Reduction of Pathological Tremor in Essential Tremor Patients Through Peripheral Electrical Stimulation of Afferent Pathways

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Por los que se fueron pero están.

UNIVERSIDAD POLITÉCNICA DE MADRID

Abstract

Escuela Técnica Superior de Ingenieros de Telecomunicación

PhD in Biomedical Engineering

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by Alejandro Pascual Valdunciel

Essential tremor (ET) is the leading cause of pathological tremor, the most common movement disorder in the adult population. This involuntary movement of one or more parts of the body can become a disabling condition for the execution of activities of daily living, which has a significant impact on patients' quality of life. Nowadays, there is no cure for ET and current treatments have limited efficacy, considerable adverse effects or they are not accessible to a large part of the population. Therefore, there is a need to develop effective and affordable solutions for the management of ET. Peripheral electrical stimulation (PES) of afferent (sensory) pathways is an emerging technique with promising results for the reduction of pathological tremor due to its usability and limited adverse effects. Despite early indications of efficacy, it is necessary to further characterize the physiological effects of PES as a tool to modulate the nervous system, as well as its potential efficacy in reducing tremor.

The main objective of this PhD thesis was the development of a technique based on PES of afferent pathways to reduce pathological tremor in ET patients by modulating neural pathways involved in the tremor circuits. To achieve this goal, a stepwise approach was employed.

First, a comprehensive review of the state of the art of PES techniques applied to reduce pathological tremor was performed, which allowed the identification of methodologies, results, advantages and limitations of the solutions proposed in scientific literature. This knowledge presented the physiological and technical bases needed to develop the consequent studies of this thesis.

The first study of this thesis demonstrated the hypothesis that stimulation of the afferent pathways of the antagonist muscle can produce inhibition of voluntary muscle activity in the wrist muscles in healthy subjects. Inhibition occurred at stimulation intensities above and below motor threshold, and the latency of this inhibition was consistent with inhibitory spinal circuits. These results added more evidence that justify the use of afferent stimulation below the motor threshold of the antagonist muscle as a tool to acutely inhibit tremor activity.

The second study presented the development and validation of a closed-loop stimulation strategy based on electromyography named Selective and Adaptive Timely Stimulation (SATS) of afferent pathways. The short-term neuromodulatory effects at the spinal level were tested after the stimulation was applied in-phase or out-of-phase with muscle activity in healthy subjects while they replicated pathological wrist tremor movements for 20 minutes. The results showed that the SATS strategy was able to deliver stimulation synchronized with muscle activity with high temporal precision. Additionally, the inhibitory spinal circuit responsible for the inhibition of the antagonist muscle was potentiated when the stimulation was applied

in-phase with the muscle activity, whereas the out-of-phase stimulation produced a depression on the same inhibitory spinal circuit. Overall, this study demonstrated the feasibility of the SATS strategy as a tool for modulating spinal circuits, and the importance of synchronizing stimulation with physiological activity to induce specific adaptations in the nervous system.

Finally, the SATS strategy was tested on a cohort of ET patients. To demonstrate the tremor reduction effects associated with the SATS strategy, patients received this stimulation applied out-of-phase with the tremor activity (stimulation of the antagonist muscle), or continuous open-loop stimulation. Both strategies were tested in two experimental sessions using minimally-invasive intramuscular electrodes or surface electrodes. The results showed that the SATS strategy applied through intramuscular electrodes acutely reduces tremor (while stimulation was active), while continuous stimulation did not reduce tremor. Furthermore, a short-term tremor reduction effect was reported at the end of the experimental session for patients receiving SATS applied with intramuscular electrodes. For some patients, this tremor reduction was maintained for 24 hours after the session. In conclusion, this PhD thesis presents evidence on the characterization and validation of PES of afferent pathways synchronized with physiological activity as a tool to reduce pathological tremor in ET patients. These advances may have a significant impact on the quality of life of patients, as they lay the foundations for the development of accessible neuroprostheses with minimal side effects for the effective acute and therapeutic treatment of pathological tremor.

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Resumen

Escuela Técnica Superior de Ingenieros de Telecomunicación

Doctorado en Ingeniería Biomédica

Reducción del temblor patológico en pacientes de temblor esencia a través de la estimulación eléctrica periférica de las vías aferentes

por Alejandro Pascual Valdunciel

El temblor esencial (ET) es la principal causa de temblor patológico, el trastorno de movimiento con mayor incidencia en la población adulta. Este movimiento involuntario de una o más partes del cuerpo puede llegar a resultar incapacitante para el desarrollo de tareas cotidianas, lo cual tiene un importante impacto en la calidad de vida de los pacientes. Actualmente no existe cura para el TE y los tratamientos actuales tienen limitada eficacia, considerables efectos adversos o no son accesibles para una gran parte de la población. Por ello es necesario el desarrollo de soluciones y eficaces y accesibles para el manejo del TE. La estimulación eléctrica periférica (EEP) de las vías aferentes (sensoriales) es una técnica emergente con resultados prometedores para la reducción del temblor patológico debido a sus limitados efectos adversos y usabilidad. A pesar de los primeros indicios de eficacia, es necesario profundizar en la caracterización de los efectos fisiológicos de la EEP como herramienta para modular el sistema nervioso, así como su potencial eficacia para reducir el temblor.

El objetivo principal de esta tesis doctoral fue el desarrollo de una técnica de EEP de las vías aferentes para reducir el temblor patológico en pacientes de TE mediante la modulación de los circuitos neuronales afectados por temblor. Para conseguir dicho objetivo, se ha empleado un enfoque gradual.

En primer lugar, se realizó una revisión exhaustiva sobre el estado del arte de las técnicas de EEP aplicadas a la reducción de temblor patológico, lo cual permitió identificar las metodologías, los resultados, las ventajas y limitaciones de las soluciones propuestas en la literatura científica. Este conocimiento presentó las bases de conocimiento fisiológicas y técnicas necesarias para el desarrollo de los siguientes estudios de esta tesis.

En el primer estudio de esta tesis se demostró la hipótesis de que la estimulación de las vías aferentes del músculo antagonista puede producir la inhibición de la actividad muscular voluntaria en los músculos de la muñeca en sujetos sanos. La inhibición se produjo con intensidades de estimulación por encima y por debajo del umbral motor, y la latencia de esta inhibición es compatible con los circuitos espinales de inhibición recíproca. Estos resultados justifican la utilización de la estimulación aferente por debajo del umbral motor del músculo antagonista como herramienta para inhibir de forma aguda la actividad del temblor.

A continuación, se desarrolló una estrategia de estimulación selectiva y adaptativa de las vías aferentes de lazo cerrado (SATS) basada en señales de electromiografía. Los efectos neuromodulatorios a nivel espinal de esta estrategia fueron testados cuando la estimulación fue aplicada en fase o en contrafase con la actividad muscular en sujetos sanos mientras estos replicaban movimientos patológicos

de temblor de muñeca durante 20 minutos. Los resultados mostraron que la estrategia SATS fue capaz de aplicar estimulación sincronizada con la actividad muscular con alta precisión temporal. Asimismo, se produjo una potenciación de la inhibición del músculo antagonista cuando la estimulación fue aplicada en fase, mientras que la estimulación en contrafase produjo una depresión de dicha inhibición. En su conjunto, este estudio demostró la viabilidad de la estrategia SATS como herramienta de modulación de los circuitos espinales, así como la importancia de la sincronización de la estimulación con la actividad fisiológica para inducir cambios específicos en el sistema nervioso, justificando así su utilización para la reducción de temblor patológico.

Finalmente, la estrategia SATS fue testada en pacientes con TE. Para demostrar los efectos de reducción de temblor asociados a la estrategia SATS, los pacientes recibieron esta estimulación aplicada en contrafase con la actividad de temblor (estimulación del músculo antagonista), así como una estimulación continua de lazo abierto. Ambas fueron aplicadas mediante electrodos intramusculares mínimamente invasivos en una sesión, y mediante electrodos superficiales en otra. Los resultados mostraron que la estrategia SATS aplicada mediante electrodos intramusculares consiguió reducir el temblor agudo (mientras la estimulación está activa), mientras que la estimulación continua no redujo el temblor. Igualmente, se reportó una reducción de temblor a corto plazo al finalizar la sesión experimental que para aquellos pacientes que recibieron la estimulación SATS aplicada con electrodos intramusculares. En algunos pacientes esta reducción de temblor se mantuvo 24 horas después de la sesión experimental.

En conclusión, esta tesis doctoral presenta evidencias sobre la caracterización y validación de la EEP de las vías aferentes sincronizadas con la actividad fisiológica como herramienta de reducción del temblor patológico en pacientes con TE. Estos avances pueden tener un gran impacto en la calidad de vida de los pacientes, ya que sientan las bases para el desarrollo de neuroprótesis accesibles y con mínimos efectos secundarios para el tratamiento eficaz agudo y terapéutico del temblor patológico.

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Vamos al territorio personal. Siempre he dicho que, de momento, tengo flor en la vida, por lo menos en lo académico y en lo profesional, y siendo sincero en lo personal también. Todo me ha salido rodado a cambio de un esfuerzo razonable, pero tampoco puedo decir que me he matado. Hasta ahora he podido dedicarme a algo que disfruto haciendo, y además se me da bien. Para poder llegar hasta aquí hay muchas personas que se han sacrificado, me han ayudado o simplemente han estado ahí, animando. Empezamos.

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List of Abbreviations

ADLs	Activities of Daily Living
CGI-S	Clinical Global Index of Severity
CGI-C	Clinical Global Index of Change
CNS	Central Nervous System
DBS	Deep Brain Stimulation
ECR	Extensor Carpi Radialis
EEG	Electroencephalography
EMG	Electromyography
EPSP	Excitatory Postsynaptic Potential
ET	Essential Tremor
FCR	Flexor Carpi Radialis
FES	Functional Electrical Stimulation
FTM	Fahn-Tolosa-Marín Clinical Scale
hdEMG	High Density Electromyography
HIFU	High-Intensity Focused Ultrasound
IMU	Inertial Measurement Unit
INP	In-Phase
IPSP	Inhibitory Postsynaptic Potential
ISI	Inter-Stimulus Interval
MT	Motor Threshold
MU	Motor Unit
PD	Parkinson's Disease
PES	Peripheral Electrical Stimulation
PSD	Power Spectral Density
OOP	Out-Of-Phase
RMS	Root Mean Square
SATS	Selective and Adaptive Timely Stimulation
SCI	Spinal Cord Injury
SCS	Spinal Cord Stimulation
sEMG	Surface Electromyography
TENS	Transcutaneous Electrical Nerve Stimulation
TMS	Transcraneal Magnetic Stimulation
VIM	Ventral Intermediate Nucleus of the thalamus

Chapter 1

Introduction

1.1 Context and motivation

Witnessing deterioration of human health with age is an ineluctable fact that everyone experiences sooner or later in their life. All human beings are subject to the remorseless natural process of aging, which is commonly associated with a progressive deterioration of health. Nonetheless, breakthroughs in medicine, nutrition, technology or modern lifestyle in the past decades have granted that the vast majority of individuals reach the third age with a health status enabling a decent and autonomous life. In spite of all mankind progress, there is still no cure for numerous diseases, including some that derive in motor or cognitive impairment. These diseases hamper the execution of activities of daily living (ADL) and increase the vulnerability to comorbidities, secondary diseases or premature death (World Health Organization, 2011). Disability does not exclusively impact on patients, but its effects also reach their social environment, as well as the economic and healthcare systems. Approximately 50% of population with disabilities in Spain claims not receiving technical or personal aid to perform ADL, a fact that evidences that both assistance and healthcare systems do not provide all necessary resources to this population. (Instituto Nacional de Estadística, 2013).

Particularly, neurological disorders have become the leading source of disability worldwide. The human nervous system is an extraordinary achievement of evolution and the full understanding of its operation is an ongoing challenge that needs to be overcome to unearth a solution for several neural diseases. An illustration of a disease with worldwide impact is Essential Tremor (ET): the main neural disorder leading to pathological tremor, which is in turn the most common movement disorder in adults (Thanvi et al., 2006). It is noteworthy that while Parkinson's disease (PD) affects an estimated 1% of the population above 60 years old, the incidence of ET is approximately 4-5% for those over 65 years old (Lau et al., 2006; Shanker, 2019). Patients suffering from pathological tremor are subjected to involuntary rhythmic movements of one or more body limbs, a condition which might be considered non severe when the amplitude of these undesired movements is low and does not interfere with the execution of some motor tasks. Regretfully, that is not the real situation for numerous patients who are affected by severe tremor during the execution of kinetic tasks such as ADL (Louis, 2001). Therefore, absence of precise motor control of the upper limb, and specifically of the hands, implies that patients are impaired for performing everyday tasks such as writing, handling their smartphone or even feeding themselves. Approximately 71% of ET patients in the US report moderate to very significant impact on their quality of life even in cases where tremors are not severe. Additionally, comorbidities related to ET are not limited to motor functions, as many ET patients experience significantly increased stress levels, lower self-esteem, symptoms of depression or unemployment. Due to worldwide aging trends, as the

incidence of ET increases with age, the socioeconomic burden grows, which has been estimated to be around 142.7 billion dollars yearly in the United States (Frost and Sullivan, 2018).

The main challenge regarding ET is the current absence of a cure for the disease. ET is a progressive neurological disease, hereditary in a vast majority of cases. However, the pathophysiological mechanisms have not been fully enlightened; even the diagnosis is purely clinical and there are no available biomarkers to determine the status of the disease (Deuschl et al., 2011). Current tremor management solutions are restricted to the mitigation of the symptoms of the disease. Pharmacological therapies, the first and most accessible line of treatment are ineffective in approximately 50% of moderate and severe ET patients (Louis, 2012). Hence, there is a need to develop alternatives for medically refractory ET patients who need a reliable and reversible tremor reduction therapy.

In the medical field novel treatments or diagnosis techniques with high research impact have not been integrated into the clinical practice as anticipated. In order to accelerate innovation from research to the clinics it is fundamental to develop proactive dialogues between engineers, researchers and physicians in order to design novel solutions that might be able to enhance prevailing ones in the clinical settings. Neural rehabilitation is a translational process that pursues the development of methodologies, interventions and devices to improve the quality of life and care for people with disabilities (Khan et al., 2017). Previous independent areas of knowledge such as neuroscience, robotics, functional rehabilitation, or machine learning converge at neural rehabilitation as a multidisciplinary process to overcome the challenges in neural recovery.

The EU Project EXTEND: Bidirectional Hyper-Connected Neural Systems (BHNS) (grant agreement Nº 779982) illustrates the necessary translational steps to develop and integrate technological tools into functional applications to address unmet needs in medicine. The main goal of the project is to design and test minimally-invasive wireless electrodes capable of bi-directionally interfacing the neural system by means of neuromuscular stimulation and sensing (Tudela-Pi et al., 2021). During the initial stage, the development of such technology requires high degree of integration between biomaterials and electronics fields. Subsequent to the development stage, the application phase befalls to test novel solutions for two neural diseases: tremor reduction in ET and PD patients, and soft control of lower-limb exoskeletons for spinal cord injury patients (SCI) (Rodrigues et al., 2020). In the course of this phase, both clinical and biomedical fields work closely to exploit the physiological mechanisms and clinical aspects that optimize the applications. Notwithstanding, the evolution of this project is not a lineal process: there are continuous and cyclic interactions among all phases to refine the technology, protocols and applications to achieve significant progress towards a final solution available for patients.

This PhD thesis was developed in the framework of the EXTEND project, particularly in the research of neural interfaces based on neuromuscular activity recording and peripheral electrical stimulation (PES). Conceptually, PES comprises the application of electrical currents aiming at recruiting the sensory or motor pathways (Trimble et al., 1991). PES is widely used in rehabilitation or sports training. However, its real efficacy or capacity to selectively modulate neural circuits aiming to achieve functional improvements has been scarcely characterized compared to other treatments (Knutson et al., 2015). Promising capabilities of PES lie in the possibility to modulate aberrant neural circuits such as those present in pathological tremor, by shifting the output of the neural system towards physiological or functional states

and, therefore, mitigating pathological behaviors. The purpose of this doctoral research is to deepen the capabilities of PES, firstly in order to contribute to the understanding of the nervous system and how we may exploit it to restore impaired neural and motor functions. Secondly, to develop a minimally invasive tremor reduction strategy suitable for a broad population suffering from disabling tremor, reducing the economic and social impact of this disorder affecting millions of people worldwide.

1.2 The basis of motor control

The capacity of movement of homo sapiens sapiens is achieved through the permanent and collaborative work of the nervous and musculoskeletal systems. Although the neuromusculoskeletal system has been estimated to be comprised of 206 bones and 650 skeletal muscles, the nervous system is capable of handling such a complex structure in a multiple, wide and even concurrent variety of tasks such as bipedal gait, reaching, painting, writing or dancing (Moore K. L., 2002). Not all the movements are planned or voluntary: most of our motor tasks are unconscious, executed without awareness; and some of them do not even require processing of the superior structures of the nervous system, being executed with extremely fast reaction times via reflex loops. Furthermore, motor control is a process in constant evolution throughout life: infants are not capable of performing complex motor tasks, yet they learn new motor skills in their first years, and these learning and adaptation capabilities accompany human beings throughout their entire lives.

The central nervous system (CNS) achieves soft motor control via a constant integration of afferent information from the peripheral neural system (PNS), which is processed in hierarchical structures in order to generate efferent commands towards the neuromusculoskeletal system (Blakemore et al., 1998). This is one of the key features of the CNS, its capability of massive parallel processing of diverse and constant flow of information from the internal body and the external environment to adapt the motor output to perform a specific goal. Precisely, afferent signals are also crucial in the learning process, since they provide the necessary error feedback to close the loop and adapt the neural system to achieve the desired output (Raymond et al., 1996). It is not surprisingly that in the artificial intelligence field, the hierarchical and feedback learning processes of the nervous system have been mimicked in the design of computational neural networks capable of learning complex behaviors (Rosenblatt, 1958).

1.2.1 Overview of the CNS

The description of anatomical, physiological and biochemical properties of the complete nervous system is an undergoing process, and yet much effort needs to be put into neuroscience research to unravel a complete (or at least approximate) model of the nervous system. Motor control is just one of the functions of the CNS along with cognition or autonomous behaviors. The scope of this work is focused on the management of pathological tremor, particularly in ET, through the use of PES as a neuromodulation tool of the CNS. Cognitive symptoms, as well as cellular and anatomical pathology of this neural motor disorder are out of the scope of this dissertation. Therefore, only a sufficient depiction of the neural structures and pathways engaged in the motor functions of the CNS and the pathophysiology of ET will be provided in order to understand the basis of the developed research.

The CNS can be differentiated in two main structures: the brain and the spinal cord. Likewise, the brain is comprised of six structures markedly differentiated: the medulla oblongata, pons, midbrain, diencephalon, cerebral hemispheres and cerebellum (Kandel et al., 2013) (Figure 1.1). The majority of neural structures and circuits present bilateral symmetry, and project into the contralateral side of the body: the right hemisphere is involved in the main control of the left side, and vice versa. Parallel to contralateral and bilateral organization, the CNS entails hierarchical processing of the efferent and afferent information. The raw information transmitted from the PNS to the CNS is firstly processed at the spinal cord, where some functions can be executed via reflex loops and spinal circuits (Dietz, 1992). Then, this information is projected towards the brain, but prior to reaching the primary sensory cortex, the ascending information is sequentially processed and integrated with other sensory pathways through the midbrain and the thalamus among other centers. Eventually, the raw basic afferent signals generated at the PNS, (e.g. the length status of one muscle or pressure receptors), has been transformed and complemented at the sensory areas of the cortex in order to create meaningful information about the proprioception of the musculoskeletal system (Wolpert et al., 2011). Hence, the afferent information is now projected to the motor system, following a similar hierarchical pathway from the motor and sensory cortex to the final muscle effectors. The brainstem groups the medulla, the pons and the midbrain, acting as a linkage between the spinal cord and the brain. Among some of the functions in which these neural structures are involved are respiratory and cardiac rhythms, motor and sensory tracts to control the head and the neck, and important coupling of motor and sensory pathways with the cerebellum and the basal ganglia and other brain structures, with the red nucleus being a key structure in motor coordination (Paxinos G., 2003).

Thalamus

The thalamus is part of the diencephalon in combination with the hippocampus, and it is a fundamental nucleus in the integration and processing of peripheral sensory information ultimately projected to the motor cortex (Steriade et al., 1988). Additionally, the thalamus is implicated in motor control through connections with the basal ganglia, the motor cortex and the cerebellum, in the thalamo-cerebello-cortical circuit. It is worth noting the effect of specific thalamic lesions and the Deep Brain Stimulation (DBS) as effective treatments of certain motor disorders such as pathological tremor or dystonia (Wichmann et al., 2006), later described in Chapter 2. Although the complete description of the fundamentals of this circuit has not been clarified yet, different surgical approaches targeting various thalamic nuclei result in dissimilar motor outcomes, which adds more evidence to the complexity and individual functions of the different thalamic nuclei (Renard et al., 2014).

Basal Ganglia

The basal ganglia are five subcortical structures with a strong engagement in fine control of voluntary movements: putamen, caudates nucleus, globus pallidus, substantia nigra, and subthalamic nucleus (Alexander et al., 1990). Striatum, composed of putamen and caudates nucleus, is the main entrance gate to the basal ganglia, receiving input from cortical premotor, motor and somatosensory areas. Then, the motor commands are projected towards the other basal nuclei via a direct and an indirect pathway, to finally reach the thalamic nucleus and the brainstem (Hoover John E, n.d.). Excitatory/inhibitory balance of every component the basal ganglia

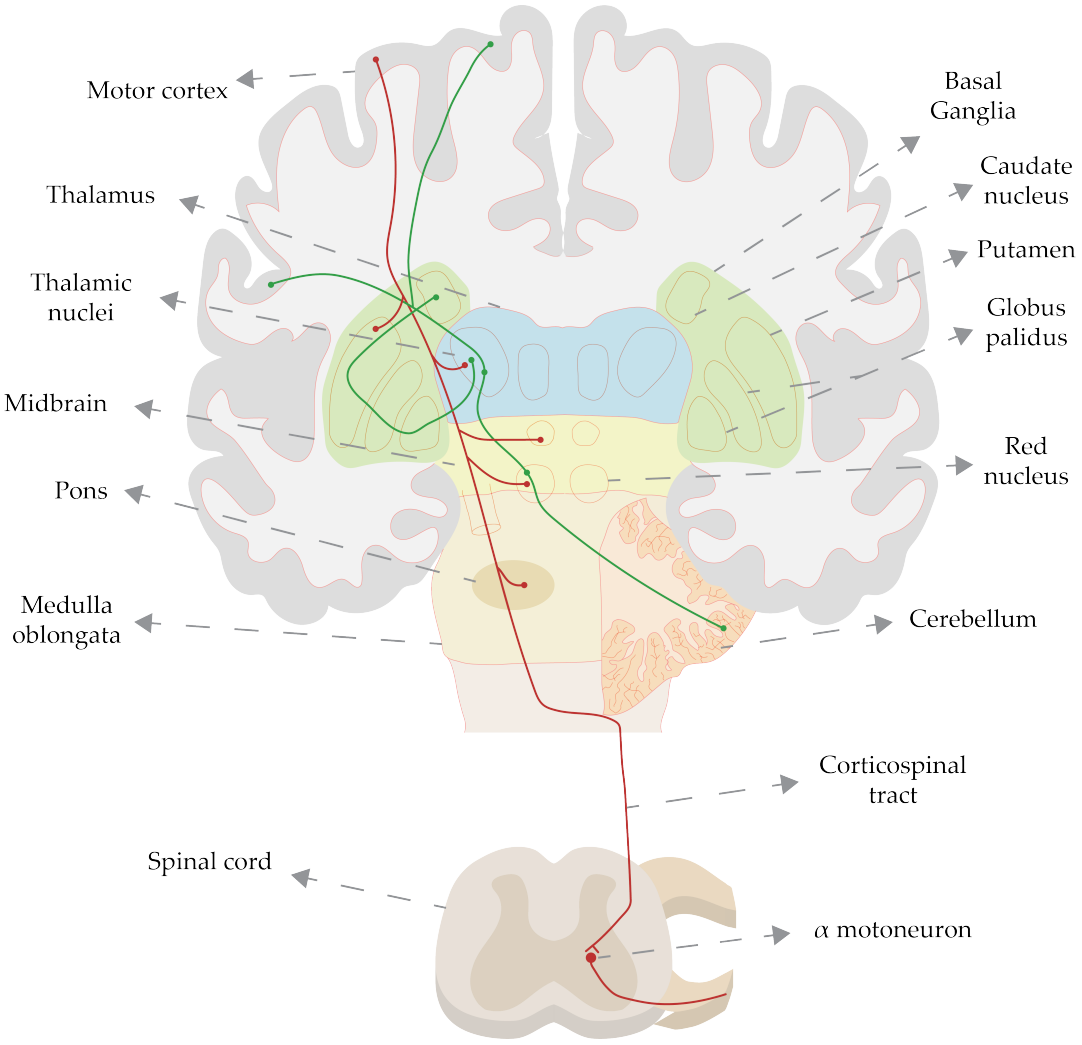

FIGURE 1.1: Simplified illustration of the main anatomic structures of the CNS. Red and green lines represent the descending and ascending pathways of the cerebello-thalamo-cortical circuits involved in motor control. Adapted from (Kandel et al., 2013)

network is required to maintain normal voluntary motor control, thus, specific nuclei degeneration lead to a whole disruption of the cerebello-thalamo-cortical circuit and the alteration of its function. As an illustration of this fact related to tremor, the neural circuitry involved in the pathophysiology of PD is well described: a reduction of the dopamine neurotransmitter caused by neurodegeneration of the dopaminergic cells of the substantia nigra unbalances the basal ganglia circuit and results in an abnormal motor function motor with symptoms like tremor (among others), included as parkinsonism (Galvan et al., 2008).

Cerebellum

This neural structure is hypothesized to be involved in motor control through the comparison of intended movements and the actual afferent feedback to provide corrective and predictive control of the motor output to fulfil the desired task. The cerebellum receives projections from the thalamo-cortical circuit and primary afferents from the spinal cord in order to adjust the motor response which is sent back to the thalamus and then to the motor cortex (Medina et al., 2008). Similar to other brain

structures described, lesion of cerebellar tissue originates motor control abnormalities such as pathological tremor, since the cerebellum fails at rapidly correcting the movement incurring in aberrant oscillations. Furthermore, the cerebellum is thought to be a major learning center due to this capability to process real and planned inputs used to provide feedforward control (Carey et al., 2002). For instance, when performing a reaching movement towards a moving object, the cerebellum is able to continuously integrate the inputs from the sensory pathways to generate an adaptive motor response which minimizes the approach error of the limb effector.

Motor cortex

The primary motor cortex, as well as the sensory cortex, is organized following a topographic distribution mapping the different parts of the body. However, this representation is not equally balanced among the different body limbs since the parts requiring finer motor control such as the hands or the fingers entail larger innervation areas (Rizzolatti et al., 2001). The motor cortex is the final site of integration of sensory and cognitive information to generate the output commands sent to the neuromusculoskeletal system, mainly via the corticospinal or pyramidal tract. Around 40% of fibers of the corticospinal tract arise from the motor cortex, descend and cross the midline in the medulla and reach the motoneurons at the spinal cord through monosynaptic connection (Cheney et al., 1980). Yet, not all the fibers from the corticospinal tract make monosynaptic connection with the motoneurons: some fibers synapse with interneurons in the spinal cord, being fundamental for the coordination of muscle groups.

1.2.2 The Motor Unit

The motor unit is the basic functional unit of the neuromuscular system, comprised of an alpha motoneuron innervating several muscles fibers (Farina et al., 2016). The efferent command is transmitted along the spinal cord and reaches the motoneurons: if the motor stimuli exceeds the motor threshold, the motoneuron is activated and an action potential is propagated through the motor axon until the neuromuscular junction (Figure 1.2). Then, the neurotransmitter is released and an action potential is propagated in the sarcolemma, which causes the contraction of the muscles fibers and the change of the mechanical output of the musculoskeletal system (Adrian et al., 1929). The number of motor units is highly variable across muscles and even subjects (Enoka et al., 2008). Motor units can be classified according to their contraction properties in slow motor units and fast motor units. The slow motor units, which are comprised of slow contracting type I fibers, generate lower but steady forces and are resistant to fatigue; while the fast motor units use fast contractile type II fibers, are capable of producing higher forces but are more susceptible to fatigue (Enoka et al., 2008).

The final control of the mechanical properties of the body limbs is achieved by the transduction of the neural inputs to the motoneurons into activity spike trains which reach the muscle and generate the muscle forces (Farina et al., 2014b). Since the motoneurons are threshold activated, the synaptic input is non-linearly processed in order to modulate the force by means of adapting the discharge rate of the motoneurons. This non-linear response leads to variable behaviors in which the common input to two motoneurons with different properties might result in dissimilar firing rates. Although the complete understanding of the motor control theory is still in permanent headway, some basis of the neural commands translation code

into muscle output have been enlightening so far. Such is the size principle of motor neuron recruitment, directly influenced by the surface or size of the motor unit. The smallest and fatigue-resistant slow motoneurons are recruited first, then followed by the increasing size motoneurons until the largest ones are activated, which present the highest fatigue sensibility (Dideriksen et al., 2012).

