio?

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 porescrito 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 y publicados en diferentes conferencias y revistas médicas, sin aportar datos personales.

Su participación en este estudio es voluntaria por lo que puede negarse a participar o revocar su consentimiento en cualquier momento sin que precise dar ninguna razón para ello.

ANNEX E: INFORMED CONSENT FORM OF BOTH STUDIES

CONSENTIMIENTO INFORMADO PARA PARTICIPAR EN LA FASE 1 DEL PROYECTO DEINVESTIGACIÓN:

"SISTEMAS NEURONALES BIDIRECCIONALES HIPERCONECTADOS PARA MANEJO DEL TEMBLOR ESENCIAL Y DEL TEMBLOR DE LA ENFERMEDAD DE PARKINSON: PROYECTOEXTEND"

MEDIANTE EL USO DE ELECTRODOS DE SUPERFICIE

DECLARACIÓN DEL PARTICIPANTE

Yo, D/Dña. _____ con DNI _____, participante del presento estudio, en pleno uso de mis facultades, y de forma libre y voluntaria, expongo que he sido debidamente informado por _____. Afirmo que he recibido explicaciones tanto de forma verbal como escrita sobre la naturaleza y propósitos del protocolo, beneficios, riesgos y medios con los 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 lo he entendido y estoy satisfecho/a de todas las explicaciones y aclaraciones recibidas sobre el proceso citado.

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

Madrid, a día_____ de _____ del 20 ____

Firma del participante:

ANNEX F: EXPERIMENTAL PROTOCOL STUDY 1

1. PRE stage

Equipment preparation

- Create subject file and check the list of names
- Check the connections between Quattrocento, Arduino M0 Pro board and the Digitimer.
- Connect everything to the computer
- Soak wristband and felt pads (of the bar electrode)
- Gather all the electrodes to be used

Volunteer preparation

- Summarise the procedure and goals of the study to the participant
- Answer all doubts
- Inform consent form
- Clean the skin with a cotton soaked with alcohol
- Find by palpation the ECR and FCR muscles
- Place bipolar sEMG electrodes over the ECR and FCR muscle bellies
- Place the wristband on the elbow
- Check EMG quality and sEMG positioning with OT Biolab+
- Ask the subject to comfortably seat with the arm stretched
- Search for the radial nerve with the bar electrode
- Place surface stimulation electrodes on these points
- Connect stimulation electrodes to Digitimer

H-reflex recruitment curves

- Ask the volunteer to slightly extend the wrist to facilitate ECR H-reflexes
- Obtain the H- and M-wave recruitment curves in a range of 5 or 6 intensity values until Mmax
- Ask the participant to slightly flex the wrist
- Search for FCR MT

MVC protocol

- Adjust the pulling strap for the volunteer
- Find the MVC of the volunteer
- Record MVC activity with OT Biolab+
- Repeat 3 times and calculate the mean

Reciprocal inhibition

- Explain the procedure
- Load Arduino script Stimulation_protocol_stim_and_force__v01.
- 4 trials of 30 pulses each (MT) with a rest period of 1 minute between each
- Record EMG activity during the procedure
- Ask the participant to maintain 20% of the MVC

2. POST30' stage

30 minutes after PRE stage

H-reflex recruitment curves

- Ask the volunteer to slightly extend the wrist to facilitate ECR H-reflexes
- Obtain the H- and M-wave recruitment curves in a range of 5 or 6 intensity values until Mmax

Reciprocal inhibition

- 4 trials of 30 pulses each (MT) with a rest period of 1 minute between each
- Record EMG activity during the procedure
- Ask the participant to maintain 20% of the MVC

3. POSTVIB stage

Immediately after POST30' stage

Prolonged vibration

- Ask the subject to comfortably seat with the arm stretched
- Place the muscle massager on the wrist tendons
- Fasten the massager to the participant's arm with two elastic straps
- 20 minutes of prolonged vibration

H-reflex recruitment curves

- Ask the volunteer to slightly extend the wrist to facilitate ECR H-reflexes
- Obtain the H- and M-wave recruitment curves in a range of 5 or 6 intensity values until Mmax

Reciprocal inhibition

- 4 trials of 30 pulses each (MT) with a rest period of 1 minute between each
- Record EMG activity during the procedure
- Ask the participant to maintain 20% of the MVC