Electromyography (EMG)

The electrical currents generated by the action potentials of the motor units at the muscle fibers can be detected by means of the technique named electromyography (EMG) (Farina et al., 2014c). Specifically, the motor unit action potentials (MUAPs) occurring at the tissues are collected into diverse kinds of electrodes, from which the electrical signals are amplified and recorded, similarly to other techniques such as electrocardiography or electroencephalography (EEG). Hence, recording individual MUAPs from all the motor units within the muscles, known as the neural drive, would ultimately contribute to the understanding of motor control and the decryption of how the neural signals are translated into muscle contractions and forces (Farina et al., 2014a). Surely, a deeper enlightenment of the motor control will allow to identify physiological or normal behaviors, as well as pathological mechanisms in neural diseases, enabling the design of specific therapeutic solutions aiming at restoring the neuromusculoskeletal system towards physiological and functional balance (Bronzino, 2000).

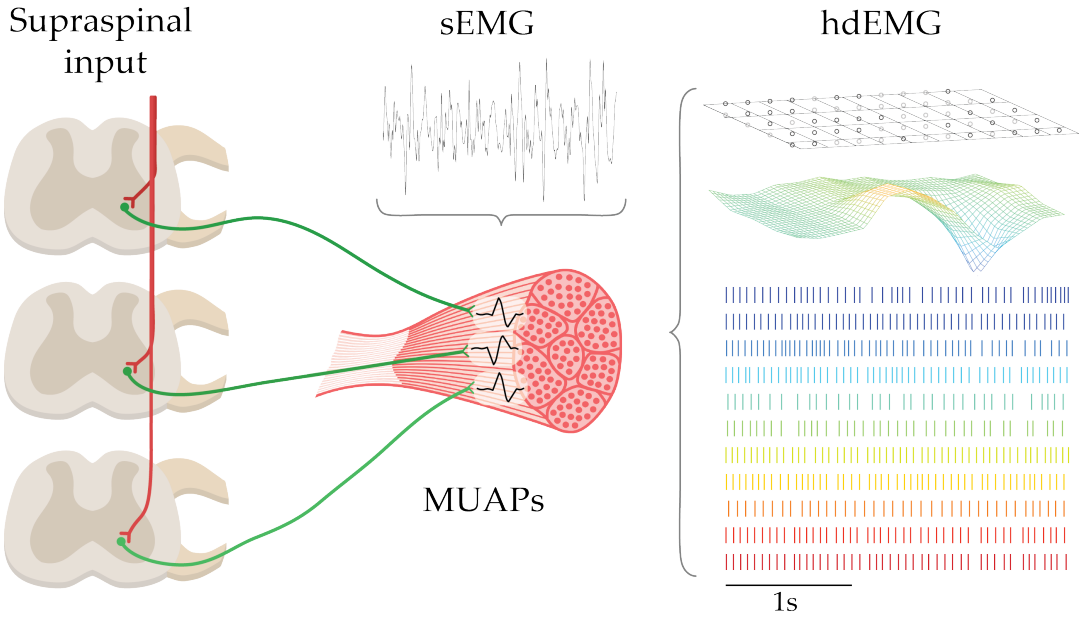

FIGURE 1.2: Schematic of the corticospinal transmission of motor commands (left panel) which are translated into MUAPs (neural drive). MUAPs ultimately elicit contraction of muscle fibers and are detected through the use of the EMG technique. The sum of MUAPs comprises the signal detected with sEMG electrodes (central panel). The use of hdEMG allows decomposition of signals into single MUAPs (right panel). Adapted from (Merletti et al., 2019)

Since muscle contractions involve the firing of a wide number of motor units, the spatial and temporal sum of those MUAPs is the signal reflected in the EMG (Figure 1.2). Therefore, the neural drive of a muscle or group of muscles can merely

be estimated due to the intricate spatial and temporal distribution of the electrical properties of the MUAPs within the tissues (Merletti et al., 2019). The use of different sorts of electrodes determines the features of the acquired signals. Traditionally, surface electrodes (sEMG) are the most widespread interface due to their ease at positioning and cost-effectiveness. Particularly, surface disposable Ag-AgCl gel-based patches are commonly placed over the muscle belly in monopolar or bipolar configuration to record the overall activity of the target muscle (Figure 1.3-B). However, spatial resolution of bipolar/monopolar sEMG is limited and due to the volume conduction phenomena, the recorded activity might not be specific to the target, but rather the result of the contribution of adjacent muscles, a phenomenon known as crosstalk. The amplitude or envelope of the sEMG signal, commonly measured as the Root Mean Square value of the signal, provides illustration of the overall activity of the muscle, which is a valuable piece of information for the control of neuroprostheses or wearable devices (Zecca et al., 2002); the extraction of muscle synergies, a term used to describe the coordinated functional activation of a group of muscles as a motor control strategy (Barroso et al., 2015); or the measurement of evoked potentials produced after the application of external stimuli (San Agustín et al., 2019), among other applications. However, the amplitude of the sEMG signal is far from being an optimal estimator of the neural drive and the motor unit properties owing to volume conduction and crosstalk, the low-pass spatial filtering effect of large electrodes, and the amount of MUAPs amplitude cancellation (Farina et al., 2014b).

On the other hand, needles or wires are commonly used to record intramuscular EMG (iEMG) signals in order to increase spatial selectivity of the recorded MUAPs (Figure 1.3-A) (Merletti et al., 2009). By placing the electrodes closely to the motor units, the volume conduction effect is minimized and the MUAPs can be identified readily compared to sEMG, although the spatial resolution might be excessively constrained to the surrounding motor fibers, merely being sensitive to a non-sufficiently representative portion of the entire muscle. Both sEMG and iEMG have been extensively used in research and clinics as a diagnosis and descriptive technique of the neuromuscular system (Lee et al., 2004).

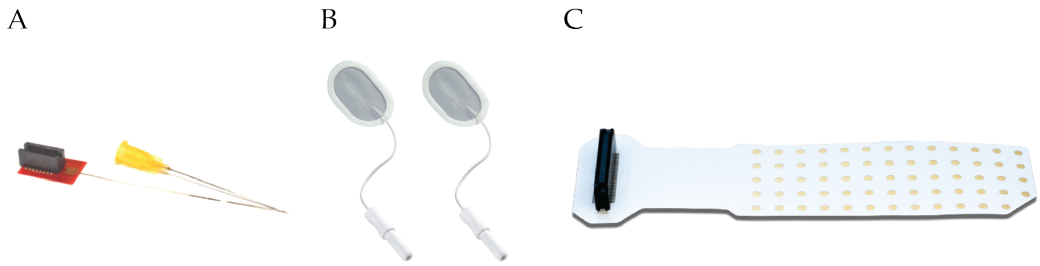

FIGURE 1.3: Examples of EMG electrodes used throughout the studies presented in this PhD dissertation. A. Intramuscular electrode. B. Surface electrodes. C. hdEMG grid.

As an alternative to traditional large sEMG electrodes, the use of arrays or matrices of electrodes, namely as high-density EMG (hdEMG) is becoming more popular in research (Figure 1.3-C). By means of increasing the spatial sampling with surface multichannel recordings and the application of advanced blind source separation algorithms, it is possible to decompose the EMG signal into MUAPs firings (Holobar et al., 2007). Consequently, hdEMG allows non-invasive estimation of the neural drive and the motor unit properties, despite the fact that the estimation cannot assume a complete representation of the motoneuron pool, since only a reduced number of

the total number of existing motor units is identified (Merletti et al., 2008). Additionally, due to the electrical distribution of the MUAPs across the tissues and the skin, the application of hdEMG decomposition algorithms has been mostly constrained to submaximal isometric tasks (Farina et al., 2014a). In spite of these limitations, through the decomposition of hdEMG in composite spike trains of MUAPs, it is possible to extract several features of individual and groups of motor units across different experimental conditions, such as the firing rate, the conduction velocity of the muscle fibers, and other descriptors of motor control as intramuscular or corticomuscular coherence (Avrillon et al., 2021).

1.2.3 The spinal cord

It is deeply known that the spinal cord is a complex structure with neural processing functions, not being limited to the containment of 31 descending and ascending pathways (Kandel et al., 2013). Indeed, the neurons at the spinal cord comprise the first and last computational layer of the CNS for afferent and efferent information, respectively. Anatomically, it is divided into four different longitudinal areas (cervical, thoracic, lumbar and sacral) containing the innervation of the different body parts. In the axial plane, the spinal tracts are separated in different sections of white matter, whereas the grey matter contains the efferent motoneurons and interneurons, grouped in the ventral horn; and the afferent or sensory fibers grouped in the dorsal horns (Peters A, 1991). In the previous description of the CNS, the fundamental role of sensory feedback to achieve functional motor control was emphasized. This principle is latent as well in the spinal cord through the spinal reflexes, the most primitive motor responses triggered by afferent signaling. Essentially, spinal reflexes are fast and patterned movements with few neural processing as a response to certain afferent information from periphery receptors (Pierrot-Deseilligny E., 2012). Notwithstanding, reflex responses are not limited to the spinal cord since the afferent pathways do not synapse only to spinal motoneurons, but also to supraspinal circuits and long-latency reflex loops (Wolpaw, 2007). Additionally, the spinal reflexes are modulated by supraspinal inputs to particular motor tasks, creating adaptive responses to specific situations (Marsden et al., 1981). The interneurons located in the spinal cord play a key role in neuromodulation of afferent information, projecting it to the brain or directly to the spinal motoneurons, and therefore adjusting the excitatory/inhibitory balance of specific pools of motor units of the same or different group of muscles (Fetz et al., 2000).

Ia fibers and the stretch reflex

The stretch reflex is the simplest instance of a spinal reflex, and despite its simple monosynaptic mechanism, it is a fundamental circuit involved in motor control. Ia afferent fibers drive sensory information about the muscle fibers length from the muscle spindles to the homonymous motoneurons (Liddell et al., 1924). When the muscle fibers are suddenly stretched, for example due to a tendon jerk, the Ia fibers activate the motoneurons to elicit the contraction of the muscle and prevent it from over lengthening and potential damage (Renshaw, 1940) (Figure 1.4).

Muscle spindles, which are inserted at the non-contractile central region of the intrafusal fibers, are the receptors sensing the change of length of muscles and connected to the Ia fibers (Hunt et al., 1951). When the extrafusal muscle fiber is stretched, the spindle is also stretched and increases its firing rate, signaling a change of muscle length closely related to the position of the muscle in relation to the joint.

Gamma motor neurons innervate the intrafusal fibers and control the sensitivity of the spindles to length variations by means of contracting the ending of such fibers. It is worth noting that the intrafusal fibers contraction is not relevant to the final force exerted by the muscle. Muscle spindles and Ia fibers are particularly sensitive to both fast and small changes in muscle length in order to provide accurate proprioceptive feedback to the CNS (Prochazka et al., 1988).

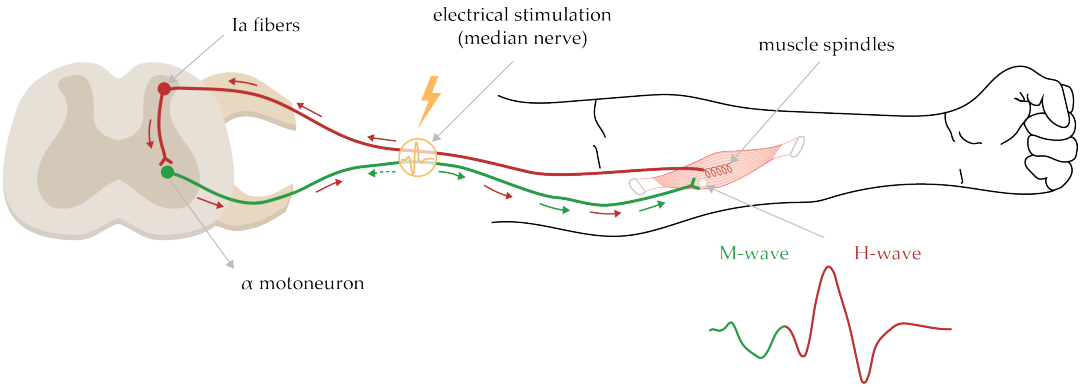

FIGURE 1.4: Illustration of the simplified stretch reflex spinal pathway. Arrows represent the direction of the action potential propagated through the afferent (red) and efferent (green) axons after the electrical stimulus is applied. The typical M and H waves elicited due to the direct and reflex motor responses are depicted in green and red lines, respectively.

The physiological response of the stretch reflex can be evoked via electrical stimulation of the Ia afferent fibers, bypassing the sudden muscle lengthening and giving rise to the H-reflex (Burke, 2016). Thus, an electrical stimulus applied with an intensity above the recruitment threshold of the Ia fibers causes an excitatory postsynaptic potential (EPSP), propagated towards the homonymous motoneuron through monosynaptic connection and eliciting an action potential which will ultimately elicit the contraction of the muscle (Figure 1.4). The evoked electrical potential is recorded with EMG electrodes placed over the target muscle. Although both efferent and afferent fibers often are anatomically grouped in the same nerve, it is possible to recruit exclusively the Ia fibers independently from motor axons since they present larger axon diameter and lower recruitment threshold or rheobase (Lin et al., 2002). Furthermore, the physiological recruitment principle is preserved in the H-reflex: slow motor unites are recruited first, followed by larger motor units while increasing the stimulation intensity (Buchthal et al., 1970). Nonetheless, the electrical stimuli can evoke action potentials in the motor axons, which travel towards the muscle fibers and elicit a motor response, known as M wave. Moreover, the H-reflex is commonly used as diagnosis tool in neurophysiology, since certain neuropathies and motor disorders modify the features of the reflexes compared to healthy subjects (Schieppati, 1987).

By means of the H-reflex assessment technique, it is possible to assess the excitability of certain spinal circuits. A common paradigm implies the use of EMG recordings of the target muscle; electrical stimulation of the nerve containing Ia fibers to elicit the H-reflex (test stimulus); and stimulation of other afferent fibers projecting to the motoneurons of the muscle tested (conditioning stimulus). When both test and conditioning stimuli are synchronously applied, the evoked EPSP or IPSP (inhibitory post-synaptic potential) can converge on the target motoneuron at

the spinal cord, so the specific neural circuit activated can be assessed (Day et al., 1984).

Reciprocal inhibition and disynaptic Group I inhibition

Proprioceptive information from Ia fibers is not only directed to the homonymous muscle to control the monosynaptic stretch reflex. Instead, Ia fibers project with excitatory connections to other motoneurons from heteronymous or synergist muscles, favoring the functional response of the muscle group to the stimulus (Pierrot-Deseilligny E., 2012). On the contrary, for a pair of agonist-antagonist muscles, Ia fibers also make excitatory connection with inhibitory interneurons (named Ia inhibitory interneurons) at the spinal cord projecting on the motoneurons from the antagonist muscle, which is the basis of the reciprocal inhibition principle (Figure 1.5-A). Due to this innervation mechanism, when one of the muscles is stretched, this disynaptic pathway allows the relaxation of the antagonist muscle to favour the movement, a functional motor behavior involved in many tasks (Baldissera et al., 1983). However, Ia fibers and Ia interneurons within the spinal cord are strongly modulated by the activity of supraspinal input and other afferent pathways, which can modify the excitatory/inhibitory balance, reinforcing or depressing reciprocal inhibition (Shindo et al., 1984). Therefore, the output of these spinal circuits is task-dependant and variable across muscle groups. For instance, for most hand muscles, the flexor and extensor muscles controlling the wrist joint do not act as pure antagonists, but as synergist muscles in order to provide joint stabilization and fine control. Admittedly, while the Ia reciprocal disynaptic inhibition between a pair of antagonist muscles is well described in physiological studies for the soleus and tibialis anterior (lower limb), or biceps brachii and triceps brachii (upper limb), for the wrist flexor and extensor muscles there are evidences about the contribution of other neurons rather than disynaptic Ia interneurons, named as disynaptic Group I interneurons (Figure 1.6-B) (Wargon et al., 2006).

Presynaptic inhibition

One of the neural mechanisms contributing to the adaptive behavior of spinal reflexes is presynaptic inhibition. Through a set of circuits at the spinal cord controlled by other afferents, interneurons and descending signals, the output of Ia fibers is modulated prior to reaching the motoneurons, thus, the strength of the Ia input is adjusted to specific tasks in order to optimize motor performance (Figure 1.5-B) (Meunier et al., 1998). Despite the fact that presynaptic inhibition can serve as a similar purpose control strategy to reduce the excitability of the antagonist muscle, the neurons involved, the longer latency and the effect duration distinguish it from reciprocal inhibition (Pierrot-Deseilligny E., 2012).

Ib fibers

The tendon reflex or autogenic inhibition is another well-known spinal circuit aiming at regulating excessive tension on the muscles by eliciting muscle relaxation as a protective response (Tortora G. J., 2017). Proprioceptive information of the muscle tension is signalled by Golgi tendon organs (sensory receptors located at muscle-tendon junctions). Ib afferent fibers coupled to Golgi tendon organs modulate their firing rate directly related to the tension of the tendon and synapse with Ib inhibitory interneurons at the spinal cord (Swett et al., 1975). The predominant effect of Ib reflex pathways entails inhibition of homonymous and heteronymous motoneurons

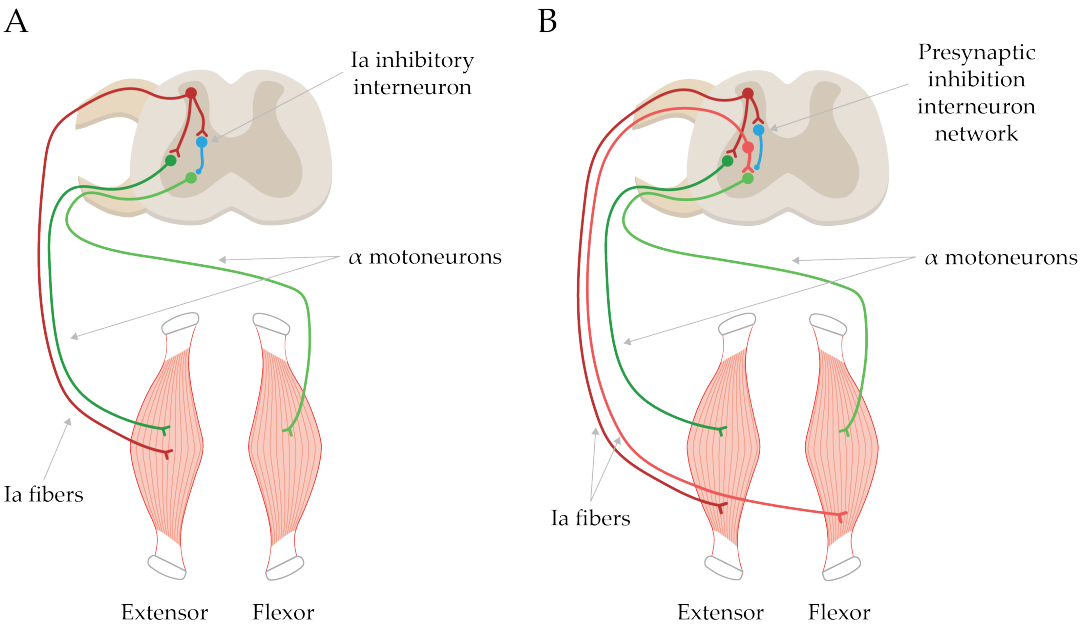

FIGURE 1.5: A. True reciprocal Ia inhibition between a pair of antagonist muscles. B. Simplified Ia presynaptic inhibition circuit.

via disynaptic and trisynaptic connections respectively; and excitation of the motoneurons from the antagonist muscle through a trisynaptic pathway (Figure 1.6-A). However, the arc reflexes are adaptive as described before, thus, Ib inhibitory interneurons are subjected to receiving inputs from supraspinal centers, propriospinal interneurons and other afferent fibers such as Ia or cutaneous (Cavallari et al., 1985). The modulation of interneurons depending on the task might have a deep effect on the spinal pathways, allowing a total reversal of the reflex circuit for given tasks such as locomotion, where the Ib inhibitory disynaptic effect on the homonymous motoneurons is depressed.

Other afferent pathways and the propriospinal system

As a whole, stretch and tendon reflexes operate in coordination in order to achieve fine motor control of each joint for each particular task. Both pathways are mediated by type I fibers, which are the afferent fibers with the largest diameter and the most rapid in terms of transmission velocity. Notwithstanding, other group of afferents from different sensory mechanisms are also involved in motor control at the spinal cord, although their contribution to motor control has not been as deeply described as Group I afferents (Pierrot-Deseilligny E., 2012). Majority of Group II fibers project from secondary muscle spindle endings, providing information about the muscle length, while other group II fibers project from the non-spindle endings being sensitive to deep pressure (Laporte et al., 1959). Group II monosynaptic connections to motoneurons are not strong and their major projections are towards spinal interneurons (Lundberg et al., 1977). Because of the fact that Group II fibers present higher recruitment threshold and are located within the same nerve branch than Ia fibers, there are limited stimulation techniques available to test these pathways in human beings. In addition to the so far described afferents, other sensory pathways such as pain, temperature, chemical stimuli or skin cutaneous afferents have an important role as feedback information to the CNS through spinal reflexes and sensorimotor integration at the brain level (Pierrot-Deseilligny E., 2012).

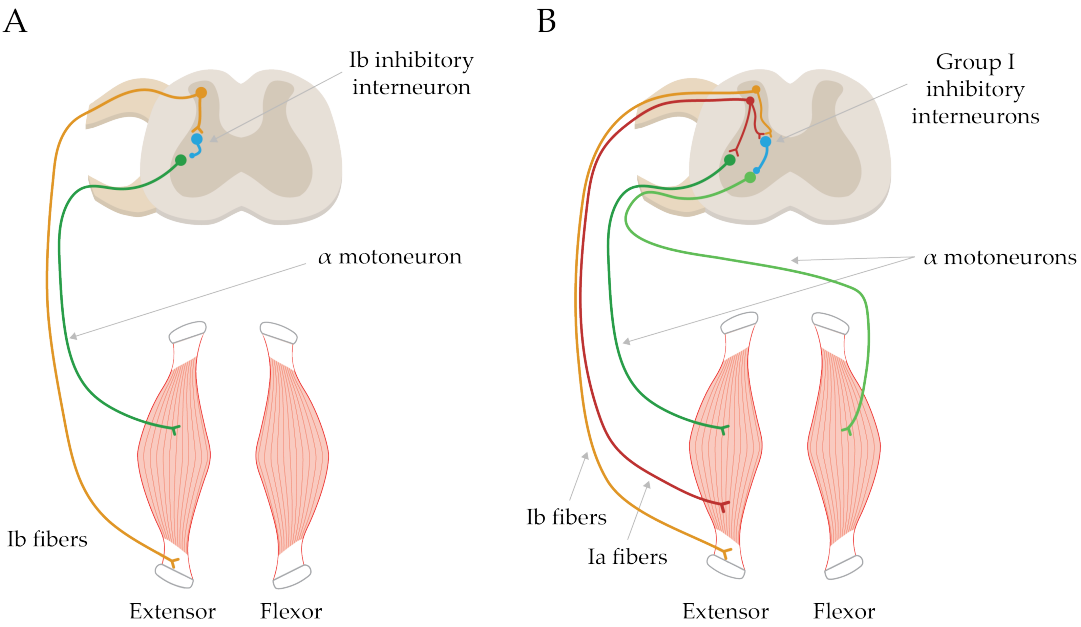

FIGURE 1.6: A. Autogenic inhibitory reflex circuit mediated through Ib fibers. B. Simplified disynaptic Group I inhibitory circuit for the wrist flexor and extensor muscles.

Propriospinal neurons are a set of interneurons located within the spinal cord and are fully connected to other neurons from different layers. The main function of the propriospinal system relies on the integration of sensory signals and supraspinal commands to continuously optimize the control of movements with the most recent proprioceptive feedback information prior to reaching the effector motoneuron (Gracies et al., 1991). Particularly, in the upper-limb there are evidences of the implication of the cervical propriospinal system in the transmission of cortical signals towards the motoneurons (Pierrot-Deseilligny, 1996). Propriospinal interneurons receive inhibitory projections from different peripheral afferents such as Group I, Group II and cutaneous fibres which can depress the excitatory input from supraspinal commands, then allowing a selection of the relevant motoneurons required for the movement and fine tuning of the speed and force of the movement (Stinear et al., 2004).

1.3 Pathophysiology of tremor

The clinical definition of pathological tremor refers to involuntary movement of a body part caused by abnormal neural commands projected to the neuromusculoskeletal system (Shanker, 2019). The frequency of the oscillations ranges from 4 to 9 Hz, showing variability across different neural disorders and subjects (Lenka et al., 2021). Pathological tremors, commonly referred to as tremor, should not be confused with physiological tremors, which are considered involuntary movements as well, yet they typically oscillate in the frequency band between 8 and 10 Hz and are present with a low amplitude in healthy individuals (McAuley et al., 2000). Additionally, physiological tremors do not present an aberrant neural activation of supraspinal centers, neither do they lead to alteration of ADL.

Tremor is traditionally related to a dysfunction of the basal ganglia, the cerebellum and other structures projecting to these systems such as the inferior olive. The subthalamic nucleus stands as a key structure involved in tremor generation

or propagation by connecting the basal ganglia with the cerebellum (Helmich et al., 2013).

1.3.1 Essential Tremor

ET is the main disorder producing pathological tremor and the most common motor disorder in the world (Bhatia et al., 2018). Despite its wide incidence and social impact, there is no scientific consensus about the etiology and physiopathology of this disease. Clinically, ET is defined as "isolated tremor syndrome of bilateral, upper-limb action tremor of at least 3 years' duration, with or without tremor in other locations (e.g., head, voice, or lower limbs), and absence of other neurological signs, such as dystonia, ataxia, or parkinsonism" (Louis et al., 2020b). Therefore, upper-limb action or kinetic tremor is the main manifestation of ET, and it is present in around 95% of patients (Louis, 2013). Other tremor manifestations, such as intention, rest, lower-limb, voice or head tremors are not common in all the ET patients, however, longer duration of the disease typically favors the development of these symptoms (Shanker, 2019). Additional symptoms derived from ET are hearing loss, mild cognitive impairment and balance difficulties. Psychiatric symptoms are often reported in ET patients, particularly, depression, anxiety, sleep disturbances or fatigue (Shanker, 2019). The severity of tremor is highly variable across patients and along the course of the disease. It is also known that emotional factors contribute to tremor exacerbation or mitigation out of control of the patient. In addition, the consumption of some drugs such as nicotine, increases tremor, while alcohol intake often reduces tremor (Shiffman et al., 1983). Despite the variety of clinical manifestations found in ET, tremor is the main symptom which deeply impairs the normal life of the patient. Common daily activities such as cooking, eating or drinking are compromised by the tremor oscillations of the body limbs.

The main diagnosis tool for ET is clinical assessment based on medical records and exploration. Recent discussion on the Movement Disorder Society lead to the definition of a new concept: essential tremor plus (ET-plus) (Louis et al., 2020b). Patients who are diagnosed with ET, primarily due to the presence of upper limb postural or kinetic tremors, commonly show additional clinical manifestations such as balance, gait impairment or mild cognitive impairments. This heterogeneity in clinical symptoms, and the shortfall of pathological evidence and biomarkers imply ET might not be a single disorder (Shanker, 2019). Considering ET is likely to be a heterogeneous disorder, two main hypotheses are considered to be the pathophysiological cause of ET:

Neurodegenerative hypothesis

Some authors support the hypothesis that ET is neurodegenerative disease, strongly associated with genetic factors and a progressive course with age (Deuschl et al., 2009). Moreover, an increased risk of developing other neurodegenerative disorders such as PD or Alzheimer's Disease is associated with ET (Elble et al., 2007). Most of the evidence gathered from the few pathology studies published converge to ET as a cerebellar disease. A decrease in cerebellar Purkinje cells was the main finding in ET patients compared to control subjects (Axelrad et al., 2008). Additional evidence suggests that neurodegeneration of the locus coeruleus is associated with ET (Shill et al., 2008), but no further strong evidence have been reported for other neural structures. However, other authors suggest these findings might not be the cause of

tremor, instead, they suggest it might be the attributed to the neural damage caused due to prolonged abnormal dynamic oscillations.

Non-degenerative hypothesis

Following this premise, ET is not considered a primary neurodegenerative disease, but a dynamic oscillatory disturbance caused by both genetic or non-genetic factors, which, in theory, could be reversed if properly addressed before neural damage is caused (Rajput et al., 2012). Among the plausible causes for this disturbance, the alteration of the inhibitory neurotransmitter GABA stands as the main candidate due to several evidence. The deep cerebellar nuclei neurons receive inhibitory GABAergic input from Purkinje cells and project into the thalamocortical circuit. Some of the most effective drug treatments in reducing tremor in ET (primidone, gabapentin or ethanol) increase GABAergic transmission (Deuschl et al., 2001). Besides, some patients show a reduction of GABA in the cerebellum and the locus coeruleus. The hypothesis states that a depletion of GABA receptors could induce abnormal hyperactivity in the cerebello-thalamo-cortical circuits contributing to tremor oscillations.

Both hypotheses, although different, are not reciprocally exclusive, and they coincide in the alteration of cerebello-thalamo-cortical circuits as the common source of tremor in ET (Figure 1.7) (Helmich et al., 2013). Along with the ET-plus concept, a classification with three subtypes of ET has been proposed in order to explain some of the clinical differences among some subgroup of patients. This classification group comprises Hereditary ET, Sporadic ET and Senile ET. Tremor is known to have a marked hereditary component (Tanner et al., 2001), although epigenetic factors contribute to the final development of the disease, which must occur before age 65 to be considered a Hereditary ET diagnosis. Senile ET is defined as the subgroup for patients developing ET after age 65. Finally, Sporadic ET is the classification for the subgroup of patients who do not have immediate familiar history of ET (Deuschl et al., 2009).

Neither consensus has been reached in relation to whether there is a single primary tremorgenic oscillator or instead, several structures of the network cooperate and act as dynamic oscillators. In recent years, this later hypothesis is raising evidences against the single oscillator model (Raethjen et al., 2012). Lesions or stimulation of different structures at the cerebello-thalamo-cortical pathways share a common result in the reduction of tremor, arguing in favor of the presence of a complex oscillating network.

The role of the spinal cord in Essential Tremor

In ET pathophysiology, the cerebellum, the basal ganglia, the inferior olive, the red nucleus or the Guillain-Mollaret triangle are structures involved in the tremor oscillatory network (Deuschl et al., 2009; Haubenberger et al., 2018). The ultimate alternating activation of antagonist muscles producing mechanical tremor oscillations is not fully explained by the sole contribution of corticospinal inputs. Thus, some studies have suggested that the supraspinal input to the motoneurons projecting to the muscles are not the only contributors to the tremorgenic oscillations at the effector muscles (Figure 1.7) (Deuschl et al., 2001).

The study of the coherence between the motor unit firings at the wrist flexor and extensor muscles and the cortical activity measured through EEG in ET patients showed a common cortical input to the motoneurons at the tremor frequency (Gallego et al., 2015b). However, the magnitude of the coherence was uncorrelated with

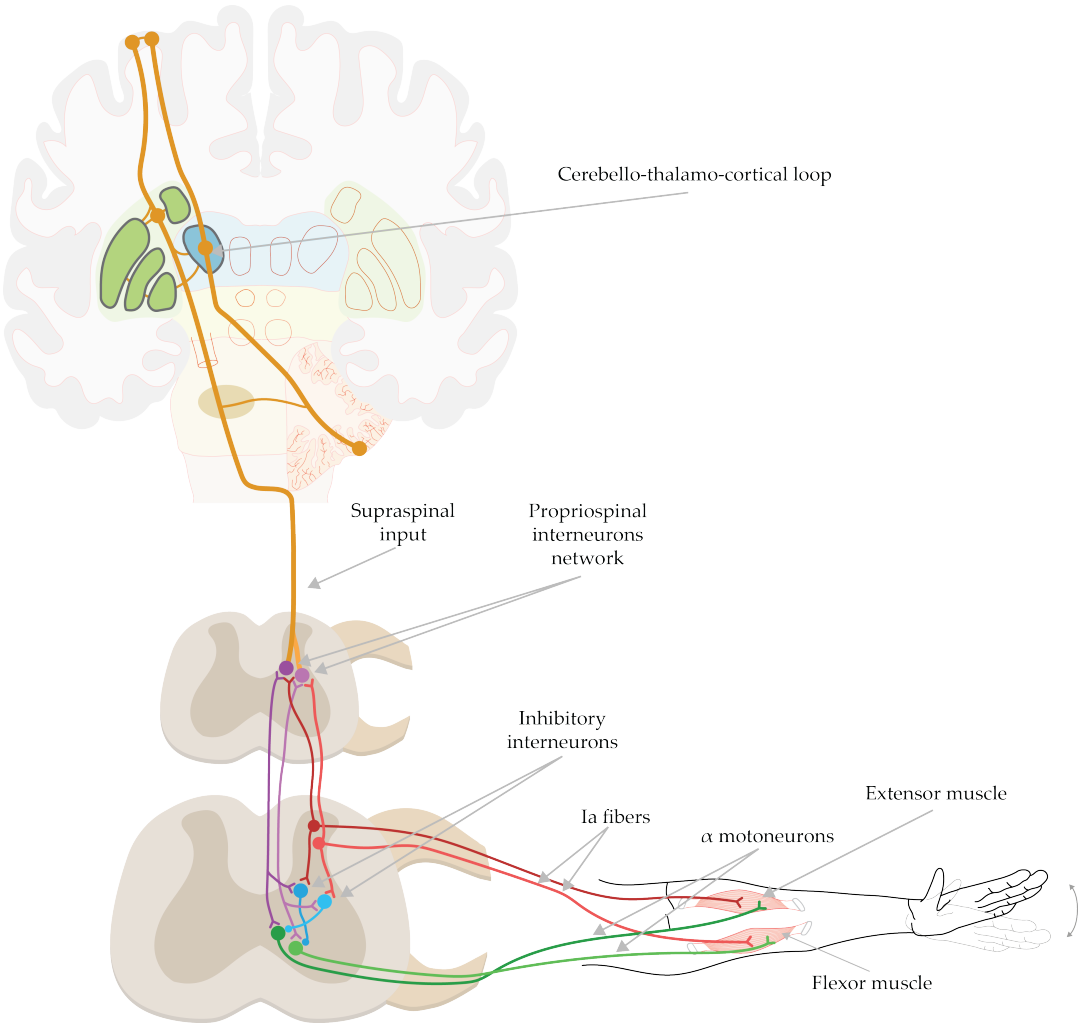

FIGURE 1.7: Simplified schematics of the anatomy of pathological tremor. Tremorgenic oscillatory signals are generated within the cerebello-thalamo-cortical loop and transmitted towards the spinal cord. According to some studies, tremorgenic signals are amplified and maintained at the spinal cord through the propriospinal and other spinal reflex pathways such as reciprocal inhibition. The final tremorgenic signals are transmitted to the muscles causing the mechanical oscillations.

the strength of the supraspinal input, which suggests the existence of other inputs to the motoneuron pool contributing to tremor (Gallego et al., 2015a). A later study confirmed that a pair of antagonist muscles share common tremorgenic supraspinal input, but the phase difference in tremor activation bursts is associated to the relative strength of that input in addition to the contribution of afferent pathways. Ia afferent fibers, responsible for driving the length muscle information from the muscle spindles, were proposed as contributors to the tremor oscillations through the monosynaptic reciprocal inhibition pathway (Puttaraksa et al., 2019).

The role of the spinal cord circuitry in motor control is not completely understood. Propiospinal neurons are a group of interneurons interconnecting different segments across the spinal cord, receiving excitatory and inhibitory inputs from supraspinal structures, but also from afferent fibers (Alstermark et al., 2007). As a complementary hypothesis to the Ia afferent fibers implication in the contribution

of tremorgenic oscillations at the muscle level, propiospinal neurons have been suggested to be responsible for the alternating tremor bursts in pairs of antagonist muscles (Hao et al., 2013). According to this model, the transmission of cortical tremorgenic activity would reach the motoneuron through the monosynaptic corticospinal tract, as well as through oligosynaptic pathways via the propiospinal interneurons. This propiospinal system, also fed by afferent inputs would contribute to the final tremor oscillations projected to the muscles.

1.4 Statement of Need and Impact

1.4.1 Impact in the socio-economic environment

ET is a motor disorder which might severely condition the execution of ADL. Additionally, the visual effects of tremor combined with a disabling condition may cause social stigma for the patients and lead to a deterioration in their mental health. Thus, the consequences of this disorder are both physical and psychological. On the other hand, due to the strong relation between tremor and age, in addition to its large incidence, pathological tremor leads to great economic impact in society when considering the healthcare systems and the care delivery to disabled patients. By understanding how the tremor components are generated at the CNS and how they can be managed from the periphery, it might be possible to develop non-invasive and cost-effective solutions as alternatives to existing therapeutic options. PES has the potential to become a transformative approach for tremor reduction interventions. Compared to medication, which might have broad bodily and CNS effects, and to more invasive approaches such as DBS or High Intensity Focus ultrasound (HIFU), PES allows more precise delivery of the intervention through closed-loop control strategies and can also be discontinued at any time. In the long term, the research performed in this thesis is expected to set the basis for the development of wearable devices capable of delivering customized PES therapy in the home setting. This might have a significant positive impact in the quality of life of individuals affected by tremor and their relatives, as well as a significant positive impact in the healthcare system.

1.4.2 Contribution to the research field

Despite the large worldwide incidence of ET, the mechanisms underlying tremor are not fully understood. This research has the potential to contribute to our understanding of neurological disorders causing tremor, demonstrating that PES of afferent pathways may induce short-term and long-term neuroplasticity and adaptations at the CNS level. By means of characterizing the induced neuromodulation, evidence of some of the neural circuitry involved in tremor generation could also be created. This knowledge will not only be useful for research on ET, but it will also contribute to the advance of neurophysiology and motor control understanding. Furthermore, providing evidence about the use of PES of afferent pathways as an effective neuromodulation technique when timely applied with physiological events might be applicable to other motor disorders presenting a misbalance in an excitatory/inhibitory circuit, such as spasticity or clonus, which can potentially benefit from this non-invasive approach via modulation of the affected pathways towards physiological or functional state.

Finally, the development of neurorehabilitation techniques should be combined with neurophysiological and kinematic studies that provide information on the

changes produced at the neuromusculoskeletal system. During the research performed for this PhD thesis, a wide array of techniques was applied in order to characterize the physiological and functional status of healthy and ET populations: kinematics assessment, neural drive estimation, clinical rating scales, and reflex loops assessments. A key technique explored through this work was the assessment of reciprocal inhibition during voluntary muscle activity to monitor the status of the afferent pathways and spinal circuitry.

1.5 Goals and hypotheses

This doctoral thesis is substantiated by the main hypothesis that PES can be applied to selectively modulate some afferent pathways involved in the regulation of motor control in order to disrupt involuntary tremor oscillations in ET patients. A stepwise approach has been designed to achieve individual objectives that will provide a comprehensive framework about the characterization and use of PES as a neuromodulation tool suitable for the reduction of pathological tremor.

Goal 0. Complete a comprehensive review about the state of the art of PES as a tremor reduction tool. Deepening the knowledge regarding the technical, experimental and physiological aspects of previous research will allow a better understanding of the breakthroughs, limitations and future trends of this technology, which ultimately may be used to design, implement and validate alternative tremor reduction strategies in ET patients.

Hypotheses 1. *PES of the afferent pathways can acutely inhibit the voluntary muscle activity of the antagonist muscle for the wrist flexor and extensor muscles in healthy participants.*

Goal 1.1. Validate and master the electrophysiological techniques of PES and neurophysiological assessment of the CNS in the upper-limb, which will be used over the course of this PhD dissertation.

Goal 1.2. Demonstrate that PES below motor threshold can be successfully applied to reduce the contribution of voluntary neural drive of the antagonist muscle through spinal reflexes as a preliminary step towards its application in tremor reduction strategies.

Hypotheses 2. *Selective and adaptive timely stimulation of afferent pathways induces short-term neuromodulatory effects on the spinal circuits controlling the wrist muscles in healthy subjects.*

Goal 2.1. Develop a closed-loop PES strategy capable of delivering specific electrical stimuli synchronized with the muscle activity estimated through EMG.

Goal 2.2. Characterize the short-term neuromodulation effects at the spinal cord level induced by phase-dependent stimulation strategy applied in-phase and out-of-phase with the neural drive in healthy subjects.

Hypothesis 3. *Selective and adaptive timely stimulation of afferent pathways applied with intramuscular electrodes induces acute and short-term tremor reduction in ET patients.*

Goal 3.1. Demonstrate that timely stimulation of the afferent pathways with the physiological activity is a fundamental principle to elicit tremor reduction mechanisms at the CNS.

Goal 3.2. Prove the safety and efficacy of thin-film intramuscular electrodes to bi-directionally interface the nervous system and reduce tremor as a preliminary step towards the development of minimally-invasive neuroprostheses.

1.6 Document organization

This doctoral thesis is organized as follows:

Chapter 1 introduces the health problem of essential tremor that motivates the research work performed in this thesis. Additionally, an overview of the main physiological principles and research techniques involved in this work is provided. Finally, the hypothesis and goals for each of the stages are presented.

Chapter 2 provides an extensive review of the state of the art on PES to reduce pathological tremor. Throughout this chapter, the methods, physiological principles and results published in literature are revisited in order to provide a deeper comprehension of the current status of PES. The knowledge gathered in this review serves as the cornerstone for developing and testing stimulation strategies in the following chapters.

Chapter 3 presents the first of the studies performed in this thesis. The acute inhibitory effects of the stimulation of afferent pathways on the antagonist muscle for the wrist flexors and extensors are characterized in a cohort of healthy subjects. This work serves as an initial step towards the design of tailored PES strategies for the mitigation of tremor.

Chapter 4 comprises the second study conducted in this work. Here, a novel closed-loop PES strategy named as Selective and Adaptive Timely Stimulation (SATS) is presented. The potential neuromodulatory capabilities of SATS is explored through assorted neurophysiological assessment tools which are used to characterize the neural adaptations induced after two phase-dependent SATS interventions in healthy subjects.

Chapter 5 presents the third and last research stage of this thesis in which different strategies of PES of afferent pathways were tested in ET patients in order to characterize their tremor reduction effects. The achieved results validate the use of PES (and SATS, specifically) to reduce pathological tremor acutely and in the short-term, emphasizing the timely synchronization of the stimulation with the tremorgenic activity, and the potential of intramuscular electrodes to robustly interface with the human body.

Chapter 6 summarizes the conclusions of this thesis through the different studies and revisits the goals and hypothesis stated in Chapter 1. This chapter additionally provides suggestions for future research in the field and overviews the scientific contributions arisen from this thesis.

Chapter 2

Peripheral Electrical Stimulation for the management of tremor

The contents from this chapter have been previously published in the following journal articles, in which the author of this PhD dissertation contributed as first author:

Pascual-Valdunciel, A.*, Hoo, G*. W., Avrillon, S., Barroso, F. O., Goldman, J. G., Hernandez-Pavon, J. C., Pons, J. L. (2021). Peripheral electrical stimulation to reduce pathological tremor: a review. Journal of Neuroengineering and Rehabilitation, 18(1), 33.

Pascual-Valdunciel A., Rajagopal A., Pons J. L., Delp S. (2022). Non-Invasive Electrical Stimulation of Peripheral Nerves for the Management of Tremor. Journal of the Neurological Sciences, 435, 120195.

2.1 Abstract

Approximately 50% of patients suffering from ET do not receive any effective treatment due to significant side effects, decline in effectiveness over time, or clinical contraindications for both of clinical standard treatments: pharmacotherapy and neurosurgical interventions. Thus, a need has been identified: an effective, affordable and extensible treatment to suppress tremor. Peripheral electrical stimulation (PES) poses as an alternative solution for tremor reduction, but further research is required to gather evidences of the effectiveness and wide application of this tool. The studies using this technology on those with tremor claim that its adverse effects are minimal compared to orthotic devices or the current clinical therapeutic options, and therefore, it has the potential of becoming an extensible effective treatment. A comprehensive and exhaustive review of the state-of-the-art tremor reduction solutions, specifically in PES, was performed. A total of 29 studies were included in the analysis according to inclusion criteria. Two main PES approaches were identified: Functional Electrical Stimulation (FES) and stimulation of afferent pathways. Several methodological aspects of the studies were revisited: stimulation strategies, stimulation parameters, electrodes, experimental designs, reported results and tremor reduction hypotheses. The fundamentals of PES as a technique to reduce tremor were unveiled. High variability across the analyzed studies in relation to the stimulation strategies, study designs or results was identified. Drawing from the existing evidences, a standardized and more detailed methodology for the future studies is suggested as a must-have to assess the real potential of the different PES strategies to be translated from bench to bedside. Limitations, advantages and future steps of

PES are pinpointed in order to set the guidance for the development of a minimally-invasive and economic solution to manage tremor in a broad population with the potential to transform our understanding of tremor generation and reduction.

2.2 Goals

The purpose of this chapter is to provide a comprehensive review on the state of the art of PES as a solution to reduce pathological tremor. Reviewing in detail the previous research on the use of PES is a fundamental preliminary step to understand the capabilities and limitations of PES, to further design, implement and test PES-based tremor reduction strategies with more robust technical and physiological background.

2.3 Introduction

Currently, there is no cure for ET. Moreover, the available solutions to manage ET are therapeutic approaches that only treat the symptoms. The partial understanding of ET physiopathology, not fully determined, hampers the development of specific drugs targeting the cellular mechanisms implied in the abnormal involuntary and cyclic firings (Shanker, 2019). The course of the disease typically starts with mild symptoms which do not interfere severely with Activities of Daily Living (ADLs), and therefore, a wide population of patients decide not to receive any treatment during this period. While the disease progresses, tremor often interferes with ADLs, and patients seek medical treatment. Pharmacological treatments have been the first line of treatment for the last few decades. Secondly, surgical interventions are considered for patients who are non-responsive to drugs. Finally, there is a growing interest in developing alternative solutions to pharmacological and surgical procedures based on mechanically devices or minimally-invasive stimulation of the central and/or peripheral nervous system (Chalah et al., 2015).

2.3.1 Pharmacological therapies

Oral medication is the first therapeutic option to reduce tremor. Limited efficacy in symptoms relieving and undesired side effects are the main drawbacks of the drugs developed so far. It has been estimated that around 50% of ET patients do not receive any effective treatment (Louis et al., 2010). The combination of multiple drugs to optimize the tremor reduction is a common practice. However, although efficacy might be increased, the adverse effects and the development of tolerance remain as limiting factors. There is still a need of development of new drugs based on the later findings on physiopathology of tremor generation in ET, particularly aiming at the cellular mechanisms involved in the progression of the disease, and not only targeting symptoms relief.

Beta-Blockers

Drugs referred to as beta-blockers are competitive antagonist of adrenaline and noradrenaline, acting by blocking the receptor sites for both neurotransmitters of the sympathetic nervous system (Tamargo et al., 1990). Beta-blockers are commonly used in the clinical practice to treat cardiovascular disorders, such as hypertension, but when applied in ET, it is thought that beta-blockers act by reducing

tremor at peripheral site. Propranolol is one of the first and main medications prescribed to attenuate tremor. The overall efficacy has been averaged approximately at 50% of hand tremor amplitude reduction, while common side effects are bradycardia, fatigue, depression or sexual dysfunction (Ondo, 2020). Tremor reduction efficacy is dose-dependent, and typically starts to have an effect 1 hour after intake. Other beta-blockers have different efficacy rates and adverse effects across patients, although non-responsive patients to propranolol will not respond to other beta-blockers (Shanker, 2019).

Nervous system depressors

Several drugs with sedative, anxiolytic or depressive effects on the nervous system have been used to manage tremor. Primidone, along together with propranolol, are the first pharmacological options prescribed to manage ET due to their proved efficacy. Adverse effects commonly described are fatigue and malaise, which lead to some patients discontinuing the treatment. Gabapentin and Topiramate are other common drugs used in ET management whether the tremor reduction outcomes or adverse effects of propranolol or primidone are not suited to the patient (Shanker, 2019). Primidone, Gabapentin and Topiramate are illustrations of drugs involved in GABAergic transmission and a consequent reduction in tremor, which support the hypothesis of the alteration of this pathway as a potential tremor source (Helmich et al., 2013). Benzodiazepines and antipsychotics are additional treatment options often used in the ET management with moderate efficacy (Ondo, 2020).

Botulinum toxin

Beside oral medication, botulinum toxin injections have been a common solution to reduce refractory tremors during the last 20 years (Shanker, 2019). Botulinum toxin and derived toxins act as acetylcholine inhibitors, reducing the neurotransmitter release at the neuromuscular junction and therefore preventing the muscle contraction. When botulinum toxin is injected in the tremorgenic muscles, the muscle is weakened, thus the tremor input is attenuated. This approach is strongly dependent on the targeted muscles, being typically more effective for hand or head tremor rather than proximal or finger tremor (Ondo, 2020).

2.3.2 Surgical procedures

The progress of ET through years might yield to a rise in the tremor amplitude and affected joints. As tremor severity increases, pharmacological therapies turn less effective in relieving symptoms and fail in improving the quality of life of the patient. In addition, there is a considerable number of ET patients who do not respond to medication or do not tolerate the side effects. Over the last few decades, surgical interventions have become a solution for these populations, showing higher effectiveness compared to current medication options.

Deep Brain Stimulation

DBS is an invasive non-destructive treatment based on the application of electrical stimuli through microelectrodes targeting brain structures in order to disrupt the tremorgenic oscillatory network and therefore prevent the tremor input to be projected to the corticospinal circuit. The electrode placement involves a stereotactic

surgical procedure, in which a craniotomy is performed to implant the microelectrodes at the target structure, requiring high spatial accuracy to maximize the outcomes and minimize the side effects.

Despite its widely proven efficacy and its standardization during the last 20 years, the mechanisms underlying tremor reduction caused by DBS have not been fully clarified. Unilateral or bilateral thalamic ventral intermediate nucleus (VIM) stimulation is the most extended and tested target for stimulation, reporting contralateral tremor reduction of over 68% for up to 100% of patients (Ondo, 2020). The underlying hypothesis states that high-frequency stimulation of the VIM induces selective neuronal inhibition, allowing the uncoupling of cerebello-thalamo-cortical oscillations responsible for tremor (Milosevic et al., 2018). Tremor reduction efficacy often decreases with time, possibly associated to stimulation tolerance, progression of the disease or suboptimal electrode implantation (Ondo, 2020). DBS is non-exempt of side effects and comorbidities. Dysarthria, paraesthesia, dystonia, ataxia, weakness or balance disturbance are common side-effects, which might be attenuated by adjusting the stimulation parameters. Additional complications derived from intervention are intracranial bleedings, stroke or depression. Around 25% of DBS procedures suffer hardware-derived complications, and mortality rate after 30 days of the intervention is estimated at 0.4% (Voges et al., 2007). DBS is an invasive but reversible approach with impressive outcomes for the majority of patients compared to traditional medication. However, the number of patients eligible for this procedure is very low, since they must meet strict inclusion criteria, such as manifesting incapacitating tremor, intolerance or non-responsiveness to medication, be under 80 years of age, and have a suitable health condition in order to undergo a moderate-risk neurosurgery.

Thalamotomy

Prior to the DBS approval as a clinical procedure to manage tremor, thalamic lesioning was the main therapeutic intervention for those among ET patients with disabling tremor who could not benefit from the effects of medication. The principle of this kind of intervention relies on the ablation to destroy a neural structure at the thalamus to disrupt the oscillatory network and prevent the tremorgenic commands from reaching the neuromusculoskeletal system (Dallapiazza et al., 2019). This basis is shared by the DBS approach, as well as the preference for the VIM as the most common targeted structure. Contrary to DBS, thalatomies are non-reversible procedures, which commonly develop into a higher probability of suffering uncontrolled adverse effects.

Stereotactic Radiofrequency Thalamotomy was the first widely used thalamic lesioning technique, consisting of using radiofrequency energy to selectively ablate the target. The development of alternative procedures with higher spatial resolution and diminished risks are leading to the discontinuation of this technique in the last recent years (Ondo, 2020). Gamma knife thalamotomy is based on the use of external gamma radiation focused on the VIM and guided via stereotactic and imaging surgical procedures. Several studies have been reporting great benefits in long-term tremor reduction comparable to DBS. However, other studies have found reduced efficacy and higher risks (Lim et al., 2010).

Finally, high-intensity focused ultrasound (HIFU), also known as MRI guided high intensity focused ultrasound (MRIgFUS), is the newest surgery approach for tremor reduction. This technique exploits the use of directional ultrasound waves towards the target area, commonly the VIM, raising the temperature of the area to

induce a permanent lesion. The use of MRI allows great spatial resolution, estimated around 1mm, resulting in better outcomes and limited size effects compared to previous thalamotomy procedures (Dallapiazza et al., 2019). The number of clinical studies reporting the overall efficacy of HIFU is still limited, but current evidence indicates it can be similar to DBS. The main advantage of HIFU is that it is a minimally-invasive procedure, it is cost-effective and a wider population can be eligible for this treatment compared to DBS. The main downsides of this approach are the limited evidence of long-term side effects of this non-reversible intervention, and a decrease in tremor reduction efficacy over the years, probably due to inaccurate targeting and a reduction of the lesion area (Shanker, 2019). Overall, the widespread application of thalamotomies is low, probably due to a compendium of factors. The surgery, which has associated risks, combined with the irreversible and uncertain side effects caused by the destruction of a neural structure, prevents its extension to ET populations with tremor that hampers ADL but that are not be considered completely disabling.

2.3.3 Alternative solutions

In recent years, there has been a rising research on alternative tremor reduction solutions aiming at overcoming current pharmacological and surgical intervention outcomes.

Non-Invasive CNS Stimulation

Several neuromodulation techniques have been proposed and tested as potential tools to reduce tremor by disrupting the oscillatory central network involved in the propagation of tremor signals. These techniques use magnetic or electrical energy to induce currents which aim at shifting the excitability of the network towards a normal physiological state. Diverse structures of the CNS accessible to non-invasive stimulation and involved in the tremorgenic network, such as the motor cortex, cerebellum or spinal cord have been targeted.

Transcranial Magnetic Stimulation (TMS) consists of the application of high-intensity electrical current through a special-shaped coil placed over the target structure, which produces a magnetic field, which in turn induces small currents within the neural populations and produces either changes of excitability or the actual firing of some neuron populations. The application of several pulses of TMS, known as repetitive TMS (rTMS), has been used to modulate some neural networks and reduce the tremor, not only in ET patients, but also in other disorders producing tremor such as PD (Taib et al., 2019; Shih et al., 2017). The reduced number of studies (<10), the shortage and disparity of reported efficacy, and the discomfort caused by rTMS to some patients prevent these techniques from becoming an alternative therapy for ET populations at the present time (Chalah et al., 2015).

Transcranial direct or alternate current stimulation (tDCS, tACS) are based on the delivery of low-amplitude electrical currents applied with surface electrodes inducing excitability changes in the targeted areas. Compared to rTMS, very few studies have explored this technique in ET or tremor reduction, with results being heterogeneous and not-conclusive. It is worth mentioning the study conduced by Schreglmann et al., 2021, in which the reduction of tremor for some patients was correlated with the stimulation phase applied over the cerebellum, showing that timely stimulation is a fundamental condition to achieve effective disruption of the oscillatory network.

Spinal cord stimulation (SCS) to reduce tremor aims to alter the excitatory balance at the spinal cord and the recruitment of afferent pathways reaching the supraspinal tremor network (Lamy et al., 2021). Epidural SCS implies a surgical procedure to implant epidural electrodes at the target spinal segment, increasing recruitment selectivity of the potential fibers interfering with the tremor circuits (Barroso et al., 2019a). The number of studies researching this technology in ET populations is even lower compared to other non-invasive CNS stimulation techniques, hence, nowadays it cannot be considered an evidence-based candidate solution for tremor management.

Wearable orthoses

The tremor reduction solutions described so far shared the common principle of actively interfacing with the body in order to interfere with the neural commands preceding the mechanical tremorgenic oscillations. On the other hand, tremor oscillations might be attenuated mechanically, i.e., using external devices which counteract or impede the joints to oscillate at the tremor frequency (Mo et al., 2021). It is known that mechanical loading of the tremorgenic limb leads to tremor reduction. Several factors might be considered to explain this behavior, such as the change of mechanical properties of the neuromusculoskeletal system by the increase of inertia and damping (Adelstein, 1981); or a change of the proprioceptive input disrupting the tremor oscillatory network. Following this approach, several wearable devices have been developed and tested, including active or passive orthoses or assistive devices (Castrillo-Fraile et al., 2019).

On balance, current wearable orthoses do not offer an effective solution to reduce tremor in home environments. Prototypes developed so far are still excessively heavy and large-sized to become solutions with broad acceptance among ET patients. An intermediate approach is the development of some assistive devices targeted at reducing tremor in some specific ADLs, such as feeding (Zhu Y, 2014). In addition, no clinical studies with large patient populations have been conducted, thus, real efficacy has not been fully demonstrated. No therapeutic effect has been reported or considered of the daily use of these wearable orthosis, not outweighing the standard clinical and surgical procedures.

Peripheral Electrical Stimulation

In recent years, PES has been considered as a suitable intervention to reduce tremor since this technique might bypass some of the limitations for pharmacological and surgical interventions (e.g., candidacy, side effects, among others), and for other alternative solutions (e.g. usability, efficacy) (Gil-Castillo et al., 2020). PES comprises the application of electrical currents delivered through transcutaneous or percutaneous electrodes to recruit efferent or afferent neural pathway, and it has been used in research and clinical rehabilitation for decades (Gil-Castillo et al., 2020; Resquín et al., 2016; Helm et al., 2021). Given the emergence of PES as a clinical tremor management solution, a review investigating PES interventions for tremor reduction in ET is timely in the field. Therefore, throughout this chapter a review on the PES literature is provided aiming at discussing key elements across the studies such electrical stimulation approaches, stimulation parameters, experimental designs, efficiency in reducing tremor, and hypothesized physiological sources of tremor reduction. Ultimately, limitations and strengths of the revisited studies are identified in order to provide guidance about the development of new PES interventions.