ANNEX G: EXPERIMENTAL PROTOCOL STUDY 2

1. PRE stage

Equipment preparation

- Create subject file and check the list of names
- Check the connections between Quattrocento, Arduino M0 Pro board and the Digitimer.
- Connect everything to the computer
- Prepare input connections of EAST (brown (1)= radial, red (2)= median, black (3)= ground, and white with adapters= EMG recording)
- Soak wristband and felt pads (of the bar electrode)
- Gather all the electrodes to be used

Volunteer preparation

- Summarise the procedure and goals of the study to the participant
- Answer all doubts
- Inform consent form
- Clean the skin with a cotton soaked with alcohol
- Find by palpation the ECR and FCR muscles
- Place bipolar sEMG electrodes over the ECR and FCR muscle bellies
- Place surface ground electrode on the olecranon
- Place the wristband on the elbow
- Check EMG signals quality and sEMG positioning with OT Biolab+
- Ask the subject to comfortably seat with the arm stretched and wrist flexed
- Search for the radial nerve with the bar electrode
- Search for the median nerve with the bar electrode
- Place surface stimulation electrodes on these points
- Connect stimulation electrodes to Digitimer
- Find the ECR MT while flexing the wrist

MVC protocol

- Adjust pulling strap for the volunteer
- Find the MVC of the volunteer
- Record MVC activity with OT Biolab+
- Repeat 3 times and calculate the mean

Reciprocal inhibition

- Explain the procedure
- Load Arduino script Stimulation_protocol_stim_and_force__v01.
- 4 trials of 30 pulses each (MT) with a rest period of 1 minute between each
- Record EMG activity during the procedure
- Ask the participant to maintain 20% of the MVC

2. SATS intervention

Equipment preparation

- Connect EAST stimulator to the computer
- Check that all input cables are properly connected
- Turn off the Quattrocento and Digitimer

Volunteer preparation

- Disconnect all Quattrocento and Digitimer cables
- Connect EAST input cables to the recording, stimulation, and ground electrodes
- Explain the procedure
- Record 30 seconds of EMG activity while the subject was mimicking wrist flexion-extension tremor
- Set stimulation parameters: stimulation intensity, tremor threshold

SATS intervention

- 2 trials of 10 minutes each that consisted of 5 sub-trials of 120 seconds each. Total recording window of 600 seconds.
- Conduct either out-of-phase/in-phase stimulation
- Ask the subject to start simulating tremor
- Start the first 10-minute trial of EAST while simulating tremor
- 1 minute rest
- Start the second 10-minute trial of EAST while simulating tremor

3. POST stage

Immediately after SATS intervention

Equipment preparation

- Disconnect EAST stimulation from computer
- Turn on the Quattrocento and Digitimer
- Set MVC on OT Biolab+
- Set MT on Digitimer

Volunteer preparation

- Disconnect all EAST cables
- Connect Quattrocento and Digitimer cables to the recording, and stimulation electrodes

Reciprocal inhibition

- 4 trials of 30 pulses each (MT) with a rest period of 1 minute between each
- Record EMG activity during the procedure
- Ask the participant to maintain 20% of the MVC

4. POST30’ stage

30 minutes after POST stage

Reciprocal inhibition

- 4 trials of 30 pulses each (MT) with a rest period of 1 minute between each
- Record EMG activity during the procedure
- Ask the participant to maintain 20% of the MVC

ANNEX H: DATA SHEET STUDY 1

Subject information	
Name of the subject file	
Date of the experiment	
Age	
Gender	

Experimental parameters (PRE, POST30' and POSTVIB)	
Values of the range of intensities	
Mmax	
MT intensity	
MVC	
20% MVC	
Sleepy fingers after prolonged vibration?	

ANNEX I: DATA SHEET STUDY 2

Subject information	
Name of the subject file	
Date of the experiment	
Age	
Gender	

Experimental parameters (PRE, POST and POST30')	
Values of the range of intensities	
Mmax	
MT intensity	
MVC	
20% MVC	
Sleepy fingers after prolonged vibration?	

SATS intervention	
Strategy	
Stimulation intensity	
Tremor detection threshold	