2.4 Methods

A literature search was conducted using four databases: Scopus, Embase, PubMed, and the Institute of Electrical and Electronics Engineers (IEEE) Xplore, from date of database inception until November 8, 2021. The following search query in "title or abstract" fields returned 2830 records: "electrical stimulation" OR "electrical*" OR "nerve stimulation" OR "neuromodulation" OR "muscle stimulation" OR "neuroprosthesis" AND "tremor". 1352 duplicates were removed to yield 1478 records. The following inclusion criteria were applied for this review: (1) full-text journal articles or conference proceedings with complete introduction, methods, results and discussion sections, (2) investigation of any type of peripheral electrical stimulation applied to patients with ET or PD tremor, and (3) descriptions of the level of tremor reduction by means of electromyography, kinematics or clinical scales. Although ET is the main neural disorder targeted in this PhD dissertation, the criteria of including PES strategies focused on PD is explained by the presence of some studies merging both pathologies , and the existence of tremor reduction hypotheses that might be common to pathological tremors. The following exclusion criteria were applied: (1) systematic reviews, books and book chapters; (2) manuscripts describing purely mechanical devices to reduce tremor (e.g., exoskeletons, orthoses, gloves), drug or pharmacological-based treatment, or interventions directly at the brain level (e.g., DBS, HIFU, transcranial magnetic stimulation, transcranial direct current stimulation, transcranial alternating current stimulation, etc.); (3) conference proceedings including case studies with only one participant, or studies completed on only healthy participants; (4) abstracts, posters, conference proceedings, or papers missing clearly described methods, results, or discussion sections; (5) non-English papers; and (6) studies that have been developed as the first contributor by the author of this PhD dissertation.

There were several research groups that published both conference proceedings describing preliminary results and a posterior full-text journal article on the same study; the conference proceedings were excluded from this review and only the journal articles were included. Additional searching was conducted on an as needed basis, with one paper included (Grimaldi et al., 2011). As a result, from 1478 records, 29 full-text studies met the inclusion and exclusion criteria that were considered for this review (Figure 2.1).

2.5 Results

Table A.1 summarizes the 29 studies included in this review. It describes the clinical population, strategy, stimulation parameters, tremor assessment method, main results, and hypothesized physiological mechanism for each study. Table 2.1 provides definition of several terms used to describe PES approaches and their features along this review.

2.5.1 Target populations

Among the reviewed articles, 12 studies reported the effects of peripheral electrical stimulation (PES) results on patients with ET (Munhoz et al., 2003; Widjaja et al., 2011; Bó et al., 2014; Heo et al., 2015; Heo et al., 2016; Lin et al., 2018; Pahwa et al., 2019; Kim et al., 2020; Isaacson et al., 2020; Yu et al., 2020; Barath et al., 2020; Reis et al., 2021), while 10 studies reported on patients with PD (Mones et al., 1969; Gillard et al., 1999; Jitkritsadakul et al., 2015; Jitkritsadakul et al., 2017; Xu et al., 2016; Hao

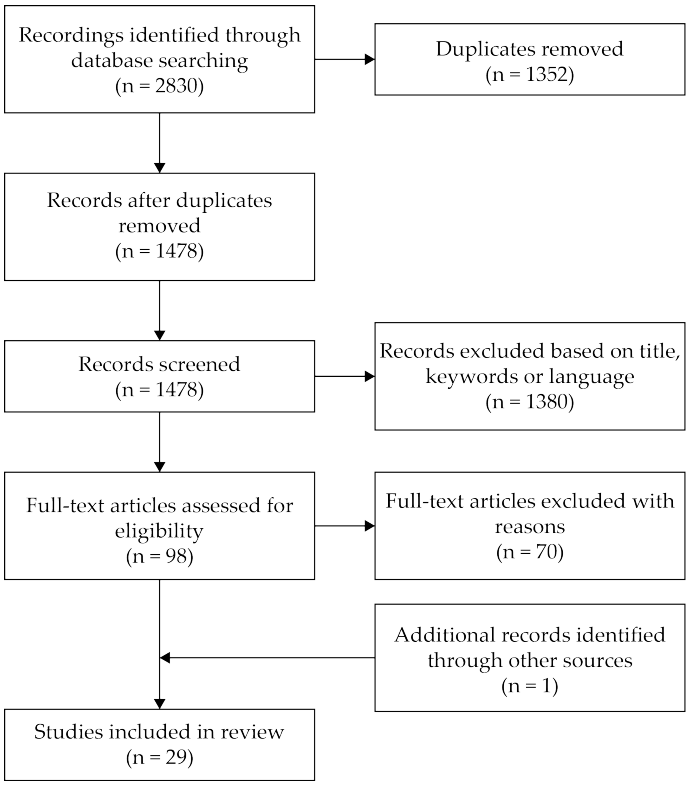

FIGURE 2.1: Flow diagram of screening process and final papers included in this review.

et al., 2017; Heo et al., 2018; Heo et al., 2019; Spiegel et al., 2002; Muceli et al., 2019) and 7 studies included both patients with ET and patients with PD (Britton et al., 1993; Dosen et al., 2015; Grimaldi et al., 2011; Gallego et al., 2013; Javidan et al., 1992; Popović Maneski et al., 2011; Dideriksen et al., 2017). In order to compare electrical stimulation strategies, some studies also included healthy volunteers that either mimicked tremorgenic activity during experimentation or were subject to artificially induced tremors for experimentation (Xu et al., 2016; Britton et al., 1993; Popović Maneski et al., 2011). Four papers reported additional results on other tremor conditions, including cerebellar ataxias (Grimaldi et al., 2011), patients with scans without evidence of dopaminergic deficits (SWEEDs) (Heo et al., 2019), peripheral neuropathy (Munhoz et al., 2003), and multiple sclerosis (Javidan et al., 1992). This review only targets PES studies tested on ET and PD populations, excluding other neural disorders. It is worth noting that the articles had a wide range of sample sizes, ranging from 1 to 263 (Widjaja et al., 2011; Isaacson et al., 2020). Articles with a relatively large sample size such as Lin et al., 2018 included 23 patients with ET; Jitkritsadakul et al., 2015 Jitkritsadakul et al. included 34 patients with PD in 2015; Pahwa et al., 2019 included 77 patients with ET; and Isaacson et al., 2020 included 263 patients with ET in a multi-center clinical trial. The rest of the articles analyzed in this review had sample sizes of 20 patients or less. In some cases, some articles can be considered case reports such as Widjaja et al., 2011 or Muceli et al., 2019, where only one experimental subject was included, or Xu et al., 2016, where only were included for experimentation, However, they were still included in this study due to the complete introduction, methods, results and discussion sections.

TABLE 2.1: Definitions of Peripheral Electrical Stimulation Approaches

"Peripheral" versus "CNS"electrical stimulation	
Peripheral electrical stimulation (PES)	Electrical stimulation of peripheral nerves to recruit efferent or afferent neural pathways. PES lies in contrast to central nerve stimulation.
Central nervous system stimulation	Stimulation of structures of the central nervous system. Methods of central nerve stimulation (e.g., DBS) are out of scope of this review.
"FES" versus "Afferent"stimulation	
FES	Electrical stimulation of efferent (i.e., motor) pathways. (Note that in efferent stimulation, the sensory pathways are also stimulated.)
Afferent stimulation	Electrical stimulation of afferent (i.e., sensory) pathways.
"Open-loop" versus "Closed-loop" versus "Calibrated" stimulation	
Open-loop stimulation	Stimulation that is delivered with a predetermined fixed waveform that is independent of any characteristic of the patient's tremor (Figure 2.2-B).
Closed-loop stimulation	Stimulation whose waveform is adjusted in real-time based on continuous sensing of the patient's tremor (Figure 2.2-A).
Calibrated open-loop stimulation	Stimulation with a waveform that is tuned (once, or repeatedly) to match characteristics (e.g., frequency) of the patient's tremor (Figure 2.2-C).
"Acute" versus "Short-term" effects	
Acute effect	Tremor reduction, measured relative to pre-stimulation levels, that is present while stimulation is applied.
Short-term effect	Tremor reduction, measured relative to pre-stimulation levels, that persists from minutes to hours after the stimulation ends.

2.5.2 PES approaches

Two main PES approaches were used to manage tremor reduction: functional electrical stimulation (FES) and stimulation of afferent pathways. The motor threshold (MT), defined as the minimal electrical current intensity that evokes a muscle twitch, is a key feature to understand the differences between these electrical stimulation approaches (Muceli et al., 2019). Seven articles used FES above the motor threshold to elicit muscle contractions, which generate forces in the musculoskeletal system and, therefore, reduce tremor oscillations (Grimaldi et al., 2011; Widjaja et al., 2011; Bó et al., 2014; Gillard et al., 1999; Gallego et al., 2013; Javidan et al., 1992; Popović Maneski et al., 2011). Alternatively, 18 studies applied electrical stimulation of afferent pathways below the motor threshold in order to neuromodulate the central nervous system (Heo et al., 2015; Heo et al., 2016; Heo et al., 2018; Heo et al., 2019; Xu et al., 2016; Hao et al., 2017; Lin et al., 2018; Pahwa et al., 2019; Isaacson et al., 2020; Yu et al., 2020; Barath et al., 2020; Kim et al., 2020; Mones et al., 1969; Britton et al., 1993; Spiegel et al., 2002; Dideriksen et al., 2017; Muceli et al., 2019; Reis et al., 2021).

Only one article compared the effects of FES versus stimulation of afferent pathways (Dosen et al., 2015). Three studies applied electrical stimulation above motor threshold with the purpose of recruiting afferent fibers, but muscle contraction was not the primary goal (Munhoz et al., 2003; Jitkritsadakul et al., 2015; Jitkritsadakul et al., 2017).

2.5.3 PES strategies

Electrical stimulation of muscles and peripheral nerves can be applied following different patterns based on mechanical and physiological models. The co-contraction strategy is based on the assumption that at a single joint, an agonist and antagonist muscle or group of muscles receive the tremorgenic input, producing involuntary mechanical oscillations (Gallego et al., 2015b). In this strategy, simultaneous electrical stimulation applied above the motor threshold in both agonist and antagonist muscles increases the impedance of the joint and, therefore, minimizes the involuntary oscillations (Grimaldi et al., 2011; Bó et al., 2014; Gallego et al., 2013).

However, the agonist-antagonist muscle pair commonly follow an out-of-phase activation pattern, as the muscles alternate contraction at the tremor frequency (Gallego et al., 2015b; Puttaraksa et al., 2019). Based on this behavior, the out-of-phase stimulation strategy stimulates the antagonist muscle when the tremorgenic agonist muscle is involuntarily activated and vice-versa. (Popović Maneski et al., 2011). This method has been applied for both FES and stimulation of afferent pathways. FES applied in an out-of-phase pattern produces opposite forces to the tremor oscillations (Dosen et al., 2015; Widjaja et al., 2011; Gillard et al., 1999; Javidan et al., 1992; Popović Maneski et al., 2011). To further refine the stimulation strategies by implementing closed-loop capability, neuromusculoskeletal models have been developed to characterize both tremorgenic and voluntary movements considering the stimulators acting as controllers of muscle activation (Gallego et al., 2015b). Closed-loop control algorithms allow real-time adaption of the stimulation to the tremor oscillations by making use of kinematics measurements (Gillard et al., 1999; Gallego et al., 2013; Javidan et al., 1992; Popović Maneski et al., 2011), electromyography (EMG) of the target muscles (Dosen et al., 2015; Dideriksen et al., 2017; Muceli et al., 2019), or both measurement types (Widjaja et al., 2011). One proposed model included electrophysiological information by means of electroencephalography and EMG recordings providing inputs to a neuromusculoskeletal model in order to isolate tremorgenic activity from voluntary movement and achieve adaptive joint impedance (Gallego et al., 2012). However, that study only involved one tremor patient as a case study for tremor reduction testing and, therefore, was excluded from this review.

Stimulating afferent pathways below motor threshold combined with the out-of-phase pattern demands precise stimulation timing with muscle activation. EMG-based algorithms have been used to drive the out-of-phase stimulation strategy, using sequential recording and stimulation windows to avoid the presence of artefacts in the EMG recordings (Dosen et al., 2015; Muceli et al., 2019; Dideriksen et al., 2017). EMG signals from the agonist-antagonist muscle pair are demodulated to extract the tremor frequency and period, which are used to predict the next tremor bursts and deliver the synchronous stimulation based on an out-of-phase pattern (Figure 2.2-A).

Alternative strategies derived from the out-of-phase pattern have also been proposed in the last three years. Tremor frequency can be estimated by means of gyroscopes to drive a calibrated open-loop sequential stimulation of radial and/or median nerves (Lin et al., 2018; Pahwa et al., 2019; Isaacson et al., 2020; Yu et al., 2020;

Barath et al., 2020) (Figure 2.2-C). This variation of the out-of-phase pattern preserves the synchronous stimulation at the mechanical tremor frequency and therefore avoids artefact issues with the EMG recordings. However, the activation of afferent pathways with the real physiological tremorgenic phase is not achieved.

Some studies applied electrical stimulation without following any time pattern or synchronization to mechanical or physiological events (Heo et al., 2015; Heo et al., 2016; Heo et al., 2018; Heo et al., 2019; Mones et al., 1969; Jitkritsadakul et al., 2015; Jitkritsadakul et al., 2017; Xu et al., 2016; Hao et al., 2017). This stimulation strategy

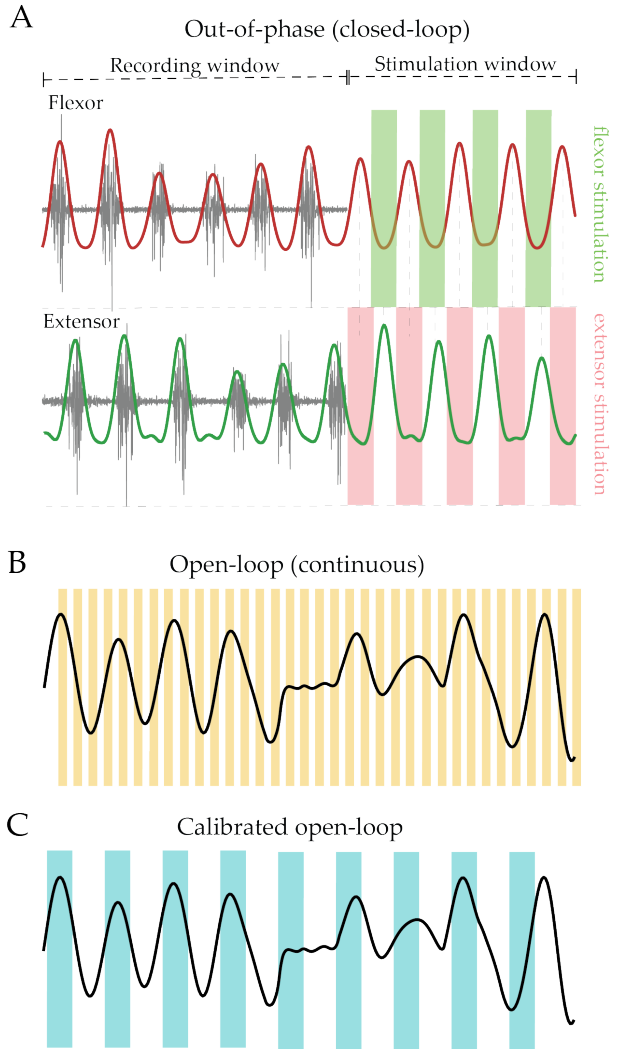

FIGURE 2.2: Illustration of some of the stimulation control strategies. A. Out-of-phase stimulation strategy. Tremorgenic EMG signals (gray lines) are demodulated for a pair of agonist/antagonist muscles during the recording window (red and green lines). If tremor is detected, the next tremor cycles are predicted based on the tremor frequency and the stimulation is timely delivered to the antagonist muscle (green and red rectangles, respectively). B. Open-loop stimulation is continuously delivered (yellow rectangles) with no relationship to tremorgenic behavior (black line). C. Calibrated open-loop stimulation is tuned to tremor (black line) features, such as tremor frequency, but does not incorporate real-time tremor measurements. As a result, stimulation (blue rectangles) may be applied even when tremor ceases.

has been labelled as continuous stimulation (open-loop) in this review (Figure 2.2-B). To limit stimulation during only tremorgenic activity instead of during voluntary movements, EMG recordings have been used to detect tremor activity and therefore enable continuous stimulation only when tremor is present (Xu et al., 2016; Hao et al., 2017).

A small number of studies tested the effects of single shock electrical stimuli in tremor features, such as amplitude, frequency or refractory rate (Mones et al., 1969; Spiegel et al., 2002; Britton et al., 1993; Reis et al., 2021). The purpose of these approaches was to study neurophysiological responses after electrical stimulation to explore possible neuromodulation outcomes, and as such are not aligned to those presented above to target exclusively tremor reduction.

2.5.4 Stimulation parameters

Stimulation intensity or amplitude (measured in mA) is a determining parameter to recruit muscle and sensory fibers. Absolute values were reported to vary from 1.5 mA in sensory intramuscular stimulation (Muceli et al., 2019) to 36 mA in some FES experiments (Grimaldi et al., 2011). Commonly, stimulation intensity is normalized for each subject to their motor threshold or their perception threshold, which is defined as the minimum current that the subject can perceive (Muceli et al., 2019), and is also referred to as the radiation or sensation threshold (Hao et al., 2017). Stimulation frequency was set in a range varying from 2 Hz to 250 Hz (Figure 2.3-A). Stimulation approaches using FES applied stimulation frequency between 25 Hz (Widjaja et al., 2011) and 100 Hz (Dosen et al., 2015). Studies targeting afferent pathways used stimulation frequencies between 2 Hz (Spiegel et al., 2002) and 250 Hz (Hao et al., 2017). There are two outliers for the minimum stimulation frequency: Spiegel et al., 2002, who tested at 2 Hz, 3 Hz, and 5 Hz, and Munhoz et al., 2003, who tested at 5 Hz, 10 Hz, 50 Hz, and 100 Hz. Certain studies have tested varying the stimulation frequencies while maintaining other stimulation parameters in order to determine optimal tremor reduction values (Munhoz et al., 2003; Kim et al., 2020). Reis et al., 2021 explored the tremor reduction effects of applying single stimuli with different phases relative to tremor.

The pulse width is another stimulation parameter and is defined as the duration of each electrical stimulus applied. Across all of the reviewed studies, the pulse width ranged from 100 to 500 μs, with the maximum value of 500 μs used by studies applying a single electrical stimulus (Mones et al., 1969; Britton et al., 1993) (Figure 2.3-B). Values between 100 and 300 μs are reported for FES applications, while it seems that higher values ranging from 150 to 400 μs are used to stimulate afferent pathways.

The duty cycle, described as the length of time comprising a train of pulses, is only indicated in some stimulation strategies (Dosen et al., 2015; Dideriksen et al., 2017; Lin et al., 2018; Pahwa et al., 2019; Kim et al., 2020; Muceli et al., 2019). This parameter is typical for out-of-phase and derivative strategies, and it usually references a portion of the tremor period. For instance, Dosen et al., 2015 fixed this parameter at 40%, which is equivalent to delivering a train of pulses during 80 ms if the tremor frequency is 5 Hz. Lin et al., 2018 and Pahwa et al., 2019 applied a duty cycle equivalent to half of the tremor period. Dideriksen et al., 2017 and Kim et al., 2020 tested the effect of several duty cycle values: 20% and 40%; and 12.5%, 25% and 37%, respectively.

The stimulus waveform is a fifth parameter that varied among the papers. The most common stimulus waveform is the squared wave, which might be monophasic

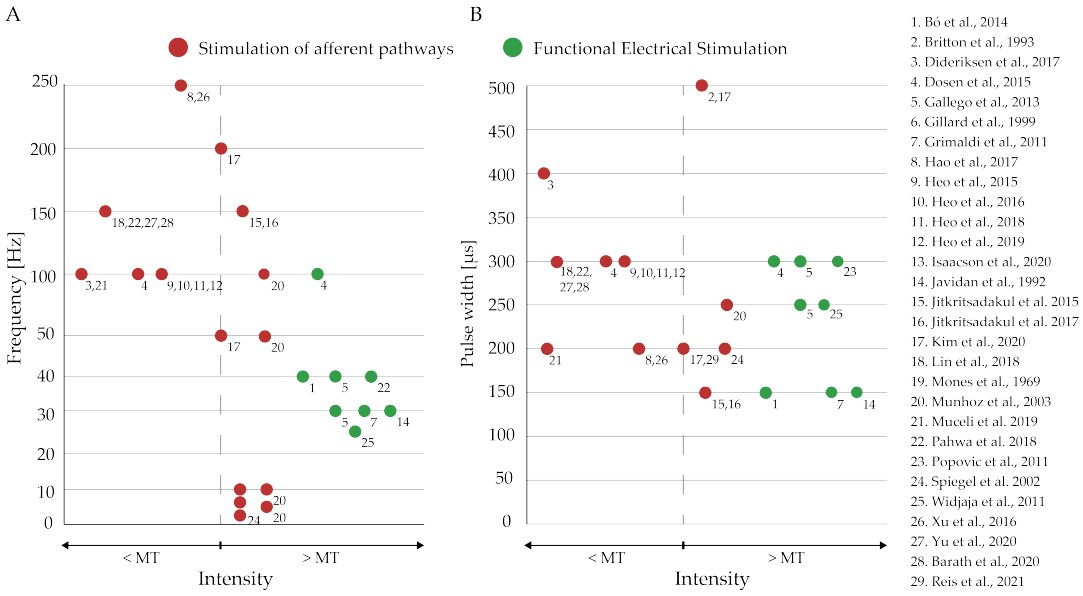

FIGURE 2.3: Stimulation parameter scatter plot. A. Frequency vs. stimulation intensity. B. Pulse width vs. stimulation intensity. Red dots represent studies using electrical stimulation of afferent pathways. Green dots represent studies using FES. Studies testing multiple stimulation parameters are represented by multiple dots. Studies performed by the same research group or replicating the same conditions are represented by the same dot. Note that four studies used stimulation of afferent pathways with stimulation intensity above motor threshold.

(Heo et al., 2015; Heo et al., 2016; Heo et al., 2018; Heo et al., 2019; Mones et al., 1969; Jitkritsadakul et al., 2015; Jitkritsadakul et al., 2017; Spiegel et al., 2002; Britton et al., 1993; Reis et al., 2021) or biphasic (Dosen et al., 2015; Widjaja et al., 2011; Lin et al., 2018; Pahwa et al., 2019; Isaacson et al., 2020; Yu et al., 2020; Barath et al., 2020; Muceli et al., 2019; Dideriksen et al., 2017; Xu et al., 2016; Hao et al., 2017; Popović Maneski et al., 2011; Gallego et al., 2013; Muceli et al., 2019). Some studies did not report this parameter (Grimaldi et al., 2011; Munhoz et al., 2003; Gillard et al., 1999; Javidan et al., 1992; Bó et al., 2014).

2.5.5 Stimulation electrodes and location

Different types of electrodes have been used to stimulate nerves and muscles across studies. Conductive pads attached to the surface of the skin, referred to as surface or transcutaneous electrodes, are widely used during muscle and nerve stimulation in all reviewed studies except in two (Dideriksen et al., 2017; Muceli et al., 2019). Table A.2 summarizes the various electrodes utilized by the studies reviewed. It is noteworthy that Dideriksen et al., 2017 first used intramuscular electrodes and compared their effect to surface stimulation. They used a pair of Teflon-coated stainless steel wires inserted into the muscle belly with a hypodermic needle. Muceli et al., 2019 tested the feasibility of thin-film intramuscular electrodes, with both EMG recording and stimulation contacts embedded in the same wire.

Tremor often manifests in the upper limbs, although it can occur in other parts of the body, and all the articles reviewed here applied electrical stimulation to upper limb structures. All the studies applied stimulation on just one arm, commonly the

side most affected by tremor. Only Mones et al., 1969 and Spiegel et al., 2002 explored the effects of stimulating contralateral ulnar or median nerves, respectively, while Munhoz et al., 2003 stimulated both sides of the brachial plexus. The studies that targeted stimulation of muscle belly commonly applied electrodes over the flexor and extensor muscles of the wrist (Bó et al., 2014; Dideriksen et al., 2017; Gallego et al., 2013). Since the anatomy of the forearm comprises a great array of superimposed muscles controlling the wrist and fingers, some studies also placed electrodes over both wrist and finger flexors and extensors (Dosen et al., 2015; Bó et al., 2014; Gillard et al., 1999). Other studies targeted both the wrist and elbow joint, placing electrodes over biceps brachii and triceps brachii (Grimaldi et al., 2011; Javidan et al., 1992; Popović Maneski et al., 2011; Heo et al., 2015; Heo et al., 2016; Heo et al., 2018; Heo et al., 2019). Jitkritsadakul et al., 2015 designed an intervention protocol and glove device to stimulate the abductor pollicis brevis as well as the first and second dorsal interosseous muscles (Jitkritsadakul et al., 2017).

Alternatively, electrical stimulation of the nerves has been explored instead of stimulation of the muscle belly. The main nerves stimulated were the distal branches of the radial nerve at the hand (Kim et al., 2020; Xu et al., 2016; Hao et al., 2017), median nerve at the wrist (Reis et al., 2021), median nerve at the elbow (Britton et al., 1993), median and ulnar nerves at the wrist (Spiegel et al., 2002), or a combined stimulation of radial and median nerves in the same session through a wearable device at the wrist (Lin et al., 2018; Pahwa et al., 2019; Isaacson et al., 2020; Yu et al., 2020; Barath et al., 2020).

2.5.6 Experimental design

Tremor is not a homogeneous condition for all patients or pathologies, and different etiologies and neural circuits involved may lead to different tremor manifestations, such as in ET and PD. Therefore, most of the experimental protocols involve patients performing a specific task or behavior to trigger their associated tremor type or measuring the effect of stimulation on the presentation of rest tremor in patients with PD (Lang et al., 1998). If needed, patients may be engaged in distracting cognitive tasks to trigger or worsen tremor. Those studies including both patients with ET and patients with PD indicated different experimental tasks to account for the different tremor presentation based on clinical diagnosis. The most common protocol for patients with ET involved delivering electrical stimulation during postural tremor, for instance, while the participants kept their arms outstretched and unsupported against gravity (Dosen et al., 2015; Grimaldi et al., 2011; Widjaja et al., 2011; Bó et al., 2014; Heo et al., 2015; Reis et al., 2021; Britton et al., 1993; Javidan et al., 1992; Dideriksen et al., 2017). In the experiments in patients with PD, participants were usually asked to keep their arms rested on a supported table (Dosen et al., 2015; Gillard et al., 1999; Jitkritsadakul et al., 2015; Jitkritsadakul et al., 2017; Xu et al., 2016; Hao et al., 2017; Heo et al., 2018; Heo et al., 2019; Muceli et al., 2019; Spiegel et al., 2002; Javidan et al., 1992; Dideriksen et al., 2017). Alternatively, Spiegel et al., 2002 asked PD patients to stretch their arms and hold them perpendicularly to the body in a position in which postural tremor was maximal. Grimaldi et al., 2011 asked all analyzed subjects, which included participants with ET or PD, to perform kinetic tasks (e.g., finger-to-finger, or index finger-to-nose) while electrical stimulation was applied. Munhoz et al., 2003 applied electrical stimulation while the patient was performing functional tasks such as writing or drinking from a glass. Heo et al., 2016 had patients completing the Archimedes spiral drawing task, and Kim et al., 2020 had patients performing the bean transfer task.

All the studies included in this review applied PES in a single session except two. Isaacson et al., 2020 and Barath et al., 2020 tested the effects of a wrist-worn wearable device in a clinical trial. Patients with ET were instructed to apply two 40-minutes sessions of stimulation per day over the course of three months. The status of the patients was assessed in three visits: at time of enrolment, one month after study onset, and study completion after three months. The majority of experimental protocols consisted of a single session acute comparison of baseline tremor periods with tremor periods while stimulation is applied (Table A.1). The stimulation time windows in the stimulation protocols varied from 5 seconds to 30 seconds, and the number of trials per condition, if specified, ranged from 1 to 13. Some of the studies included comparisons between different stimulation strategies or conditions: Munhoz et al., 2003 tested different stimulation intensities and frequencies; Spiegel et al., 2002 tested different stimulation frequencies and nerves stimulated; Dosen et al., 2015 benchmarked the effects of FES and afferent stimulation below motor threshold; Dideriksen et al., 2017 tested the out-of-phase strategy and random-timed stimulation with two stimulation intensities and two duty cycles, using both surface and intramuscular electrodes; Kim et al., 2020 assessed different combinations of frequency, duty cycle, and open/closed-loop control across trials; and Reis et al., 2021 compared the effects of phase-specific stimulation. Only three protocols included a sham group to explore the possible placebo effect of stimulation on tremor reduction (Lin et al., 2018; Pahwa et al., 2019; Jitkritsadakul et al., 2017).

Conversely, lasting effects after the application of peripheral electrical stimulation have also been explored by a limited number of studies. Hao et al., 2017 and Xu et al., 2016 explored the short-term tremor reduction effect immediately following 5 seconds after stimulation. Heo et al., 2015 assessed tremor before stimulation, during 15 second stimulation windows, and 5 minutes after stimulation ended (Heo et al., 2016; Heo et al., 2018; Heo et al., 2019).Lin et al., 2018 and Pahwa et al., 2019 proposed a 40-minute stimulation session, assessing tremor before and after the stimulation, which was performed in subsequent clinical trials (Isaacson et al., 2020; Yu et al., 2020; Barath et al., 2020).

2.5.7 PES efficiency to reduce tremor

The results of applying PES are reported in most of the analyzed articles in terms of tremor reduction, namely, how tremor is reduced during or after stimulation is applied. Primary results corresponding to each study can be visualized in Table A.1.

The most common method to assess tremor reduction in the studies reviewed is the use of kinematic measurements collected by means of accelerometers (Munhoz et al., 2003; Kim et al., 2020; Isaacson et al., 2020; Yu et al., 2020; Barath et al., 2020; Reis et al., 2021), gyroscopes (Heo et al., 2015; Heo et al., 2016; Heo et al., 2018; Heo et al., 2019; Popović Maneski et al., 2011), initial measurement units (Dosen et al., 2015; Dideriksen et al., 2017; Muceli et al., 2019) or motion capture systems (Xu et al., 2016; Hao et al., 2017). These sensors are placed onto the body segments targeted by stimulation, collecting kinematic data such as angle displacement, angular velocity, or acceleration that are offline analyzed using secondary metrics. As an example, Bó et al., 2014 used the root mean square (RMS) of the tremor kinematics. Some groups (Dosen et al., 2015; Dideriksen et al., 2017; Muceli et al., 2019) computed the tremor power from the power spectrum density (PSD) of the angle displacement in the tremor band (3-9 Hz) to assess the level of tremor reduction.

Quantitative evaluation of tremor reduction was, in a few cases, complemented or replaced by evaluation based on patient-reported or clinician-observed questionnaires or clinical scales rating the patient's tremor status while performing postural, kinetic, or functional tasks (Grimaldi et al., 2011; Munhoz et al., 2003; Heo et al., 2016; Lin et al., 2018; Pahwa et al., 2019; Kim et al., 2020; Isaacson et al., 2020; Yu et al., 2020; Barath et al., 2020; Jitkritsadakul et al., 2015; Jitkritsadakul et al., 2017). Scales used in some studies were: The Unified Parkinson's Disease Rating Scale (UPDRS) used to assess patients with PD (Jitkritsadakul et al., 2015; Jitkritsadakul et al., 2017); The Essential Tremor Rating Assessment Scale (TETRAS) (Lin et al., 2018; Pahwa et al., 2019; Isaacson et al., 2020; Kim et al., 2020; Barath et al., 2020) and Fahn-Tolosa-Marin Clinical Rating Scale (FTM) (Heo et al., 2016). In this line, Kim et al., 2020 complemented the objective tremor reduction evaluation based on the tremor PSD with a qualitative assessment based on a 7-item Likert-scale questionnaire (Kim et al., 2020); Jitkritsadakul et al., 2015 combined the use of RMS on different kinematics variables and the UPDRS tremor score to assess the effects of stimulation. In some cases, the effect of stimulation on tremor reduction was assessed based on the EMG signals collected at various muscles of interest (Mones et al., 1969; Xu et al., 2016; Hao et al., 2017; Britton et al., 1993).

Studies that applied FES for tremor reduction reported variable results. Gillard et al., 1999 achieved average acute reduction across three patients with PD of 84.5 ± 2.2%. Popović Maneski et al., 2011 reported 67 ± 13% average acute reduction in three and four patients with ET and PD, respectively. Bó et al., 2014 reported individual acute tremor reduction ranging from 37.2% to 94.7% in the RMS kinematics in 10 patients with ET. While Grimaldi et al., 2011 reported approximately a 50% improvement for one patient with ET in finger-to-finger tasks on a clinical scale (Clinical Neurophysiological Functional Tremor Evaluation Scale, CNF-TES) and kinematics PSD. The same study reported no improvements for two patients with PD and cerebellar ataxia.

High variability in terms of tremor reduction results was also found across studies applying stimulation of afferent pathways. Tremor reduction via below motor threshold stimulation was reported by Heo et al., 2016 averaging a 12% reduction in the RMS angular velocity at the distal finger segment and metacarpophalangeal joint of patients with ET during action tremors. Heo et al., 2015 reported a reduction of 90% and 77% RMS angular velocity at the same finger and metacarpophalangeal joints in postural tremors. Jitkritsadakul et al., 2015 reported average acute tremor reduction in 30 patients with PD in both tremor power peak of 49.6 ± 38.9% and UPDRS from 10.6 ± 1.7 before stimulation to 8.9 ± 2.2 during stimulation. Similar tendencies in acute tremor reduction using continuous stimulation of afferent pathways were replicated in a sham study with 30 patients with PD, reporting 60.2 ± 38.9% average acute tremor reduction in the RMS of the wrist angular velocity, statistically higher than the sham group Jitkritsadakul et al., 2017. Only Dosen et al., 2015 compared FES and stimulation of afferent pathways for tremor reduction, reporting an average acute tremor reduction of 60 ± 14% and 42 ± 5% for stimulation above and below motor threshold, respectively. It is worth noting that even though stimulation above motor threshold on average outperformed stimulation below motor threshold, the statistical tests showed no significant differences between the stimulation modalities.

Dideriksen et al., 2017 explored the use of intramuscular electrodes in comparison with surface stimulation with the average of the highest acute reduction levels across all patients at 54 ± 20% (intramuscular) and 50 ± 41% (surface), respectively.

Although results were not statistically different, the authors found that the variability across trials was lower with intramuscular stimulation, thus suggesting that intramuscular electrodes led to a more consistent effect on acute tremor reduction.

Results from different stimulation strategies are scarce and inconclusive. Dideriksen et al., 2017 did not report differences in acute tremor reduction when applying out-of-phase strategy or random time stimulation, while Kim et al., 2020 could not conclude that closed-loop stimulation achieved higher acute tremor reduction than open-loop stimulation.

All studies reporting short-term tremor reduction after stimulation sessions used stimulation of afferent pathways (Heo et al., 2015; Heo et al., 2016; Heo et al., 2018; Heo et al., 2019; Lin et al., 2018; Pahwa et al., 2019; Yu et al., 2020; Barath et al., 2020). Lin et al., 2018 and Pahwa et al., 2019 both reported that patients with ET achieved higher TETRAS clinical scale scores after 40 minutes of stimulation compared to the sham group. Although these results might be promising, differences were only found in one item of TETRAS and no objective kinematics measurements were provided. This work was continued by Isaacson et al., 2020, who reported that tremor was reduced in both kinematics and clinical scales when applying the same protocol in a three-month, open-label clinical trial. Additionally, Heo et al., 2015 reported a tremor reduction in kinematics during three stimulation trials (15 seconds per trial) and five minutes after stimulation, compared to baseline in patients with ET (Heo et al., 2016) and PD (Heo et al., 2018; Heo et al., 2019).

2.5.8 Physiological sources of tremor reduction

Tremor reduction using PES mainly involves two strategies: 1) FES, based on the generation of forces within the tremorgenic muscles to mechanically reduce tremors; and 2) the stimulation of afferent pathways, relying on disrupting central tremorgenic circuits via timely recruitment of afferent pathways projecting into the tremor oscillatory network.

FES takes advantage of electrical stimulation to recruit muscle fibers and generate force (Dosen et al., 2015; Grimaldi et al., 2011; Bó et al., 2014; Widjaja et al., 2011; Gillard et al., 1999; Javidan et al., 1992; Popović Maneski et al., 2011; Gallego et al., 2013). Specifically, the electrical current activates axons that generate action potentials and force once the action potential reaches the muscle fibers (Enoka et al., 2020). The higher the stimulation intensity, the greater the number of fibers recruited and, therefore, the higher the force produced. The co-contraction strategy achieves a reduction of tremor oscillations by means of stimulating a pair of agonist-antagonist muscles, evoking opposite forces, which increases the joint impedance (Bó et al., 2014; Gallego et al., 2013). The studies applying the out-of-phase strategy evoked antagonist forces to the tremorgenic muscle to increase joint stiffness, alternating the stimulation of tremorgenic agonist muscle and corresponding antagonist muscle (Dosen et al., 2015; Widjaja et al., 2011; Javidan et al., 1992; Popović Maneski et al., 2011). These studies also reported a risk of muscle fatigue and movement control alteration.

Alternatively, low current stimulation is also effective in activating EPSPs (excitatory post-synaptic potentials) and IPSPs (inhibitory post-synaptic potentials) from different sensory pathways that reach different circuitries at the CNS level and might modulate the tremorgenic loops (Dideriksen et al., 2015). Interestingly, some studies have found not only acute, but also short-term effect of PES on tremor reduction after the end of the stimulation session (Heo et al., 2015; Heo et al., 2016; Heo et al., 2018; Heo et al., 2019; Lin et al., 2018; Pahwa et al., 2019;

Isaacson et al., 2020; Yu et al., 2020; Barath et al., 2020; Kim et al., 2020). This implies that tremor generation at the supraspinal level or tremor transmission at the spinal level remain altered even after electrical stimulation is discontinued.

Modulation at the Spinal Cord

While the majority of the studies examining pathological tremor focus on the brain, where primary oscillators are located, some studies point to the spinal cord as being actively involved in the tremor network, suggesting that sensory PES could disrupt the tremor oscillations at this level (Figure 2.4-B). Abnormal spinal reflexes have been reported in both ET and PD patients, evidence supporting aberrant behaviour at the spinal cord (Mercuri et al., 1998; Meunier et al., 2000). Through computational models and electrophysiological data from ET patients, studies indicate that the tremor neural drive, i.e., the recurrent pathological activation of motor unit pools, is associated with one central brain network generating neural oscillations at the tremor frequency (Gallego et al., 2015b). However, the strength of this pathological neural drive is uncorrelated to the net supraspinal synaptic input at the tremor frequency (Gallego et al., 2015a). This suggests a contribution of spinal afferents and/or secondary supraspinal pathways in projecting common input to muscles at the tremor frequency. Ia afferent fibers have been proposed to contribute to tremor amplification in a pair of antagonist muscles through disynaptic reciprocal inhibition (Puttaraksa et al., 2019). Furthermore, studies using sensory closed-loop PES strategies synchronized to EMG suggest that acute tremor reduction is due to recruitment of these reciprocal inhibition loops, selectively activated with intramuscular electrodes (Dosen et al., 2015; Dideriksen et al., 2017; Muceli et al., 2019).

Propriospinal interneurons are known to be engaged in the corticospinal transmission of voluntary commands (Pierrot-Deseilligny, 1996; Stinear et al., 2004), and stimulation of cutaneous afferents can modulate their response (Nielsen et al., 1991). The propriospinal system is altered in PD patients and could potentially contribute to the tremor oscillatory network (Pol et al., 1998). Hao et al., 2013 proposed a model of corticomuscular transmission of tremor signals through propriospinal neurons in PD which was used as a hypothesis for the tremor reduction effects when stimulating cutaneous afferent fibers at the hand (Hao et al., 2017). Although the hypotheses based on spinal cord modulation might partially explain the acute effects of sensory PES, no evidence has been gathered to support the role of the spinal cord in any short-term tremor reduction effects.

Supraspinal modulation

Alternatively to the spinal cord modulation hypotheses, some studies support that tremor reduction effects are caused by modulation of supraspinal centers in the tremor oscillatory network (Figure 2.4-A). Sensory PES reaches different brain structures, as somatosensory evoked potentials used in research and clinics (Grisolia et al., 1980; Baker et al., 2021) or neuromodulation techniques in rehabilitation paradigms (Wolpaw et al., 2006; Hishinuma et al., 2019) revealed. One salient hypothesis underlying short-term tremor reduction after sensory PES posits that afferent cues reach the ventral intermediate nucleus (VIM) and disrupt tremor activity (Lin et al., 2018; Pahwa et al., 2019; Isaacson et al., 2020). This hypothesis is based on the DBS mechanism whereby high-frequency stimulation of the VIM leads to selective neuronal inhibition, disrupting cerebello-thalamo-cortical oscillations responsible for tremor (Ceballos-Baumann et al., 2001; Milosevic et al., 2018). Evidence indicating that median nerve stimulation alters firing patterns in the thalamus and subthalamic nucleus is, however, not sufficient to confirm that the modulation of the

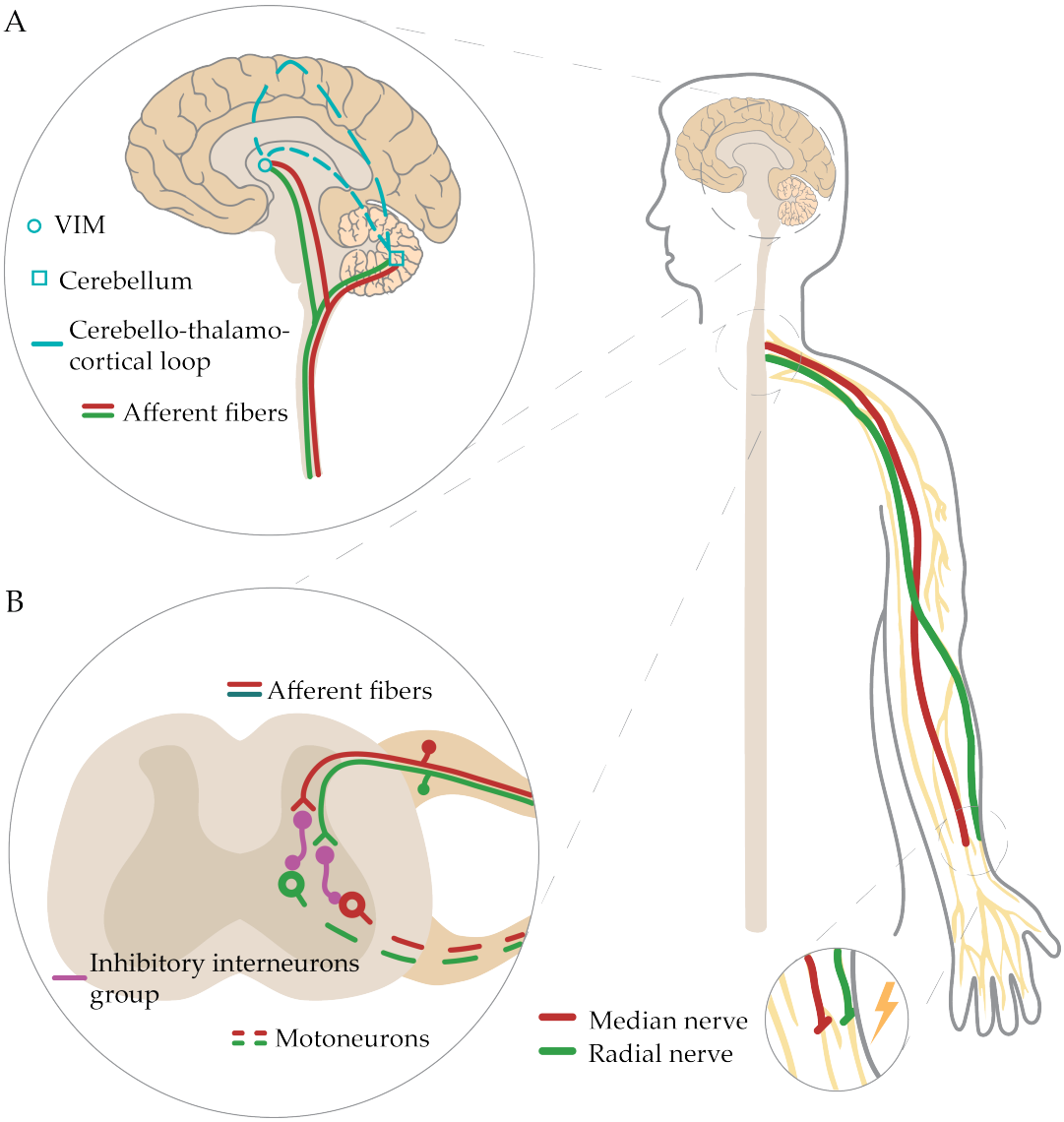

FIGURE 2.4: Schematic of tremor reduction mechanism hypotheses after PES. A. The afferent fibers activated through PES reach the tremor sources located at the brain, primarily the cerebellum and the VIM, and disrupt the aberrant tremorgenic activity. B. The recruited afferent fibers make connections with inhibitory interneurons at the spinal cord, mainly involved in spinal reflexes circuits and/or the propriospinal system, which modulate the supraspinal tremorgenic input and prevent it from reaching the muscles.

VIM activity is responsible for the short-term tremor reduction effects reported after sensory PES (Klostermann et al., 2009; Hanajima et al., 2004; Hernandez-Martin et al., 2021). Regarding PD, Heo et al., 2018 and Jitkritsadakul et al., 2015 proposed that continuous stimulation might reduce the hyper-excitability of the cerebello–thalamo–cortical circuit, which may be related to the impaired cerebellar inhibition in PD.

Although there is still debate about the pathological source of tremor, Purkinje cell degeneration at the cerebellum is one of the main hypotheses in the case of ET (Louis et al., 2020a). The cerebellum is involved in motor control and learning by

integrating projections from the thalamo-cortical circuit and primary afferents to adjust the motor response. Cerebellar injury can lead to pathological tremor, for instance, in cerebellar ataxias (Medina et al., 2008; Marsden, 2018). Consequently, it has been hypothesized that the tremor reduction reported after sensory PES might be due to alteration of cerebellar circuits, which project into the thalamo-cortical network. This hypothesis is supported by a recent study in which metabolic changes at the cerebellar region were found in SPECT imaging after three months of Transcutaneous Afferent Patterned Stimulation) (TAPS) therapy for 5 ET patients (Barath et al., 2020). An alternative study providing evidence in favour of the cerebellar hypothesis demonstrated that transcutaneous cerebellar electrical stimulation synced with the tremor phase, similar to the out-of-phase approach, could reduce tremor in ET patients (Schreglmann et al., 2021).

Mones et al., 1969, Britton et al., 1993 and Reis et al., 2021 explored the effect of peripheral nerve single shock on tremor, suggesting that afferences can reset the central tremor oscillators, but they did not provide further discussion on the specific structures targeted nor the pathways activated. Spiegel et al., 2002 explored the effect of low-frequency nerve stimulation on tremor frequency, but also did not provide any evidence on the afferent pathways activated, and only speculated about the alteration of thalamic neurons implied in tremor generation.

2.6 Discussion

Recent studies demonstrate the potential and usability of PES as an intervention to reduce tremor. However, results are highly variable across studies and patients, which points out the need for consensus and standardized procedures to allow more reproducibility and cross-comparisons. There is substantial opportunity for PES to be explored as tremor reduction intervention, given that closed-loop control strategies allow a more precise delivery of the intervention in comparison to medications that can have broad CNS or bodily effects, and to DBS or HIFU that can be more invasive or irreversible, respectively; in addition, PES can be discontinued at any time.

Several limitations of the reviewed studies have been identified: small sample sizes, lack of control or sham groups, varied stimulation parameters across studies, and in some, combined ET and PD groups. Also, the reporting of results was not always representative of the overall efficiency of the stimulation strategy (i.e., some studies report the highest reduction results for a subset of patients or single trials) and was highly variable among studies. As there is limited research on this topic, future studies can address these issues as well as the long-term effects of the application of PES.

Studies have been conducted mostly in two main pathologies: ET and PD. The pathophysiology of each of these disorders differs and, as a result, observable tremor reduction may be attributed to the modulation of different underlying tremor mechanisms. Future studies examining relationships between diagnosis (ET, PD) and tremor response to stimulation using larger sample sizes and potentially coupled with additional physiological measures may help elucidate this issue. Open-loop algorithms, which define a pre-calibrated stimulation pattern, might not be suited to fluctuating tremor status and could have limited efficacy compared to closed-loop control strategies, which show higher compliance to the tremor features (Lin et al., 2018; Pahwa et al., 2019; Kim et al., 2020; Isaacson et al., 2020; Yu et al., 2020; Barath et al., 2020). On the other hand, EMG-based strategies, mainly used by the

out-of-phase strategy, allow for superior time and spatial resolution, since the stimulation output can be synchronized with physiological tremorgenic activity from a single muscle, a desirable feature when stimulating afferent pathways. The stimulation artifacts present in the EMG signals still entail a challenge, despite some groups having developed artifacts suppression methods in the EMG (Widjaja et al., 2011), or used sequential recording and stimulation windows (Dosen et al., 2015; Dideriksen et al., 2017; Muceli et al., 2019). Finally, kinematics-based strategies allow real-time control based on limb relative position and avoid dealing with stimulation artifacts, but in exchange have inferior time and spatial resolutions, since the stimulation cannot target specific muscle movement or electrophysiological activity due to the electromechanical delay (Widjaja et al., 2011; Kim et al., 2020). The use of neuromusculoskeletal models that are able to isolate voluntary movements from tremor oscillations were not indicated in the afferent stimulation studies. The deployment of these models integrating both kinematics and electrophysiological signals may enhance the delivery of electrical stimulation of afferent pathways and thereby overcome the limitations of previous stand-alone approaches.

Stimulation parameters such as frequency, pulse width, and waveform play a role in tremor reduction, but are still to be determined. These parameters are known to have an effect on afferent stimulation efficiency (Mang et al., 2010). However, there is high variability in these stimulation parameters across different studies, which highlights the need for more research on this area. Stimulation frequencies lower than 50 Hz are used in FES applications to minimize muscle fatigue, while frequencies between 50 and 250 Hz are preferred for recruiting afferent fibers with low amplitude stimulation. FES uses high stimulation intensities to elicit muscle fiber contraction of a sufficiently large population to produce the desired forces opposing tremor. Higher stimulation frequencies are reported to rapidly increase fatigue, and therefore limit this value for FES applications (Gil-Castillo et al., 2020). Conversely, stimulation of afferent pathways allows higher stimulation frequencies with lower stimulation intensities due to the lower recruitment threshold of sensory fibers (Dideriksen et al., 2015). A similar division of pulse width parameter was observed. Shorter pulses were preferred for FES applications while longer pulses were preferred for stimulation below motor threshold. Finally, another critical step towards standardizing stimulation strategies is reporting the intensity of stimulation applied. This should be normalized either to the motor threshold or the perception threshold. Once some of these limitations are addressed, more comparisons among studies can be done and more conclusions can be drawn, not only on acute effects, but also on possible lasting or longitudinal effects.

Surface electrodes are the preferred interface to deliver PES as they are non-invasive, cost-effective and convenient to replace. However, their selectivity for targeting different structures can be limited since skin movement can adversely shift the stimulation location. Also, the variation of electrochemical properties of the electrode interface and current distribution could lead to an undesirable stimulation effect over time (e.g., impedance increase due to the gel drying out) (Muceli et al., 2019). Only two studies tested and presented results using intramuscular electrodes. Although percutaneous or intramuscular electrodes are invasive and stimulate a reduced volume of tissue, they provide a more repeatable and robust outcome due to targeting the same group of fibers during stimulation and are less affected by the movement of the skin (Muceli et al., 2019). More research is needed regarding intramuscular electrodes to provide evidence about their safety and advantages against surface electrodes in long-term use to overcome the issue of their minimal invasiveness.

The majority of studies targeted tremor at the wrist joint, and only a few studies additionally stimulated muscles or nerves controlling the elbow or shoulder. However, mechanical tremor oscillations have been proved to propagate from proximal to distal joints (Corie et al., 2019). Therefore, focusing on just one isolated joint might be insufficient to efficiently reduce tremor, as the tremor assessed at the wrist could be a product of the oscillations produced at the elbow or shoulder. The large variety in electrode locations targeting different groups of muscles or nerve branches, compounded by the limited number of studies and poor methodology descriptions in some cases, interfere with the reproducibility and comparison of results across studies.

Experimental design by some studies specified the age, sex, and duration of tremor for their subjects, but other studies did not report demographic or clinical details. Further examination of the relationships of demographics, disease-related features (e.g., duration, concomitant treatments such as medications) and other motor symptoms to stimulation response may be needed to better understand the clinical application of these stimulation strategies. Next, the posture held by each patient during stimulation trials should also be adapted according to the tremor pathology. However, some studies do not report the posture maintained by patients during stimulation trials; others used the same task (postural or rest) for different clinical populations, which would affect the tremor presentation. Only a few studies tested the effects of stimulation during basic functional tasks such as touching a finger to the nose (Grimaldi et al., 2011) or drawing a spiral (Heo et al., 2016), perhaps due to the challenge of targeting tremorgenic oscillations instead of voluntary movements by control algorithms. Despite the difficulty, including both intervention and assessment during ADL could contribute to testing the efficacy of the PES approach in real-life applications in order to determine or refine the strategy to minimize any effect on normal voluntary movement.

Fourthly, only three studies included a control/sham group, which is an important component to test the efficiency of the experiments (Lin et al., 2018; Pahwa et al., 2019; Jitkritsadakul et al., 2017). To address the lack of control groups, some studies have used different stimulation conditions across sessions (Munhoz et al., 2003; Kim et al., 2020; Spiegel et al., 2002; Dideriksen et al., 2017). These studies blinded subjects to the different stimulation strategies in order to observe which type of stimulation strategy leads to optimal tremor reduction. Finally, it is important to emphasize that all the studies in this review tested subjects during a single session except the clinical trials presented by Isaacson et al., 2020 and Barath et al., 2020. The longitudinal approach should be of consideration for future studies, and in particular could influence the shift of acute tremor interventions to the development of therapeutic approaches applied in the home setting, such as done in the study by Isaacson et al., 2020 where continuous accelerometry recordings were collected during each home therapy session to allow monitoring of progress without in-person clinician intervention or office visits.

2.6.1 Physiological sources of tremor and reduction mechanisms

Stimulation of afferent pathways below motor threshold has been explored in the past six years as an alternative without the main drawbacks of stimulation above motor threshold (Xia et al., 2004). Due to the early stage of research on this topic and the absence of precise knowledge about the neural circuitries implied in tremor generation, it is only possible to speculate on the explanations for the tremor reduction outcomes. Important clues to improve protocols for stimulation below motor

threshold at the peripheral level have been given by simulation studies. Selectively timed activation of Ia afferents and cutaneous afferents PES in synchronization with tremorgenic electrophysiological activity should be further explored to better understand and exploit spinal reflex mechanisms and inhibitory pathways for tremorgenic activity reduction (Dosen et al., 2015; Dideriksen et al., 2017; Muceli et al., 2019). It is worth mentioning that one study, out of the scope of this review, proposed mechanical vibration as an alternative method to acutely suppress tremor via stimulation of afferent fibers (Lora-Millán et al., 2019). No tremor reduction was achieved for the patients with ET, which might imply that electrical stimulation is more suitable than mechanical vibration to selectively activate afferent fibers interacting with tremorgenic circuitries.

Unveiling possible supraspinal mechanisms and eventually identifying any prolonged plastic effects will be of special importance. Thus, once the activated afferent fibers transmit signals to the spinal cord, the EPSPs and IPSPs might disperse to both spinal networks and ascending pathways to finally reach the supraspinal centers involved in tremor generation, inducing neuromodulatory effects that are maintained over some period of time. Explanations suggested by those studies reporting a short-term effect on tremor reduction after stimulation (e.g., no longer than five minutes after stimulation) require further hypothesis generation and data to fully understand the conclusions on side effects. In addition, characterizing motor threshold is critical. For instance, Kim et al., 2020 and Spiegel et al., 2002 did not explicitly report the motor threshold level in reference to the stimulation levels. Therefore, it cannot be decisively inferred that their results can all be attributed to stimulating afferent pathways. Similarly, Jitkritsadakul et al., 2015 and Munhoz et al., 2003 stated that they stimulated above the motor threshold yet claimed that afferent pathways were exploited to reduce tremor. A main advantage of stimulating afferent pathways is avoiding muscle contraction and the resulting muscle fatigue. As such, their approach minimizes this advantage.

One important step for a better understanding of results would be the standardization of assessment metrics. Some studies use kinematics (usually with accelerometers or gyroscopes) while others only report clinical scales. Clinical scales are liable to inter- and intra-rater variability and subjective judgment (Chan et al., 2019). On the other hand, they can be used to assess functional tasks and measure the impact of therapies on activities of daily living, which cannot always be assessed by kinematics. Hence, reporting kinematics and functional scores together might benefit future comparisons across studies. The majority of studies also presented limited sample sizes and high variability in results. Few studies included statistical tests to support the tremor reduction data, and some of them presented only the highest reduction results for individual trials or patients. Results should include average values across groups or patients, adding statistical tests when possible, and limiting individual case studies which have been proven to skew comparisons with their high variability.

2.6.2 Conclusions and future directions

PES of afferent pathways below motor threshold stands as a promising intervention to manage pathological tremor due to its minor adverse effects compared to FES. Usability of PES for regular and/or daily use to reduce pathological tremor has been showcased in a novel wrist-worn peripheral nerve stimulation device (Lin et al., 2018; Pahwa et al., 2019; Isaacson et al., 2020; Yu et al., 2020; Barath et al., 2020). More

sophisticated wearable devices and algorithms should be pursued, especially combining both EMG and kinematic based-control to disregard voluntary movement components while reducing tremorgenic activity, as well as the potential to target stimulation at multiple muscles or joints of the tremorous limb. The development and testing of implantable technologies using intramuscular electrodes controlled with external wireless devices could also serve as a long-term solution (Ivorra et al., 2015; Becerra Fajardo et al., 2017). The preliminary evidence of short-term tremor reduction after stimulation also opens the scope to develop longitudinal interventions towards a standardized therapy widely accessible to patients unresponsive to medication or ineligible for surgical treatments. Finally, it remains necessary to characterize the tremor reduction effects specific to tremor pathologies to personalize these strategies for ET or PD patients.

Chapter 3

Modulation of muscle activity during voluntary contraction through Peripheral Electrical Stimulation

The contents from this chapter have been previously published in the following conference article, in which the author of this PhD dissertation contributed as first author:

> **Pascual-Valdunciel A**, Barroso FO, Muceli S, Taylor J, Farina D, Pons JL. Modulation of reciprocal inhibition at the wrist as a neurophysiological correlate of tremor suppression: a pilot healthy subject study. Annu Int Conf IEEE Eng Med Biol Soc. 2019;2019:6267–72.

3.1 Abstract

It has been shown that activation of Ia and other Group I afferents, which connect to inhibitory interneurons, can inhibit muscle activity of the ipsilateral antagonist, a mechanism known as disynaptic Group I inhibition at the wrist. Stimulation of these afferents may be explored for the therapeutic reduction of pathological tremor. However, only a few studies have investigated disynaptic Group I inhibition in wrist flexor and extensor muscles. Here, the effects of the disynaptic Group I inhibition circuit on the antagonist muscle was explored for the wrist flexors and extensors by applying surface electrical stimulation to the radial and median nerves, respectively. Firstly, the direct (M) and monosynaptic (H) reflex responses to increasing median and radial nerve stimulation were recorded to characterize the recruitment curve of the flexor carpi radialis (FCR) and extensor carpi radialis (ECR) muscles, respectively. Based on the recruitment curve data, the median and radial nerves were then stimulated below and above motor threshold (MT) during a submaximal isometric task to assess the amount of inhibition on ECR and FCR muscles, respectively. The stimulation of both nerves produced inhibition of the antagonist motoneuron pool activity. On average, maximum peak of inhibition was 27 ± 6% for ECR and 32 ± 9% for FCR after stimulation below MT; maximum peak of inhibition was 45 ± 7% for ECR and 44 ± 13% for FCR when using stimulation above MT. These results validate this neurophysiological technique that demonstrates a mechanism similar to classical reciprocal Ia inhibition reported for other limb joints and that can be used to benchmark strategies to suppress pathological tremor.

3.2 Goals

The main goal of this study is to test the hypothesis that PES of afferent pathways can be applied to inhibit muscle activity of the antagonist muscles in healthy subjects. Specifically, demonstrating that PES applied below motor threshold can inhibit the voluntary neural drive of the antagonist muscle by means of modulating spinal cord circuits comprises an initial step towards the development of PES-based tremor reduction strategies. Moreover, the application and validation of electrophysiological techniques used in this study provides the technical and scientific background required to achieve the consequent goals of this PhD thesis.

3.3 Introduction

The alteration of the cerebellar circuits remains as the main pathophysiological hypothesis in ET (Deuschl et al., 2009), and several pieces of evidence support the role of cerebello-thalamo-cortical circuits in tremor generation for ET as well as for other disorders causing pathological tremor such as PD (Helmich et al., 2013). Although brain structures are the primary tremorgenic source, afferent inputs from the proprioceptive system into the tremor network is hypothesized to contribute to the oscillatory and synchronic activation of the muscles. Previous studies based on EMG and EEG data from ET patients and computational neural models have suggested the presence of a powerful interaction between central neural drive and mechanical receptors at the peripheral level. The strength of supraspinal tremorgenic input to the motoneurons is not correlated with the corticomuscular coherence (Gallego et al., 2015b). In addition, the phase difference between the wrist flexor and extensor muscles activation is not related with the sole supraspinal input to the motoneuron pool, but also to an additional afferent input (Gallego et al., 2015a; Puttaraksa et al., 2019). This hypothesis led to the development of tremor reduction strategies based on the afferent fibers recruitment via PES delivered out-of-phase with the tremorgenic activity. Dosen et al., 2015 and Dideriksen et al., 2017 reported acute tremor reduction at the wrist when the stimulation was applied below the motor threshold (MT) on the antagonist muscle to the tremorgenic activity measured through EMG (out-of-phase strategy). Nonetheless, the inhibition of muscle activity elicited through electrical stimulation of the Ia afferent fibers was not directly proved in those studies.

Through Chapter 1 the relevance of afferent contribution to achieve robust and versatile body motion was empathized. Information from muscle proprioceptors and cutaneous sensors is transmitted to the CNS (Alessandro et al., 2018) and permits not only the sense of relative position of the body in space but also coordination of muscles acting across joints (Kandel et al., 2013). Excitatory and inhibitory pathways mediating the final output that results in muscle activity can be modulated with electrical stimulation (Stowe et al., 2008). For instance, some studies have shown that the stimulation of Ia afferents inhibits antagonist α motoneurons, a general principle of motor organization known as reciprocal inhibition, which assures reduction of the activation of an antagonist when the agonist muscle is activated (Petersen et al., 1998). Reciprocal inhibition in humans has been mainly studied between the ankle extensors and flexors (Mizuno et al., 1971). However, very few studies have investigated reciprocal inhibition and spinal reflexes in the upper limb.

Several factors might explain the reduced number of studies focused on the muscles acting at wrist and hand. On the one hand, the anatomic location of muscles and

nerves is more compressed in the upper forearm, leading to crosstalk issues both in the EMG recordings and the selective electrical stimulation (Knikou, 2008). In fact, the recording of FCR H-reflex through sEMG cannot be exclusively attributed to a FCR response, but also to simultaneous contribution from other muscles innervated by the median nerve such as the palmaris longus or finger flexors. On the other hand, the nerve tracks are longer in the lower limb, leading to a clearer differentiation among the different evoked potentials elicited through electrical stimulation. For instance, FCR H-reflex recruitment using median nerve at the elbow implies average M and H wave latencies of 5 ms and 16 ms respectively, which could lead to overlapping of both potentials for some subjects and recordings (Stowe et al., 2008). Instead, when recruiting the soleus H-reflex by means of stimulating the posterior tibial nerve, the soleus M and H waves latency are approximately 9 ms and 30 ms respectively, thus, both waves can be markedly identified (Burke, 2016). Besides, interneurons and motoneurons excitability are highly time dependent and the electrophysiological studies involving test and conditioning stimuli demand experimental paradigms capable of handling milliseconds resolution. For instance, the Group I EPSPs (excitatory post-synaptic potential) of Ia fibers has been estimated to last just around 1 or 2 milliseconds (Burke et al., 1984). An additional factor that has limited the upper-limb reflexes physiological studies might be related to the anatomical and functional motor control complexity. In lower limb, during the different gait phases (a motor task widely researched in neurorehabilitation), soleus and tibialis anterior acts primary as antagonist muscles (Capaday et al., 1990). However, in upper-limb, there are few tasks requiring repetitive and oscillatory movement of a joint as the wrist. Reaching, grasping or pinching movements are common tasks requiring the coordination of several muscle groups controlling several degrees of freedom, acting as synergists and being co-activated in most of scenarios to achieve fine motor control of the hand or the fingers as final effectors (Pierrot-Deseilligny E., 2012).

Baldissera et al., 1983 and Day et al., 1984 reported inhibition of the H-reflex in wrist flexors when stimulation of the radial nerve was timely synchronized with the test stimulus. Similar to the studies reporting inhibition of the soleus after stimulation of the peroneal nerve, Ia interneurons were hypothesized to be responsible for this disynaptic reciprocal inhibition (Baldissera et al., 1983). However, true reciprocal inhibition at the wrist level has been questioned, partly because of the synergistic action of these muscles to perform certain tasks and the possibility that Ib afferents may also be involved (Wargon et al., 2006). The wrist is considered a ball joint with 2 degrees of freedom, thus, pure rotations around one axis are not common movements. For instance, it has been shown that the flexor carpi radialis (FCR) receives inhibitory projections from Ia fibers innervating the flexor digitorum superficialis (Nito et al., 2018b). Contribution of Ib fibers to the group of interneurons projecting from ECR to FCR was proved by means of the H-reflex inhibition when stimulating the radial nerve after vibration was applied to the ECR tendon, a procedure that raises the recruitment threshold of Ia fibers. This evidence favours the absence of true reciprocal Ia inhibition for the wrist flexor and extensor muscles, however, the aforementioned experiments were performed by testing the H-reflex rest condition and the results might be limited to this paradigm since it is known the reflex circuits are adaptive to the movement task. Eventually, the coexistence of inhibition mechanisms onto disynaptic Group I interneurons elicited through both Ia and Ib afferents remains a plausible hypothesis (Pierrot-Deseilligny E., 2012). More recently, inhibition of ongoing wrist extensor muscle activity has been demonstrated after stimulation of brachioradialis during mid extension (Nito et al., 2018a). As an alternative to H-reflex inhibition paradigm, the use of electrical stimuli to modulate voluntary

muscle activity measured through EMG is a simple method to assess some spinal circuits during the execution of a certain motor task (Mrachacz-Kersting et al., 2017). Therefore, further characterization of this CNS modulatory mechanism is required.

Drawing from the limited evidence about the acute effects on muscle activity of electrical stimulation of antagonist muscles at the wrist, the main goal was to test the hypothesis that inhibition of wrist flexor and extensor activity can be achieved through the activation of afferents arising from the ipsilateral antagonists. The main hypothesis was tested with a twofold methodology. Firstly, the H-reflex stimulus-response curves for FCR and ECR were obtained to define the individual MT and ensure the activation of Ia afferent fibers for each subject. Secondly, inhibition of wrist extensor and flexor muscles was assessed by stimulating nerves innervating the antagonist muscles below MT and above MT during an isometric muscle contraction. The results reported here provide an initial step in further characterizing the capabilities of electrical stimulation to elicit reciprocal inhibition at the wrist level in healthy subjects.

3.4 Methods

3.4.1 Participants

Eight healthy volunteers (3 females and 5 males; age: 27 ± 4 years; height: 173 ± 9 cm), all of them right-hand dominant and with no neurological injuries volunteered to participate in this study. Participants were informed about the procedures and possible discomfort associated with the experiments. After that, they gave their informed written consent to participate. All procedures were conducted in accordance with the Declaration of Helsinki and approved by a local ethical committee.

3.4.2 Experimental protocol and data collection

The procedure was divided into two experiments. Firstly, recruitment curves of FCR and ECR spinal excitability were obtained by modulating the direct (M) and monosynaptic (H) responses to the nerve stimulation. Secondly, stimulation of the median and radial nerves below and above MT during isometric wrist extension/flexion tasks was applied to assess the amount of inhibition on ECR and FCR, respectively. The most dominant upper limb (right side for all participants) was chosen for stimulation and recording.

Experiment 1. H-reflex recruitment curves

The goal of this experiment was to obtain H-reflexes and M-waves of FCR and ECR for each participant in order to guarantee proper nerve stimulation by defining the MT for each muscle and subject, as well as the proof of activation of Ia afferent fibers, which would be necessary to modulate inhibition of both muscles in Experiment 2. Muscle activity from FCR and ECR was recorded using surface electromyography (sEMG) bipolar electrodes (Ag-AgCl, Ambu Neuroline 720, Ambu, Ballerup, Denmark). Each pair of electrodes was placed at a 2 cm inter-electrode distance over the belly of the muscles. FCR and ECR muscles were identified by palpation during manually resisted flexion or extension (Stowe et al., 2008) and following the recommendation described in Cram's guidelines (Eleanor, 2017). A wet wristband was used as reference and preliminary tests to check the quality of the signal and proper electrode positioning were also performed. sEMG signals were recorded using a

biosignal amplifier (Quattrocento, OT Bioelettronica, Italy), with a gain of 150, acquisition frequency of 10,240 Hz and were electronically band-pass filtered (10-4,400 Hz). Final data were stored for offline analyses.

Surface stimulation was delivered via bipolar round electrodes (3,2 cm diameter and 2 cm inter-electrode distance) (ValuTrode Cloth, Axelgaard Man., Denmark), and placed over the median and radial nerves, following similar procedures to those described in Stowe et al. (Stowe et al., 2008) (Figure 3.1). To stimulate the radial nerve, electrodes were placed over the radial side of the humeral region (Figure 3.1-A). On the other hand, electrodes were placed on the median side of the humeral region to stimulate the median nerve (Figure 3.1-B). A constant current stimulator unit (Digitimer DS7A, UK) was used. Median and radial nerves were stimulated to obtain the H-reflexes curves of FCR and ECR, respectively. The order of nerves stimulated was randomized in order to avoid any bias. Proper stimulation site was checked by palpation of the muscles and visually confirmed when motor response of the target muscle was clearly evoked. Before placing all electrodes (stimulation and sEMG electrodes), the subject's skin was cleaned with alcohol.

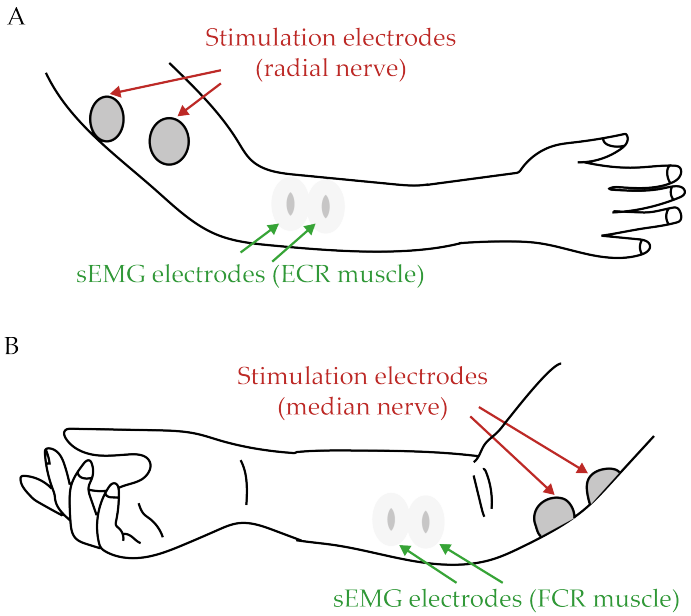

FIGURE 3.1: Electrodes placement for experiments 1 and 2. A. Surface electrodes were used to stimulate the radial nerve and elicit ECR activation. sEMG electrodes were placed over ECR muscle belly to record its activity. B. Surface electrodes were used to stimulate the median nerve and elicit FCR activation. sEMG electrodes were place over FCR muscle belly to record its activity.

To perform this experiment, participants comfortably sat on an armchair, with the dominant forearm resting horizontally on a table in front of the chair. The shoulder joint was kept at 45 degrees and the elbow joint was kept at 120 degrees (Figure 3.1-B). Participants were asked to slightly flex or extend the wrist to facilitate the development of the corresponding FCR or ECR reflexes (Baldissera et al., 1983). For each muscle and participant, 8-12 different stimulation intensities ranging from 0.5 to 26 mA were delivered to obtain a representative measurement of the excitability of the afferent and direct pathways. The order of delivery of each stimulation value was randomized. For each stimulation amplitude, 5 electrical monophasic squared pulses (1ms duration) were delivered with an inter-stimulus interval (ISI) of 5 ± 0.2

s to avoid post-activation depression (Burke, 2016). An Arduino board was used to control the ISIs and trigger the stimulator unit.

Experiment 2. Muscle inhibition through surface nerve stimulation

The goal of Experiment 2 was to modulate disynaptic Group I inhibition during a voluntary wrist flexion/extension task. Inhibition in the activity of the muscle active during the task is elicited by stimulating afferent fibers (presumably type Ia) of its antagonist muscle, evoking the Group I inhibition mechanisms. Motor threshold was defined as the minimum current that produced a muscle contraction, as detected visually and by palpating muscles (Muceli et al., 2019), and confirmed with the M-waves recruitment curves determined from Experiment 1. The stimulation and recording electrodes used previously in Experiment 1 were kept and used in Experiment 2. Experiment 2 was divided into 2 parts: i) FCR inhibition through radial nerve stimulation; and ii) ECR inhibition through median nerve stimulation. The order of nerves stimulated was randomized for each participant.

Participants were asked to keep the arm in the same position held during Experiment 1. Regarding the experiments performed in the first part (ECR inhibition), the subjects were asked to perform isometric wrist extension with the forearm pronated; for the second part (FCR inhibition), subjects were asked to perform isometric wrist flexion with the forearm supinated. The amount of muscle activation for each trial was kept around 10% of maximum voluntary contraction (MVC). This value was displayed on screen during the experiment to help subjects to maintain a constant level of muscle activity. Surface stimulation was delivered over the radial nerve (innervating ECR) or medial nerve (innervating FCR) to modulate the antagonist muscle (FCR and ECR, respectively) inhibition. Two different levels of stimulation were used: immediately below MT and above MT. In order to ensure proper afferent fibers recruitment, it was verified that the stimulation of radial and median nerves did not produce visible contraction of ECR and FCR, respectively, when stimulating below MT. For each stimulation amplitude (below MT and above MT), 3 trials were performed, each one consisting on sequences of 30 stimuli (electrical monophasic squared pulses with 1ms duration) with a randomized ISI of 2 ± 0.2 s. One-minute rest period was taken between trials in order to prevent fatigue.

3.4.3 Data analyses

All sEMG signals were processed offline with MATLAB R2018b (Mathworks, Natick, MA).

Experiment 1

Raw EMG signals were digitally band-pass filtered (2^{nd} order, zero-phase, Butterworth, 20-1000 Hz). For each participant, stimulation amplitude and muscle, filtered EMG data corresponding to 0 to 100 ms after each of the five stimuli were averaged. Peak-to-peak amplitudes of H-reflexes and M-waves were calculated from each average profile, following similar procedures to those described in (Stowe et al., 2008): H-reflexes were assessed in the time window of 16-25 ms and M-waves were assessed in the time window of 4-15 ms after stimulation (Figure 3.2-A). After that, for each participant, stimulation amplitude and nerve stimulated, the average H-reflex and M-wave was computed across stimuli and intensity. Mean values were

expressed as a proportion of maximum M-wave (Figure 3.2-B) (Knikou, 2008). M-wave recruitment curves were used to determine the stimulation levels to be used in Experiment 2: below MT and above MT. Recruitment of H-reflexes implies the activation of Ia afferent fibers since the recorded wave response is primary caused by the monosynaptic excitation of the homonymous motoneuron.

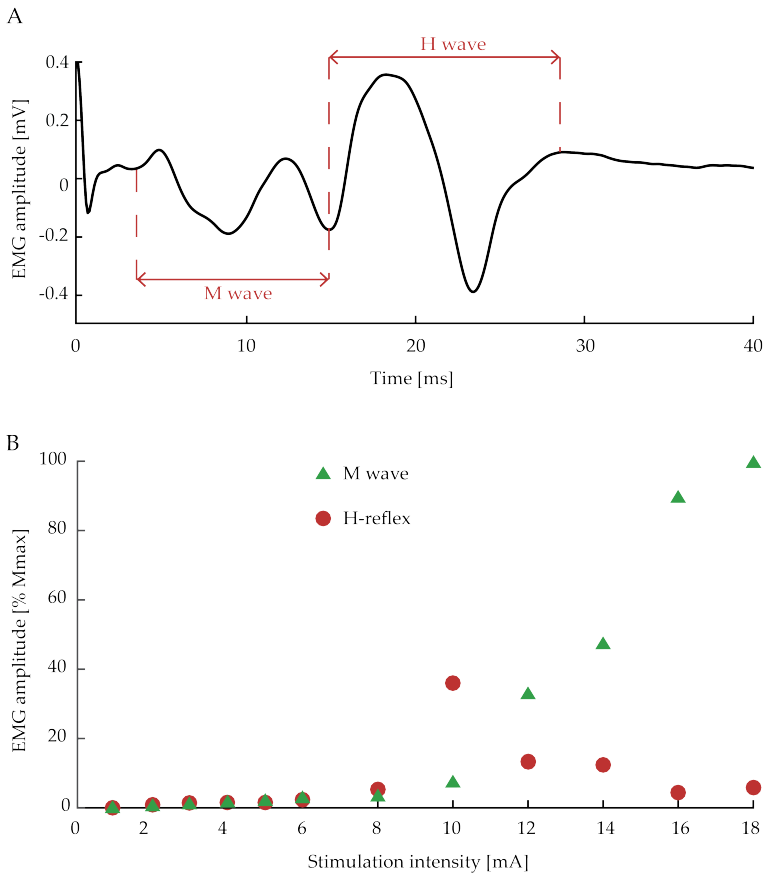

FIGURE 3.2: H-wave response and associated recruitment curve. A. Example of individual EMG data recorded from FCR after stimulating the median nerve with 8 mA. M-wave and H-reflex are clearly displayed. B. Recruitment curves for FCR. M-wave curve is depicted with green triangles and H-reflex curve is depicted with red circles and as a function of % maximum M-wave. Data from S01.

Experiment 2

Raw EMG signals were digitally band-pass filtered (2^{nd} order, zero-phase, Butterworth, 20-1000 Hz) and then rectified. Rectification of the raw EMG signals is a widespread procedure to consider the contributions from both positive and negative electrical potentials resulting from the MU firings (Pierrot-Deseilligny E., 2012). For each participant, muscle and stimulation amplitude, data corresponding to the total of 90 stimuli across the three trials were segmented (Figure 3.3) and then averaged to compute the profile of on-going EMG for a given subject, muscle and stimulation amplitude. The baseline activity was calculated as the averaged rectified EMG in the time interval from 140 to 40 ms before the stimulus (Figure 3.3-A). The beginning of inhibition was determined as the time when the signal was lower than baseline for at least 5 ms. The end of the inhibition was set as the time when the signal was

no longer lower than baseline for at least 5 ms. These timings were always confirmed by visual inspection. For each subject, muscle and level of stimulation, the amount of inhibition was calculated as the mean activity in the inhibition window previously defined (Figure 3.3-B) and normalized as the percentage of the baseline (100%) value. Additionally, the maximum peak of inhibition, defined as the lowest value in the inhibition time window, was considered as an inhibition feature.

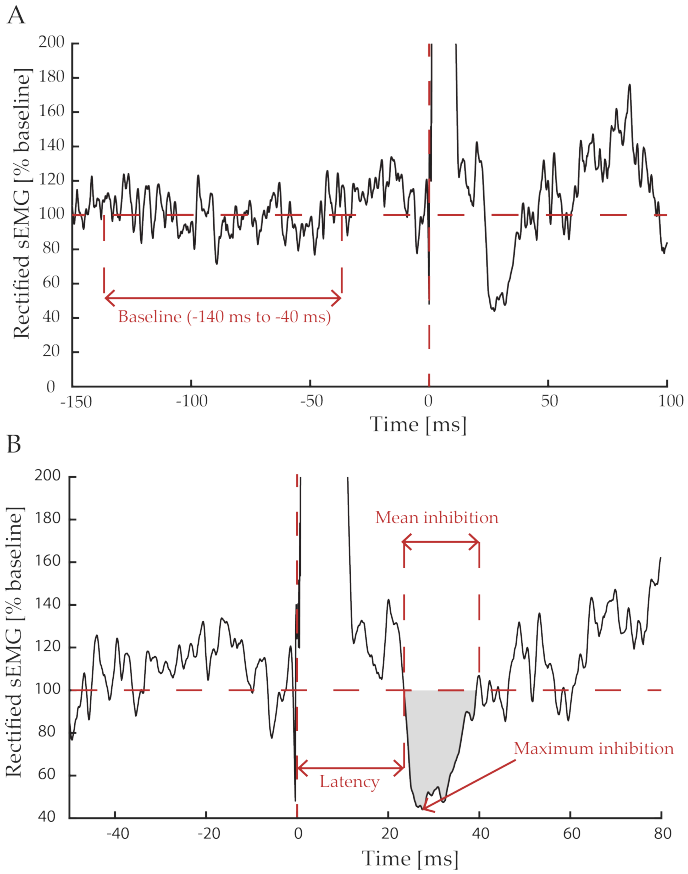

FIGURE 3.3: Individual example of a segment of data (filtered and rectified EMG from FCR) corresponding to the stimulation of the radial nerve above MT. A. The baseline activity was calculated as the average of the signal from 140 ms to 40 ms before the stimulus. B. The zoomed image shows the inhibition of FCR activity (gray area), as well as its peak, latency and duration.

3.4.4 Statistical analyses

Shapiro-Wilk's test was applied to check the normal distribution of the mean inhibition scores. For each subject, muscle and level of stimulation, paired t-tests across stimuli were performed to assess differences of the rectified EMG activity between baseline epochs and the mean inhibition computed during the selected time windows. A two-way ANOVA procedure, with stimulation intensity (below MT, above MT) and muscle inhibited (FCR, ECR) as factors, was applied to test differences and interaction of mean inhibition and maximum peak of inhibition among all subjects. Post-hoc Tukey's test was used to find statistical differences between factors. Statistical significance was set by a p-value of 0.05.

3.5 Results

3.5.1 H-reflex recruitment curves

In this experiment, H-reflexes and direct M responses were elicited in all 8 subjects for FCR and ECR muscles by means of stimulation of the median and radial nerve, respectively. Figure 3.2-A shows an individual example of a M-wave and H-reflex obtained in response to median nerve stimulation. For this specific subject, maximum H-reflex was produced when stimulating at 10 mA; and M-wave amplitude increased until stimulating up to 18 mA (Figure 3.2-B).

The stimulation intensity needed to recruit maximum FCR H-reflexes were on average 37% of maximum M-wave among subjects, while maximum stimulation intensity needed for ECR H-reflexes were on average 5% of maximum M-wave. The stimulation intensities needed to elicit the minimum H-reflex were 24 ± 12% and 46 ± 11% of the intensity needed to recruit the maximum M-waves for FCR and ECR, respectively. The H-reflexes obtained in this experiment ensured proper placement of the stimulation electrodes to recruit Ia fibers. In addition to visual inspection and muscle palpation, MT values for each participant and muscle were determined based on the stimulation amplitudes that would also elicit visible M-waves. On average, MT was 15 ± 5% for FCR, and 61 ± 15% for ECR, of the amplitude required for eliciting maximum M-wave. MT values were then used as guides for setting the stimulation values below MT and above MT in the following experiment.

3.5.2 Inhibition of ECR activity from median nerve stimulation

Two different stimulation amplitudes were applied over the median nerve to evoke inhibition in ECR activity: stimulation below MT was on average 14 ± 5% of maximum M-wave; stimulation above MT was 27 ± 9% of maximum M-wave. The stimulation of the median nerve using both amplitudes elicited an inhibition in the averaged rectified EMG signals of ECR in a time window compatible with spinal reflexes (Figure 3.4-A). For stimulation below MT, inhibition was statistically significant in 6/8 subjects ($p < 0.05$), while for stimulation above MT, all subjects (8/8) presented a significant inhibition in the ECR average and rectified EMG activity ($p < 0.05$) (Table 3.1).

For stimulation below MT, inhibition started at 22 ± 7 ms, finished at 38 ± 7 ms, with a total duration of 16 ± 7 ms. Maximum peak of inhibition was 27 ± 6% and mean inhibition was 12 ± 4%. For stimulation above MT, the onset of the inhibition happened at 21 ± 5 ms and finished at 40 ± 2 ms, with a total duration of 18 ± 5 ms. Maximum peak of inhibition was 45 ± 7% and mean inhibition was 22 ± 6%.

3.5.3 Inhibition of FCR activity from radial nerve stimulation

Electrical stimulation delivered at two different amplitudes was applied on the radial nerve to evoke inhibition in FCR activity: stimulation applied below MT was 57 ± 13% of maximum M-wave; and stimulation applied above MT was 75 ± 12% of maximum M-wave. Both stimulation amplitudes of the radial nerve produced an inhibition in the averaged and rectified sEMG signals of FCR (Figure 3.4-B). For stimulation below MT, inhibition was statistically significant in 7/8 subjects ($p < 0.05$), while for stimulation above MT, all the subjects (8/8) presented a statistically significant inhibition in the FCR rectified EMG activity ($p < 0.05$) (Table 3.2).

For stimulation below MT, inhibition started at 21 ± 4 ms, finished at 38 ± 8 ms, with a total duration of 17 ± 10 ms. The value for the maximum peak of inhibition

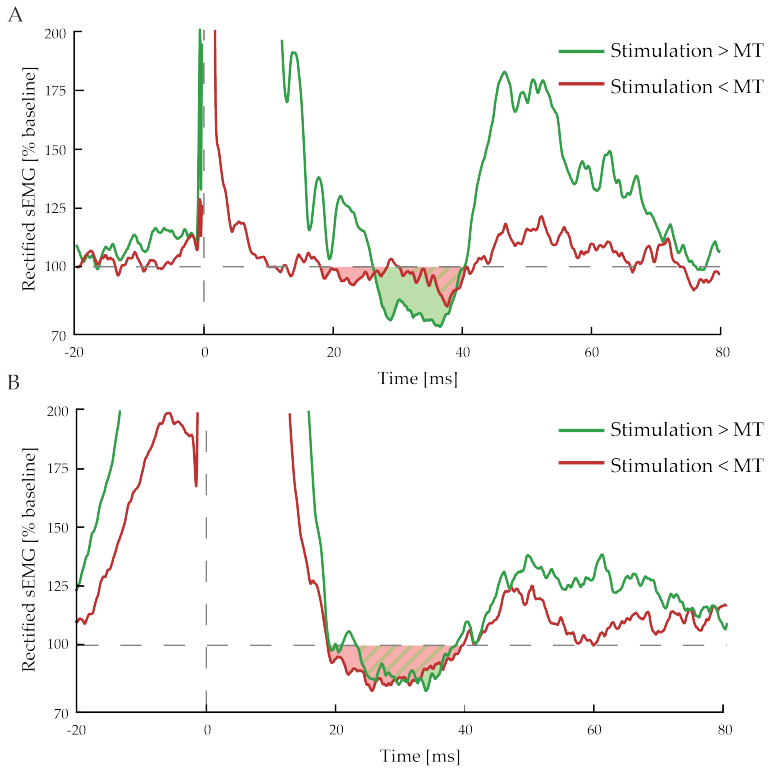

FIGURE 3.4: A. Average rectified EMG from ECR across subjects in response to median nerve stimulation < MT (red line - mean inhibition 12 ± 4%, maximum peak of inhibition 27 ± 6%) and > MT (green - mean inhibition 22 ± 6%, maximum peak of inhibition 45 ± 7%) of the median nerve. B. Average rectified EMG from FCR across subjects in response to radial nerve stimulation < MT (red line - mean inhibition 16 ± 6%, maximum peak of inhibition 32 ± 9%) and > MT (green line - mean inhibition 25 ± 10%, maximum peak of inhibition 44 ± 13%) of the radial nerve.

was 32 ± 9% and the mean inhibition was 16 ± 6%. For stimulation above MT, the onset of the inhibition was at 20 ± 4 ms and finished at 37 ± 8 ms, with a total duration of 17 ± 7 ms. The value for the maximum peak of inhibition was 44 ± 13% and mean inhibition was 25 ± 10%.

Two-way ANOVA analysis showed differences in the mean inhibition and maximum peak of inhibition between the factors muscle and stimulation intensity ($p < 0.05$). Post-hoc tests showed statistically significant differences in the stimulation intensity factor (below MT vs above MT) in both mean inhibition and maximum peak of inhibition (Figure 3.5). No differences were found for the amount of mean inhibition or maximum peak of inhibition between the FCR and ECR. Interaction between muscle inhibited and stimulation intensity presented no significant differences.

Finally, it is also worth mentioning that both ECR and FCR rectified and averaged sEMG activity increased following the inhibition time window (Figure 3.4-A,B, respectively). This observation was present for both stimulation intensities showing an increased excitation after electrical stimulation above MT.

TABLE 3.1: Summary of the stimulation parameters used to stimulate the median nerve and the resulting ECR inhibition features.

Subject	Condition	Intensity [mA]	Inhibition start [ms]	Inhibition end [ms]	Duration [ms]	Maximum inhibition [%]	Mean inhibition [%]	p-value (mean inhib)
S01	<MT	1.80	30.70	40.07	9.37	33.38	13.30	0.036
	>MT	3.60	25.42	40.56	15.14	46.83	19.74	<0.001
S02	<MT	2.00	20.54	34.50	13.96	19.65	3.24	0.424
	>MT	4.00	27.18	41.83	14.65	39.15	18.57	<0.001
S03	<MT	1.50	19.47	45.15	25.68	30.59	10.88	0.012
	>MT	3.00	18.10	38.90	20.80	33.34	13.95	<0.001
S04	<MT	4.50	17.32	32.94	15.62	22.27	8.82	0.102
	>MT	9.00	17.81	39.68	21.87	42.82	18.01	<0.001
S05	<MT	1.10	17.22	41.93	24.71	28.52	12.99	<0.001
	>MT	2.20	21.71	40.27	18.56	54.22	29.00	<0.001
S06	<MT	3.50	21.13	38.31	17.18	18.93	6.78	0.030
	>MT	7.00	11.46	37.73	26.27	55.57	20.71	<0.001
S07	<MT	1.20	32.06	43.10	11.04	32.07	20.09	<0.001
	>MT	2.60	21.91	41.83	19.92	42.50	24.11	<0.001
S08	<MT	0.80	14.68	25.54	10.86	20.05	8.20	0.049
	>MT	1.60	27.47	36.85	9.38	43.84	31.54	<0.001

TABLE 3.2: Summary of the stimulation parameters used to stimulate the radial nerve and the resulting FCR inhibition features.

Subject	Condition	Intensity [mA]	Inhibition start [ms]	Inhibition end [ms]	Duration [ms]	Maximum inhibition [%]	Mean inhibition [%]	p-value (mean inhib)
S01	<MT	10.00	18.98	30.31	11.33	26.04	12.08	0.046
	>MT	14.00	23.86	38.41	14.55	39.91	29.66	<0.001
S02	<MT	8.00	19.95	33.14	13.19	30.04	18.00	<0.001
	>MT	10.00	18.68	37.14	18.46	36.47	18.89	<0.001
S03	<MT	8.00	23.08	28.65	5.57	20.23	7.40	0.299
	>MT	10.00	18.39	37.83	19.44	59.65	31.40	<0.001
S04	<MT	11.00	30.50	39.58	9.08	25.34	11.05	0.038
	>MT	14.00	18.49	33.92	15.43	35.44	14.32	0.038
S05	<MT	12.00	21.91	33.24	11.33	31.51	19.80	<0.001
	>MT	14.00	16.05	30.60	14.55	42.34	20.61	<0.001
S06	<MT	12.00	17.22	39.29	22.07	42.54	21.81	<0.001
	>MT	14.00	17.12	43.39	26.27	52.92	27.01	<0.001
S07	<MT	8.00	18.39	42.81	24.42	37.50	12.74	<0.001
	>MT	14.00	19.17	23.37	4.20	24.38	13.29	0.017
S08	<MT	8.00	20.05	53.94	33.89	44.46	23.72	0.001
	>MT	12.00	26.40	48.67	22.27	63.61	42.60	0.001

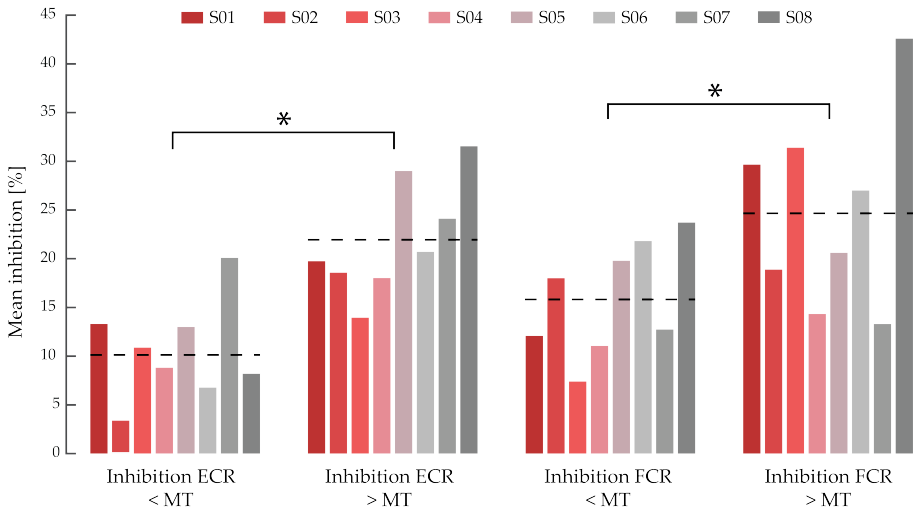

FIGURE 3.5: Mean inhibition obtained across all subjects, for each stimulation intensity and recorded muscle. Each bar and color represents the average for each subject. Black horizontal dashed lines represent the mean of the 8 subjects. * significant differences ($p < 0.05$) between conditions.

3.6 Discussion

The main goal of this study was to inhibit voluntary muscle activity of wrist flexor and extensor muscles via stimulation of afferent pathways . In order to demonstrate and characterize this type of inhibition, a twofold methodology was followed. As a first step, the H-reflex and M-wave recruitment curves for FCR and ECR to prove the activation of Ia afferent fibers. Thereafter, the antagonist muscle was stimulated through the nerve to evoke inhibition in the active muscle during wrist flexion and extension tasks. Furthermore, the secondary goal of mastering some electrophysiology techniques used for the assessment of the neuromuscular system, among which are EMG and PES, was achieved through this study. Overall, the results obtained in this study represent an important first step to characterize the capabilities of PES of afferent pathways for tremor reduction.

3.6.1 H-reflex recruitment curves

The results from Experiment 1 validated the application of electrical stimulation to assess spinal excitability through H-reflex recruitment for the wrist muscles in healthy humans. The H-reflex curves obtained following similar procedures to those described by Stowe et al., 2008 were essential to confirm proper electrode positioning for each subject and muscle, which allowed the activation of afferent pathways involved in the disynaptic Group I inhibition. Furthermore, the M-wave recruitment curves were used to determine MT of stimulation for each participant and muscle. Inter-stimulus intervals (ISIs) used in Experiment 1 were larger than those used in Experiments 2, since the muscles were almost at rest in Experiment 1 and, thus, longer ISIs were needed to prevent post-synaptic depression and obtain a reproducible reflex (Mrachacz-Kersting et al., 2017).

3.6.2 Inhibition of ECR and FCR muscle activity

The experiments performed in the second part of this study (Muscle inhibition through nerve surface stimulation) allowed to elicit inhibition of FCR and ECR muscle activity via surface stimulation of radial and median nerve, respectively. Not only voluntary muscle activity of FCR and ECR was modulated by activating spinal reflexes pathways, but it was also demonstrated that this modulation was viable with stimulation below MT and above MT, though higher inhibition was achieved with stimulation above MT. Baldissera et al., 1983 demonstrated inhibition of the H-reflex of wrist flexors through the stimulation of the radial nerve, whereas they failed to observe reciprocal effect of wrist extensors through the stimulation of the median nerve. In their experiment, the hand was kept in a resting position, with the forearm muscles relaxed. Nonetheless, in this experiment each subject performed a voluntary isometric task, so the excitability of the descending and afferent circuits were modified.

Nito et al., 2018a performed a similar protocol to the one performed in this study. Although the authors did not assess ECR inhibition when stimulating the median nerve, they assessed FCR inhibition using surface stimulation of the radial nerve below MT, targeting the recruitment of those fibers innervating the brachioradialis muscle. Mean inhibition obtained in our study was bigger (16 ± 6% vs 8.8 ± 0.9%), of a longer latency (21 ± 5 ms vs 14.7 ± 1.2 ms) and of a longer duration (17 ± 10 ms vs 4.8 ± 0.9 ms) that those reported by Nito et al., 2018a. Berardelli et al., 1987 performed a similar pilot experiment and the latency of the reported inhibition was in the range of 20-25 ms lasting around 20 ms, which is in line with the results obtained in this study. These differences may be explained by differences in the methodological testing procedures, including electrode positioning. The importance of performing individual H-reflex recruitment curves is crucial so that an adequate stimulation of Group I afferents including Ia fibers is performed.

PES applied below MT is an effective neurophysiological testing procedure to activate Group I afferents without recruiting motor nerves (Barroso et al., 2019a). However, exclusive recruitment of Ia fibers is not possible by means of transcutaneous or surface stimulation. Wargon et al., 2006 provided consistent evidence about the absence of true Ia reciprocal inhibition between flexor and extensor muscles of the wrist. However, their results were limited to the assessment of H-reflex, and therefore, should not be extrapolated to other experimental paradigms considering dynamic or isometric tasks. It has been demonstrated that the organization of the spinal circuits of the upper-limb, and particularly those controlling the wrist muscles are different from the circuits of the lower limb. Pure and isolated spinal reflexes such as Ia reciprocal inhibition are likely a simplified representation of the real circuits for such complex joints as the wrist or the fingers involved in fine motor control. For instance, results presented by Nito et al., 2018a when stimulating the radial nerve below MT suggested that Group Ia afferents should mediate the inhibition of FCR motoneurons, as shown through the application of a vibration protocol.

Contribution from Ib fibers to elicit the reported inhibition cannot be ruled out. Additionally, for the stimulation above MT, it is possible that other pathways rather than Group I afferents (Ia, Ib) are activated including cutaneous afferents that may contribute to the inhibition of muscle activity (Mrachacz-Kersting et al., 2017). EMG averaging method does not provide enough temporal resolution to determine the contribution of each of these pathways to the inhibition profile. Nonetheless, our study also shows that muscle inhibition is possible when applying stimulation below MT. Previous studies have shown that an intensity of 1.1 times motor threshold,

can activate Ia afferents and spinal pathways for reciprocal inhibition at rest (Fuhr et al., 1993). These intensities are sufficient not to saturate the reciprocal inhibitory pathway (Petersen et al., 1999). The first phase of the long-duration inhibition most likely reflects disynaptic and presynaptic Ia inhibition, while the late phase may reflect activity in long-loop or transcortical reflexes. Indeed, cortical control of the wrist is relayed through cervical propriospinal interneurons (Gracies et al., 1991; Pierrot-Deseilligny, 1996), which are also activated during unilateral arm movements (Stinear et al., 2004). Based on these previous reports, we hypothesize that inhibition evoked from the median and radial nerves on ECR and FCR, respectively, is partially mediated by type Ia fibers involved in disynaptic Group I inhibition. Other spinal mechanisms such as Group I interneurons activated via Ib fibers, and the propriospinal neurons, are also presumably involved in the reported inhibition. The late excitation observed after the muscle activity inhibition noted for both FCR and ECR (Figure 3.4) has been described by Berardelli et al., 1987. One hypothesis relies on the synchronized firing of all the silenced motoneurons after the effect of the IPSPs (inhibitory post-synaptic potential) responsible for the reported inhibition is lapsed. Further studies are required to characterize the specific modulatory mechanisms involved in both reciprocal inhibition and facilitation, especially at the wrist level.

3.6.3 Limitations and future work

This study provides a first step to benchmark novel tremor reduction strategies by modulating the voluntary muscle activity through low-threshold stimulation. There is evidence of the potential application of PES to activate afferent pathways triggering inhibitory mechanisms which could ultimately disrupt the tremorgenic activity at the CNS (Dosen et al., 2015). The results here described are aligned with the acute tremor reduction hypothesis based on the out-of-phase stimulation strategy, by which the alternating stimulation of the antagonist muscle to the tremorgenic activity can lead to acute inhibition of the tremor input at the spinal cord level (Gallego et al., 2015a).

Precise characterization of the afferent fibers that might be involved in the inhibition mechanisms has not been addressed in this study. Though the absence of this knowledge does not prevent the development of tremor reduction strategies based on PES, unveiling all the fibers and spinal circuits participating in the elicited inhibition of the antagonist muscle would allow better characterization of the modulatory effects of PES, which could be applied to other pathologies. The use of intramuscular stimulation electrodes would probably provide a solution to achieve greater selectivity for afferent activation, since the stimulation applied within the muscle belly would allow better isolated recruitment of Ia fibers. Finally, the stimulation parameters here applied were set in order to obtain measurable changes in the ongoing EMG after delivering single stimulus. In following studies, particularly those aiming at designing a tremor reduction intervention, stimulation parameters should be tuned to provide an optimal therapeutic treatment of pathological tremor activity.

Chapter 4

Selective and Adaptive Timely Stimulation of afferent pathways

4.1 Abstract

Peripheral electrical stimulation (PES) of afferent pathways is a tool commonly used to induce neural adaptations in some neural disorders such as pathological tremor or stroke. However, the neuromodulatory effects of closed-loop stimulation strategies have been scarcely researched in the upper-limb. Here, the short-term spinal effects of a 20-minute stimulation of afferent pathways protocol applied with a closed-loop strategy named Selective and Adaptive Timely Stimulation (SATS) was explored. The SATS strategy was applied to the radial nerve in-phase (INP) or out-of-phase (OOP) with respect to the muscle activity of the extensor carpi radialis (ECR). The neural adaptations at the spinal cord level were assessed for the flexor carpi radialis (FCR) by measuring disynaptic Group I inhibition, Ia presynaptic inhibition, and Ib facilitation from the H-reflex, and estimation of the neural drive before, immediately after, and 30 minutes after the intervention. SATS strategy was proved to deliver synchronous stimulation with the real-time measured muscle activity with an average delay of 17±8 ms. SATS-INP induced an increase of the disynaptic Group I inhibition (77±23% of baseline conditioned FCR H-reflex), while SATS-OOP elicited the opposite effect (125±46%). Not all the subjects maintained the changes after 30 minutes. Additionally, no other significant specific neural adaptations were found for the rest of measurements. These results suggest that the short-term modulatory effects of phase-dependent PES occur at the specific targeted spinal pathways for the wrist muscles in healthy individuals. Overall, timely recruitment of afferent pathways with the muscle activity is a fundamental principle which should be considered in tailoring PES protocols for the specific neural circuits to be modulated.

4.2 Goals

The main goal of this study was to demonstrate that the SATS strategy induces induces short-term neuromodulatory effects on the muscles controlling the wrist in healthy subjects. On the one hand, the SATS strategy was designed and tested as a closed-loop stimulation strategy using real-time EMG measurements to apply stimulation synchronously with the physiological activity. Additionally, the phase of the stimulation with respect to the muscle activity was researched in order to determine its impact on the modulation effects at the spinal cord level. The results from this study would serve as the basis for testing the SATS strategy in patients suffering from ET as solution to manage pathological tremor.

4.3 Introduction

Motor control is regulated by the central nervous system (CNS) through the interplay between afferent inputs from different sensory systems and supraspinal commands, which produces coordinated and adaptive neural instructions directed to the neuromusculoskeletal system (Blakemore et al., 1998). By means of neural plasticity, synaptic transmission between neurons is potentiated or depressed in order to acquire or maintain stability of functions, a fundamental mechanism of learning and adaptation following neural injury or acquisition of new behaviors (Wolpaw, 2007). Plasticity at the spinal cord level plays a fundamental role in coordinating adaptive motor response to specific tasks (Wolpaw et al., 2001): interneurons are actively involved in the neuromodulation of afferent information, projecting it to the brain or directly to the spinal motoneurons and, therefore, adjusting the excitatory/inhibitory balance of specific pools of motor units (Fetz et al., 2000).

Muscle spindles, which are stretch receptors, convey information on muscle length changes to the CNS through Ia afferent fibers, one of the main pathways involved in proprioception (Kröger et al., 2021). Particularly, reciprocal inhibition pathway mediated by Ia fibers enables relaxation of the antagonist muscle to favor a functional movement. Nevertheless, as discussed in Chapter 3, some studies suggest that true reciprocal Ia inhibitory interneurons are replaced by the disynaptic Group I inhibitory interneurons, which receive major contribution from Ib fibers, and Ia fibers as well, and this mechanism mediates the inhibition of the antagonist muscle rather than the pure Ia pathway reported for the elbow or ankle joints (Wargon et al., 2006).

There is still no consensus on whether short-term (from minutes to hours) spinal cord plasticity is exclusively activity-dependent (i.e., plastic changes at the spinal cord level are associated with movement) or instead might also involve the CNS across different behaviors (Giboin et al., 2020). Several studies have supported the idea that plastic changes are induced during interventions involving training of specific motor tasks (Geertsen et al., 2008). Conversely, other studies have provided results supporting alterations of some CNS circuits even without engaging in any particular behavior or motor tasks (Mazzocchio et al., 2006; Perez et al., 2005).

In the past few years, peripheral electrical stimulation (PES) of afferent (sensory) pathways has been tested as a strategy to modulate the CNS aiming at restoring impaired functions in patients suffering from neural disorders such as spinal cord injury (SCI), stroke or essential tremor (ET) (Barroso et al., 2019a; Gil-Castillo et al., 2020). Specifically, some studies have applied PES to enhance activity-dependent plasticity of the CNS by recruiting afferent fibers paired with physiological activity (Mrachacz-Kersting et al., 2012). For instance, patterned stimulation of tibialis anterior was shown to enhance reciprocal inhibition of the soleus in healthy subjects (Perez et al., 2003). In other study, PES of the first dorsal interosseous muscle paired with single pulse of transcranial magnetic stimulation (TMS) at the corticospinal-motoneuronas synapses improves corticospinal transmission in healthy and SCI patients (Urbin et al., 2017). Plastic changes induced after PES interventions are not only limited to neurophysiological measurements: functional improvements have been reported in different motor disorders (Mrachacz-Kersting et al., 2012). Regarding pathological tremor, PES of afferent pathways targeting the antagonist muscle to the tremorgenic activity at the wrist level has been proved reduce pathological tremor in ET patients (Dosen et al., 2015; Dideriksen et al., 2017).

Despite the recent translation from bench to bedside of several PES-based therapeutic interventions in the upper-limb, including tremor management solutions,

none of the prior studies have explored the short-term neural changes elicited after a PES intervention onto the muscles controlling the wrist (Chapter 2). The main goal of this study was to assess short-term spinal effects of a novel phase-dependent closed-loop PES strategy named Selective and Adaptive Timely Stimulation (SATS). To achieve this goal, the afferent fibers innervating extensor carpi radialis (ECR) were recruited by stimulating the radial nerve in-phase or out-of-phase with the muscle activity measured in real-time through EMG. This was done while healthy volunteers performed fast wrist flexion-extension movements (mimicking pathological tremor). A set of neurophysiological techniques including disynaptic Group I inhibition of the H-reflex, Ia presynaptic inhibition, Ib fibers facilitation and estimation of the neural drive, were applied to assess the effects of the intervention immediately after and 30 minutes after the stimulation ceased. It was hypothesized that in-phase and out-of-phase stimulation would elicit an increase and decrease in wrist disynaptic Group I inhibition of the H-reflex, respectively, similarly to what has been observed for the soleus and tibialis anterior muscles in the lower limb. By unravelling some of the spinal mechanisms underlying neural plasticity after time-dependent PES of afferent pathways, this study provides fundamental knowledge that can be used to develop therapeutic approaches for some neural impairments, including pathological tremor.

4.4 Methods

4.4.1 Participants

Eleven healthy subjects (aged 22-27 years, 6 male, 5 female) volunteered to participate in the study. All procedures were approved by an institutional review board and participants signed a written informed consent in accordance with the Declaration of Helsinki.

4.4.2 Experimental paradigm

The short-term effects on FCR after the selective and adaptive timely stimulation (SATS) strategy applied in-phase (INP) or out-of-phase (OOP) with respect to the extensor carpi radialis (ECR) muscle activity were assessed (Figure 4.1-A). The study consisted of two experimental sessions for each participant, separated by at least 3 days (wash-out period). In each of the two sessions, one stimulation strategy randomly assigned (SATS-INP or SATS-OOP) was applied during the intervention phase. The subjects were blinded to the stimulation condition. The neurophysiological assessments were similar across visits, and included three time points: baseline (PRE), immediately after the stimulation (POST), and 30 minutes after the stimulation (POST30').

Experimental setup

Subjects comfortably sat on an armchair throughout the experimental procedures, during which they were asked to rest their dominant forearm on an armrest, keeping their shoulder extended and the elbow extended at approximately 45 degrees and 120 degrees in supination, respectively (Figure 4.1-B).

To record muscle activity during the neurophysiological assessments, high-density EMG (hdEMG) grids (13×5 electrode grid, 8 mm inter-electrode distance;

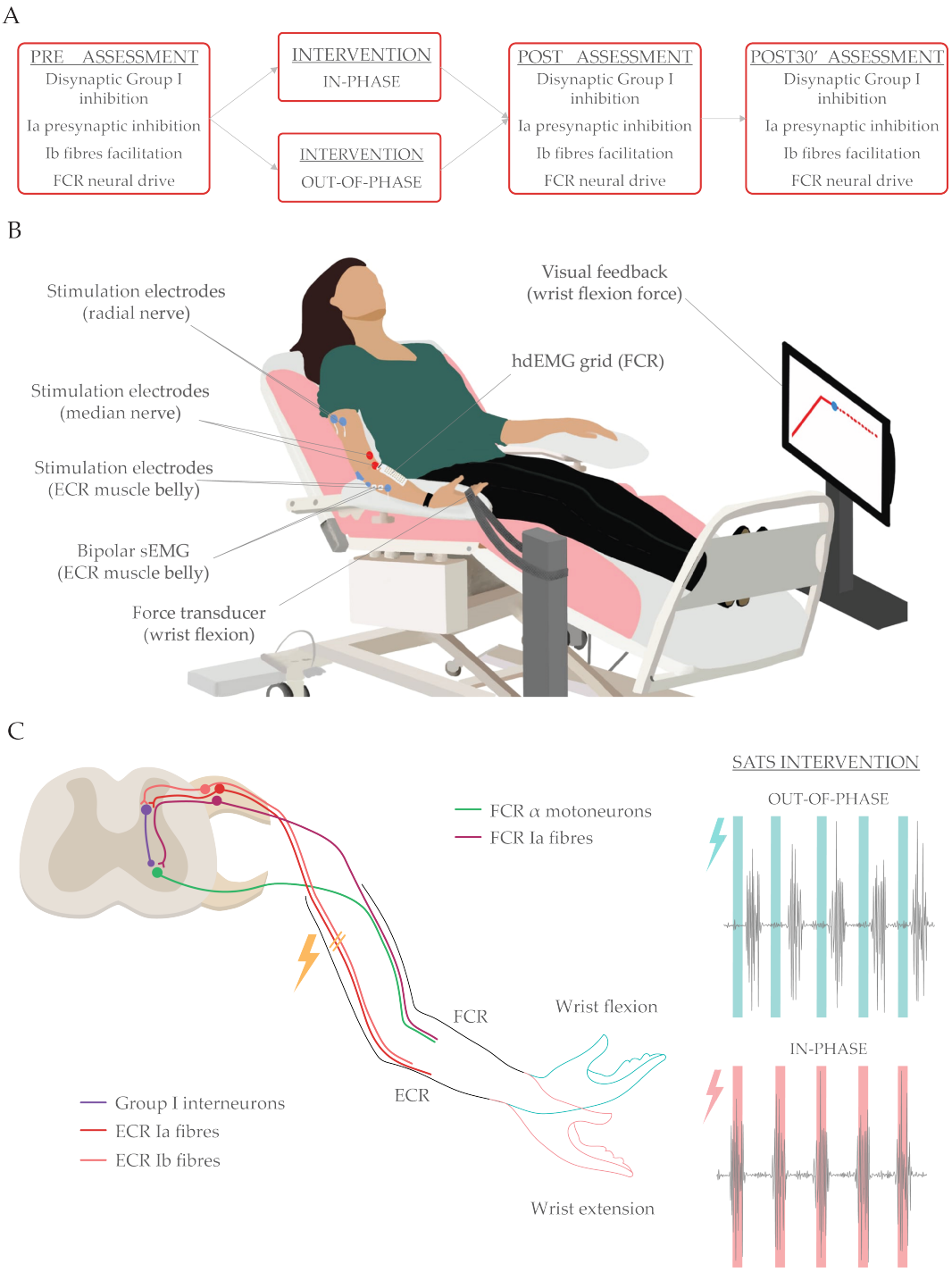

FIGURE 4.1: A. Experimental protocol. Subjects underwent two experimental sessions in different days, in which the either SATS-INP or SATS-OOP strategy was applied during 20 minutes. Neurophysiological assessments were performed before, immediately after and 30 minutes after the intervention. B. Illustration of the experimental setup used in this study. C. Simplified schematics of the spinal circuits involved in the experimental hypothesis. On the right, grey lines represent EMG activity of the ECR muscle, while colored rectangles represent the applied stimulation. Note that during the SATS-INP intervention, the stimulation was delivered to the radial nerve when the ECR muscle was active, while during the SATS-OOP intervention, the stimulation was delivered out-of-phase to the ECR activity.

OT Bioelettronica, Italy) were placed over the muscle belly of the flexor carpi radialis (FCR). Monopolar EMG signals were acquired at 2,048 Hz with a biosignal amplifier (Quattrocento; OT Bioelettronica, Italy) and stored for offline analysis. In addition to hdEMG assessments, bipolar surface (sEMG) electrodes were placed on the ECR muscle belly and the wrist flexors group (20 mm inter-electrode distance) in order to record muscle activity as input for the EMG-based stimulation strategies. A customized embedded system (OT Bioelettronica, Italy) with processing, stimulation and biosignal amplification capabilities was used to acquire the sEMG signals at 2,000 Hz. A wet wristband was used as reference for both EMG recordings modalities.

Two surface round stimulation electrodes (2 cm diameter, Axelgaard, Denmark) were placed over the median nerve at the cubital fossa, and two over the radial nerve at the spiral groove (Wargon et al., 2006). Prior to positioning the surface electrodes for stimulation, a stimulation bar was used to find the optimal location to elicit wrist extension when applying stimulation to the radial nerve, as well as to elicit wrist flexion and a reproducible FCR H-reflex when applying stimulation to the median nerve. Additionally, two bipolar surface stimulation electrodes (3.2 cm diameter, Axelgaard, Denmark) were placed over the ECR muscle belly (cathode proximal) to directly recruit the muscle fibers during the Ib fibers facilitation assessment. Two constant current stimulators (DS5, Digitimer, UK) were used to deliver the electrical stimuli during the neurophysiological assessments. The customized embedded system was used to run the stimulation strategies during the intervention.

During the neurophysiological assessments, a force sensor was used to measure the force exerted by the subject, which was displayed through visual feedback to guide the subject while performing the submaximal isometric flexion task (Figure 4.1-B).

4.4.3 Stimulation interventions

Subjects were asked to mimic typical tremorogenic movements by performing fast wrist flexion/extension with their dominant arm for 20 minutes. In each session, one of the two stimulation strategies (SATS-INP or SATS-OOP) was applied while subjects mimicked tremor movements (Figure 4.1-C). The intervention was divided into two periods of 10-minute stimulation each and 2 minutes of rest between them to minimize fatigue of the wrist muscles. SATS consists of a closed-loop EMG-based stimulation in which tremorgenic bursts are detected in a pair of antagonist muscles through the EMG signals and stimulation is consequently applied to one of the muscles (antagonist or agonist) to activate afferent pathways timed with physiological activity (Figure 4.1-C). Stimulation was delivered through the surface electrodes placed on the radial nerve and a common ground electrode placed on the olecranon. Stimulation frequency was set to 100 Hz to optimize recruitment of Ia afferent fibers (Dideriksen et al., 2015), pulse width was set to 400 μs (Dosen et al., 2015), and duty cycle was set to 20% of the tremor cycle to allow fast adaptation to rapid tremor movements. Stimulation intensity was calibrated for each subject prior to the intervention, being set immediately below motor and discomfort thresholds. SATS strategy operation was the following: 1 s recording windows were sequentially acquired and demodulated in order to determine the movement frequency (Figure 4.2). If the estimated frequency was within the tremor bandwidth (3-12 Hz), a 4 s stimulation window would be enabled. Along the stimulation window, the root mean square (RMS) of short EMG windows ($\sim$15 ms) for each muscle were computed in real-time and compared to an adaptive threshold computed as the RMS during the

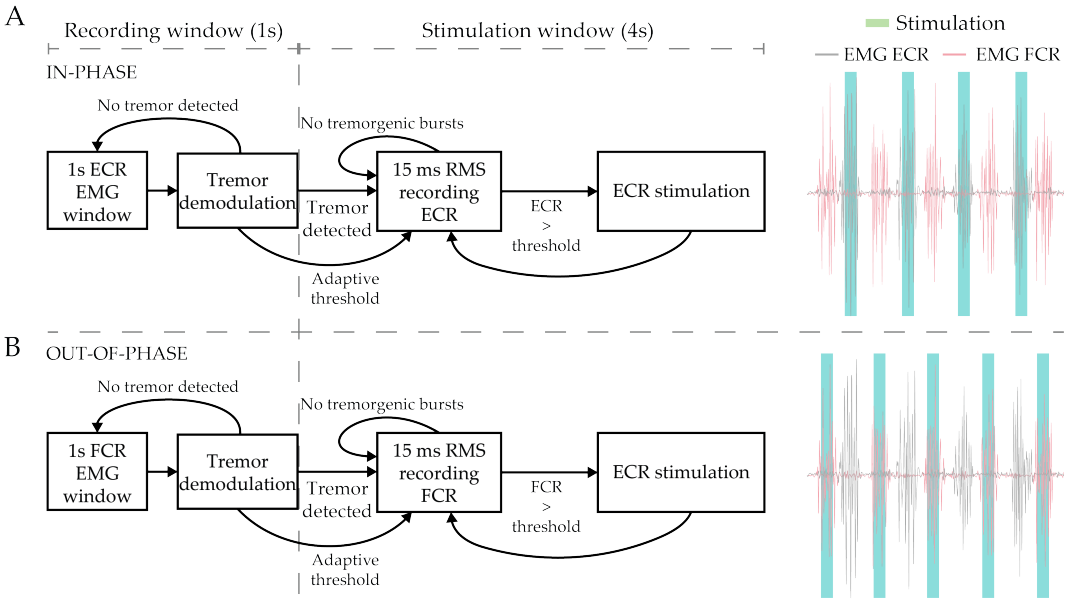

FIGURE 4.2: A. Control flow diagram of SATS-INP (in-phase) strategy. B. Control flow diagram of SATS-OOP (out-of-phase) strategy. When tremor is detected in the 1 s recording window, the stimulation is activated for the next 4 s. If tremor is not detected, the recording window restarts. Stimulation of the radial nerve is applied if tremor activity is detected in the ECR RMS windows (SATS-INP) or if tremor is detected in the FCR RMS windows (SATS-OOP).

recording window and multiplied by a gain factor. For the SATS-INP strategy, the radial nerve was stimulated when the simulated tremorgenic activity was detected in the ECR muscle (Figure 4.2-A). For the SATS-OOP strategy, the radial nerve was stimulated when the simulated tremorgenic activity was detected in the FCR muscle (Figure 4.2-B). After delivering a train of stimuli with a duration of 20% of the tremor cycle, the RMS and stimulation epochs were sequentially repeated until the stimulation window was over. Then, the 1 s recording window was repeated again to assess the presence of tremor and to update the adaptive threshold to the tremor amplitude.

4.4.4 Neurophysiological assessments

Disynaptic Group I inhibition and Ia presynaptic inhibition

Disynaptic Group I inhibition was assessed through the FCR H-reflex conditioning paradigm. Basal FCR H-reflexes were obtained by applying monopolar rectangular pulses of 1 ms over the median nerve (Wargon et al., 2006). The stimulation intensity of the test stimuli was set to elicit a FCR H-reflex with an amplitude on the ascending slope of the recruitment curve. The conditioned FCR H-reflexes were obtained by stimulating the radial nerve with 1 ms monopolar rectangular pulses prior (-1 ms), during (0 ms) or after (+1 ms) the application of the test stimuli (Baldissera et al., 1983). The stimulation intensity of the conditioning stimuli was set immediately below motor threshold. For each condition, ten H-reflexes were elicited, with an inter stimulus interval (ISI) of 5 seconds to avoid post-activation depression (Burke, 2016). The order of the test and conditioned H-reflexes was randomized. To facilitate the reflex response, subjects were asked to keep a slight wrist flexion during

the assessment (Baldissera et al., 1983). Additionally, presynaptic inhibition was measured following the same procedure by applying a conditioning stimulus on the radial nerve 20 ms prior to the test stimuli (Berardelli et al., 1987).

Ib facilitation

A similar protocol to the one described by Aguiar et al., 2018 was followed to assess the evoked ECR Ib fibers excitability. Stimulation of the ECR eliciting a visible tendon pull increases excitability of the FCR H-reflex by means of activation of Ib fibers and Ib interneurons. The FCR H-reflex was conditioned by applying a single stimulus (30 ms prior to the stimulation of the median nerve, 1 ms monopolar rectangular pulse) of the ECR muscle belly with an intensity 3 times above motor threshold. Ten unconditioned and ten conditioned FCR H-reflex responses were averaged to obtain representative measurements.

Neural drive

The excitability of the FCR motoneuron pool was assessed via hdEMG while the subjects performed a voluntary submaximal isometric contraction of the FCR muscle. At the start of the experiment, the subjects were asked to perform three maximum isometric voluntary contractions (MVC) of the FCR during 3 seconds while the elbow joint movement was constrained. The assessment consisted of one isometric wrist flexion at 10% of the average maximal exerted force during the MCV trials. The contraction involved a 5 seconds ramp up, a 30 seconds plateau (10% force of MVC) and a 5 seconds ramp down.

4.4.5 Data analysis

SATS performance

The performance of the SATS strategy was estimated in order to demonstrate the phase difference between SATS-INP and SATS-OOP stimulation interventions. sEMG signals recorded during the intervention for ECR and FCR and were processed offline, in order to extract the components relative to the stimulation artefacts and mimicked tremor muscle activity. The isolation of the stimulation artefact component and exclusion of muscle activity was performed by applying a second order zero-phase Butterworth high-pass filter (500 Hz) to the sEMG signals, which resulted in the extraction of the stimulation harmonics without muscle activity since the stimulation was delivered at 100 Hz. On the other hand, the muscle activity component was extracted by applying a Notch filter at 60 Hz to remove the power line interference, and a set of Notch filters at the stimulation frequency and its harmonics (100 Hz, 200 Hz, 300 Hz... 900 Hz). Then, a second order zero-phase Butterworth band-pass filter (10-90 Hz) was applied to extract an estimation of the muscle activity component. Consequently, both muscle activity and stimulation components were band-pass filtered in the tremor band ±1 Hz with respect to the estimated tremor frequency in the power spectral density (PSD) computed for the ECR muscle (Le Van Quyen et al., 2001). The instantaneous phase was estimated by means of the imaginary part of the Hilbert transform (Dideriksen et al., 2011). The absolute phase difference between the muscle activity and the stimulation artefacts was computed and normalized to the range [0 2π]. Circular polar histograms with 20º bin resolution were produced with the phase difference between the muscle activity for ECR and FCR, and the SATS-INP and SATS-OOP stimulation artefacts (Puttaraksa et al.,

2019). Then, the absolute mean delay between the stimulation and the tremor activity was estimated based on the circular mean and the instantaneous frequency following a similar approach to that described in (Gallego et al., 2015b). The ECR activity was selected as the reference muscle for computing phase difference and mean delays characterizing the stimulation performance. Ultimately, the phase difference and mean delay between the ECR and FCR mimicking tremor activity was estimated following the similar procedure for the OOP-SATS intervention in order to characterize the out-of-phase pattern.

Disynaptic Group I inhibition, Ia presynaptic inhibition and Ib facilitation

FCR H-reflexes recorded via monopolar hdEMG were digitally filtered with a third order Butterworth band-pass filter (20-500 Hz). For each stimulation condition, ten responses to stimulation were averaged channel by channel. The intensity of the M-wave and H-reflex responses were estimated by computing peak to peak amplitude of the evoked potentials (Perez et al., 2003). The hdEMG grid not only covered the FCR muscle belly, but also the surrounding muscles, thus, it was necessary to identify a region of interest (ROI) with the more prominent reflex activity elicited through the stimulation. Besides, though the same positioning protocol was followed for all the participants, the anatomic distribution of the muscles covered by the grid was variable across subjects (Vieira et al., 2010). Therefore, an automatic segmentation algorithm was applied to compute a two-dimensional activation map and determine a ROI for each condition. The matrix containing the average H-reflex amplitudes for each channel was used as input as a grayscale image to the algorithm based on the morphological segmentation techniques described by Rojas-Martínez et al., 2012. The resulting map was an umbralized or binary image containing the ROI of the H-reflex (Figure 4.3). The ROI defined for the basal or unconditioned H-reflex at the PRE assessment was used to extract the H-reflex response for the different conditioned and unconditioned H-reflex across time assessments (POST and POST30'). Ultimately, the channels within the ROI were averaged to provide the final H-reflex response. The conditioning time stimulus (-1 ms, 0 ms or 1ms) showing greatest inhibition of the FCR H-reflex at the PRE assessment was selected to assess the change in disynaptic Group I inhibition for the POST and POST30' measurements.

The same analysis procedure described in the disynaptic Group I inhibition assessment was applied with the reflex responses recorded during the Ia presynaptic inhibition and Ib fibers facilitation assessment protocols.

Neural drive

Monopolar signals were digitally filtered with a second order Butterworth band-pass filter (20-500 Hz) and a Notch filter (60 Hz) to remove the power line interference. A convolutive blind-source separation algorithm was applied to decompose the monopolar signals into motor unit spike trains following the procedure described in Negro et al., 2016. Then, a manual inspection on the automatically identified spike trains was applied to remove duplicates, false positives and false negatives (Hug et al., 2021). After manual edition, all the motor units (MUs) showing pulse-to-noise ratio above 30dB, which ensures sensitivity higher than 90%, were kept for the subsequent analysis. Instantaneous discharge rate was computed for each MU during the 30 seconds plateau of the submaximal contraction at 10% force of the MVC by isolating the MU firing times associated with the plateau. Isolated MU firing times were used to compute inter-spike intervals, which were then

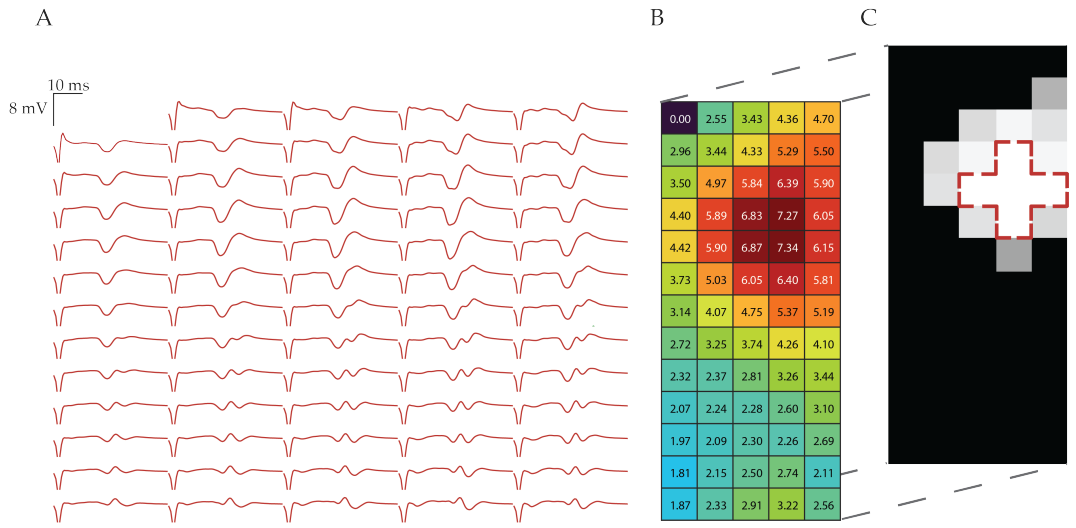

FIGURE 4.3: Illustration of the ROI segmentation of the basal H-reflex responses recorded for one of the subjects. A,B. The peak to peak amplitude of the FCR H-wave was estimated for each of the 64 channels. C. The resulting values were transformed into a grayscale image and a morphological segmentation method was applied to extract the ROI (red dashed lines).

smoothed and inverted to obtain instantaneous discharge rate, following the procedures described in (Mottram et al., 2005). Mean discharge rate for each MU was computed by averaging the instantaneous discharge rate for the given plateau.

The global EMG amplitude was used as an estimation of the neural drive (Farina et al., 2014c). The EMG filtered signals from the 64 hdEMG channels recorded during the MVC trials were segmented and the average RMS was computed by using a 250 ms moving window around 3 seconds containing the maximum peak of exerted force. The average RMS values for all channels were used as input to a segmentation algorithm to extract the ROI of the FCR, similar to the one used to extract the ROI for the FCR H-reflex (Rojas-Martínez et al., 2012). Then, the EMG recorded during the 10% MVC isometric wrist flexion task was normalized channel by channel to the RMS computed for the MVC task. The RMS values were computed over a 5 second window around the middle of the 30 s plateau, and the averaged RMS representing the EMG amplitude were computed for the channels contained within the ROI.

The force produced by the subjects during the submaximal wrist flexion was also analyzed. Similar to the analysis of the EMG amplitude, the force signals were normalized to the maximum force produced during the MVC task. A representation of the force level was estimated by means of the RMS applied over a 5 seconds window around the middle of the 30 seconds plateau. Alternatively, the PSD of the 30 s plateau was estimated using Welch's periodogram method. The power of the force exerted corresponding to the frequency band related to the frequency band of the mimicked pathological tremor and physiological tremor was estimated by averaging the PSD in the [3-6] Hz and [5-12] Hz frequency bands, respectively (Jalaleddini et al., 2017). The goal of this analysis was to explore the potential effects of the phase-dependent stimulation in the specific frequency bands of motor control related to the stimulation applied and the physiological tremor.

Statistical analysis

All statistical analyses were performed using linear mixed effect models in R. Post-hoc comparisons between assessments (PRE, POST, POST30') and stimulation phase (SATS-INP or SATS-OOP) were applied using Holm-Bonferroni corrected t-tests. Distributions were inspected for normality for all the data examined here. Firstly, it was evaluated whether there was a significant difference between conditions for different phases. Then a mixed model analysis was performed of the disynaptic Group I inhibition, Ia presynaptic inhibition, Ib facilitation and mean discharge rate of the MUs during isometric wrist flexion with fixed effects of condition, phase, and interaction between condition and phase, and random slopes and intercepts for each subject. Paired t-tests were also applied to investigate the frequency difference for the mimicked tremor movements and the phase difference between the stimulation and muscle activity between experimental sessions.

4.5 Results

A total of 11 subjects completed both experimental sessions (SATS-INP and SATS-OOP). All the stimulation interventions were delivered in the range between 4 and 9 mA (always below motor threshold), with no adverse effects being reported by any of the subjects. Subjects were able to perform rapid flexion/extension wrist movements mimicking tremor, with the estimated frequency for ECR muscle activity being similar during both phase-dependent interventions ($p<0.05$; 4.3±0.5 Hz for the SATS-INP intervention, and 4.4±0.7 Hz for the SATS-OOP intervention).

4.5.1 SATS performance

Figures 4.4-A,B show the circular histograms with the phase difference values (°) between the stimulation and the ECR muscle activity for SATS-INP and SATS-OOP interventions. The absolute mean phase difference between the ECR activity and the stimulation applied across subjects for SATS-INP intervention was 26±10° (equivalent to a delay of stimulation of 17±8 ms), while for SATS-OOP, the absolute mean phase difference was 127±29° (equivalent to a delay of 81±21 ms). These results showed that the applied stimulation strategy was different ($p<0.05$) in both interventions. The delay results between the stimulation timing and the ECR muscle activity for the SATS-OOP strategy were not completely aligned with an out-of-phase pattern of 180°. Therefore, the average phase difference and delay between the ECR and FCR muscle activity was estimated in order to characterize the physiological out-of-phase pattern of mimicked tremor. Mean phase difference between ECR and FCR was 155±14°, equivalent to 98±18 ms delay (Figure 4.4-C).

4.5.2 Disynaptic Group I inhibition, Ia presynaptic inhibition and Ib facilitation

Figure 4.3 illustrates the procedure followed to extract the average FCR H-reflex peak to peak amplitude from the hdEMG grid for each set of conditioning stimuli. Basal (unconditioned) FCR H-reflex at the PRE assessment was on average 57±62% and 50±64% of maximal M-wave for the SATS-INP and SATS-OOP sessions respectively. Neither SATS-INP nor SATS-OOP elicited statistically significant changes in the basal FCR H-reflex amplitude at the POST and POST30' assessment (fixed effects: stimulation phase $p=0.92$; time $p=0.55$; interaction, $p=0.59$), reflecting that the

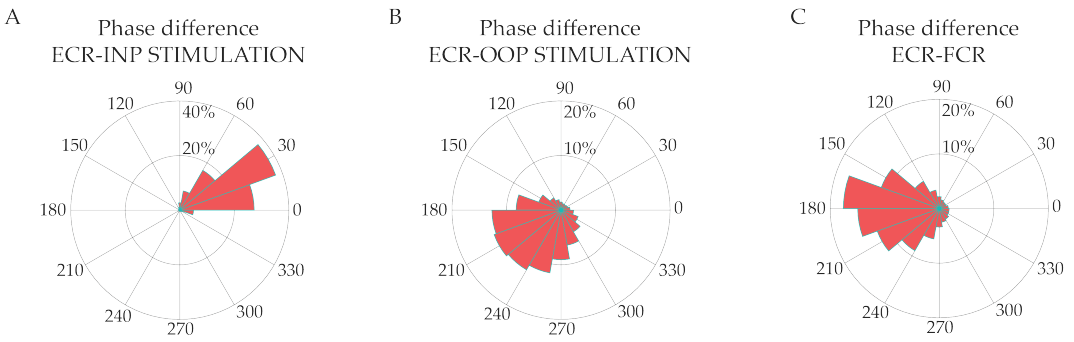

FIGURE 4.4: Circular histograms containing the estimated phase differences. The size of the bins represents the cumulative count of bins representing the phase difference normalized between 0 and 100% from all the subjects. A. Phase difference between the mimicked tremor activity of the ECR and the stimuli trains delivered using the SATS-INP strategy. B. Phase difference between the mimicked tremor activity of the ECR and the stimuli trains delivered using the SATS-OOP strategy. C. Phase difference between the mimicked tremor activity of the ECR and FCR during the SATS-OOP interventions.

reflex response remained stable across time and interventions. Figures 4.5-A,B,C,D illustrate the disynaptic Group I inhibition measured at the conditioned H-reflex for one of the subjects during the SATS-INP experimental session. Average disynaptic Group I inhibition increased at POST (77±23% of conditioned FCR H-reflex at PRE) and POST30' (84±18% of conditioned FCR H-reflex at PRE after the SATS-INP intervention, while the SATS-OOP intervention elicited the opposite effect, resulting in decreased inhibition at POST (125±46% of conditioned FCR H-reflex at PRE), but not at POST30' (101±26% of conditioned FCR H-reflex at PRE) (Figures 4.5-E,F). Linear mixed effect analysis for disynaptic Group I inhibition found that there was no significant main effect of the time assessment ($p = 0.529$) and stimulation phase ($p = 0.051$), but found a significant main effect of the interaction between time assessment and stimulation phase ($p < 0.001$). The post-hoc comparisons revealed statistically significant differences between SATS-INP POST and SATS-OOP POST ($p < 0.05$), and SATS-INP PRE and SATS-INP POST ($p < 0.05$), while there were no significant differences between any of the other comparisons.

Ia presynaptic inhibition measured through the conditioning of FCR H-reflex with a stimulus delivered at the radial nerve 20 ms prior to the test stimulus was not modified by any of the two interventions (Figures 4.6-A,B). Following SATS-INP intervention, average Ia presynaptic inhibition resulted in 99±27% and 94±28% of the PRE values for the POST and POST30' assessments, respectively, while following SATS-OOP intervention, it resulted in 107±29% and 117±39% of the PRE values for the POST and POST30' assessments, respectively. Linear mixed effect analysis found no significant main effect of the time assessment ($p = 0.814$), stimulation phase ($p = 0.455$), and the interaction between both factors ($p = 0.291$).

SATS-OOP intervention elicited an increase of average Ib facilitation for the POST (147±92% of conditioned H-reflex at PRE) and POST30' (111±57% of conditioned H-reflex at PRE) assessments compared to the PRE assessment, while for the SATS-INP, the mean Ib facilitation was 93±38% (POST) and 94±37% (POST30') compared to the PRE assessment (Figures 4.6-C,D). However, due to the great variability the linear mixed effect analysis for Ib facilitation found no significant main effect of the time assessment ($p = 0.768$), stimulation phase ($p = 0.880$), and the interaction

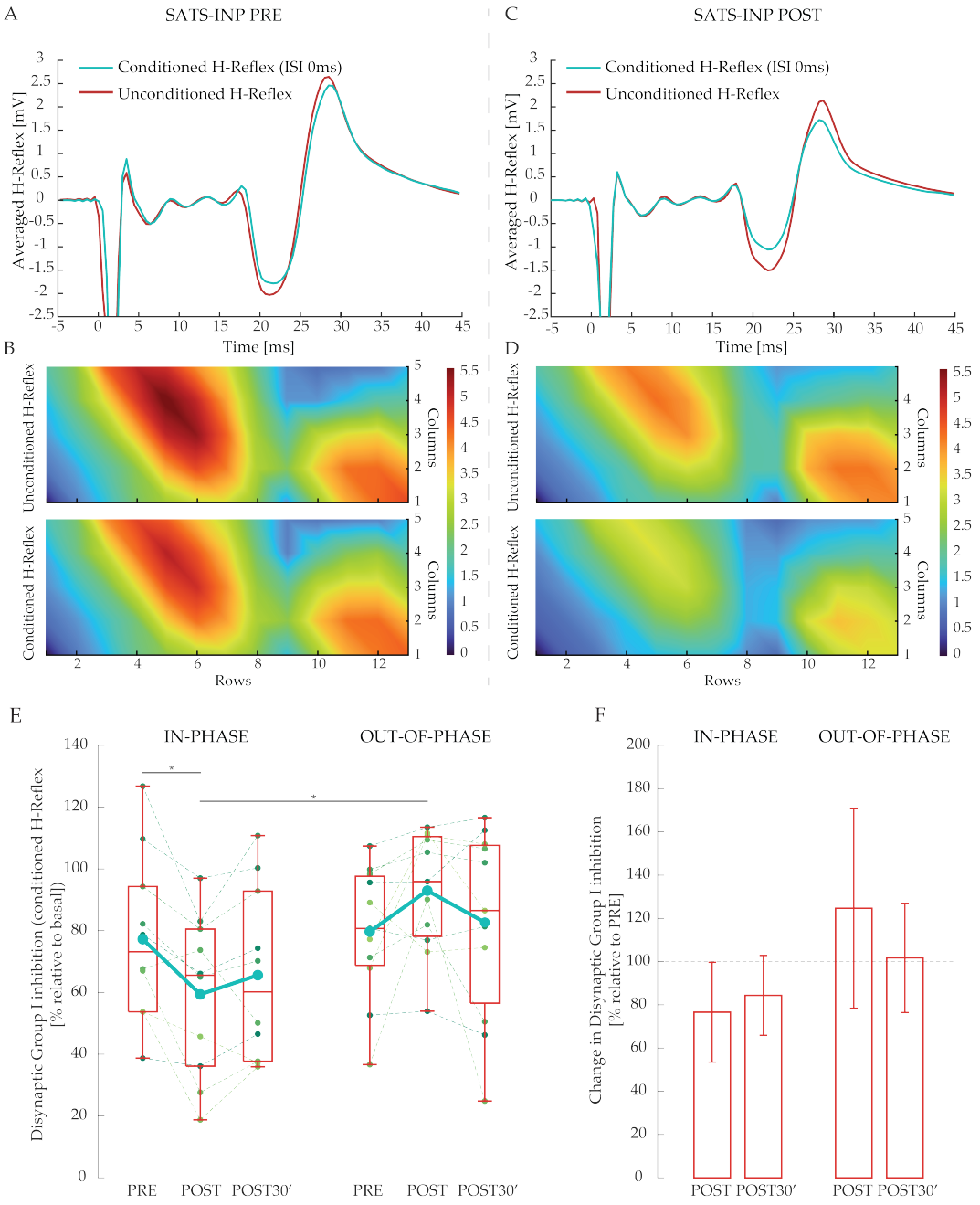

FIGURE 4.5: Disynaptic Group I inhibition measured with the FCR H-reflex conditioning paradigm. A-D. Example of FCR H-reflex responses of one of the subjects. A, C. average of ten baseline and ten conditioned FCR H-reflex responses of the ROI recorded at the PRE and POST assessments during the SATS-INP session, respectively. B, D. 2D colormap representing the peak to peak FCR H-reflex amplitude through the hdEMG grid. E. Boxplots representing the disynaptic Group I inhibition across time assessments and stimulation conditions. Small dots and dashed lines represent individual subject data. Solid green lines represent averaged values across subjects, while red horizontal lines within the boxes represent the median values. F. Average change and standard deviation in disynaptic Group I inhibition at the POST and POST30′ assessments compared to the PRE assessment. * p-value < 0.05; Holm-Bonferroni corrected t-test

between time assessment and phase (p = 0.414).

4.5.3 Neural drive

Neither SATS-INP nor SATS-OOP interventions induced changes in FCR neural drive estimated through global EMG amplitude, as shown in Figures 4.7-A,B (fixed effects: stimulation phase p=0.863; time p=0.420; interaction, p=0.273). Similarly, the force level production remained unchanged during the isometric wrist flexion task after applying both stimulation interventions (fixed effects: stimulation phase p=0.241; time p=0.064; interaction, p=0.938), meaning the submaximal wrist flexion task protocol was reproducible across time assessments (Figures 4.7-C,D). The linear mixed models did not show any significant effect in the power of the force produced in the stimulation frequency band (fixed effects: stimulation phase p=0.898; time p=0.245; interaction, p=0.842, Figures 4.7-E,F) or in the power of the physiological tremor frequency band (fixed effects: stimulation phase p=0.938; time p=0.943; interaction, p=0.953, Figures 4.7-G,H). It is noteworthy that in 18 out of 22 experimental sessions, the spectral power of the force produced in the stimulation frequency band was increased after the intervention (Figure 4.7-E).

The neural drive estimated through the MUs mean discharge rate during the plateau of the isometric wrist flexion task did not change across time due to either SATS-INP or SATS-OOP interventions, as shown in Figures 4.7-I,J (fixed effects: stimulation phase p=0.569; time p=0.082; interaction p=0.296).

4.6 Discussion

The main goal of this study was to assess short-term spinal effects of applying phase-dependent PES. To achieve this goal, the radial nerve was stimulated in-phase or out-of-phase with the ECR muscle activity while healthy volunteers performed fast wrist flexion-extension movements (mimicking pathological tremor). Neurophysiological changes were assessed before, immediately after and 30 minutes after stimulation trials. Results supported the hypothesis that in-phase and out-of-phase stimulation would elicit an increase and decrease in disynaptic Group I inhibition, respectively. Further assessments measuring specific neural circuits such as Ib fibers facilitation, Ia presynaptic inhibition or the estimation of the neural drive during submaximal flexion task did not provide robust evidence about the presence of additional neural mechanisms altered after the interventions. These results suggest that the neural adaptations derived from the stimulation of the antagonist muscle might be specific to the predominant spinal circuit activated (disynaptic Group I inhibition) and modulated during the task performed.

The hand is the end effector of the upper limb and requires fine motor control, which is ultimately achieved by means of synergistic activation of the intrinsic and extrinsic extensors and flexors of the hand (Geng et al., 2020). Thus, pure agonist/antagonist muscle behavior might be limited to a few motor tasks, being the rapid and cyclic wrist flexion-extension contractions among them (Wargon et al., 2006). It has been hypothesized that the fusimotor system adapts the gain of the muscle spindles to the stretching of the antagonist muscle (Prochazka et al., 1988). Therefore, the PES intervention here tested was delivered while the subjects performed voluntary rapid flexion-extension movements as if they were mimicking hand tremor. The selection of this task allowed the activation of the wrist flexor

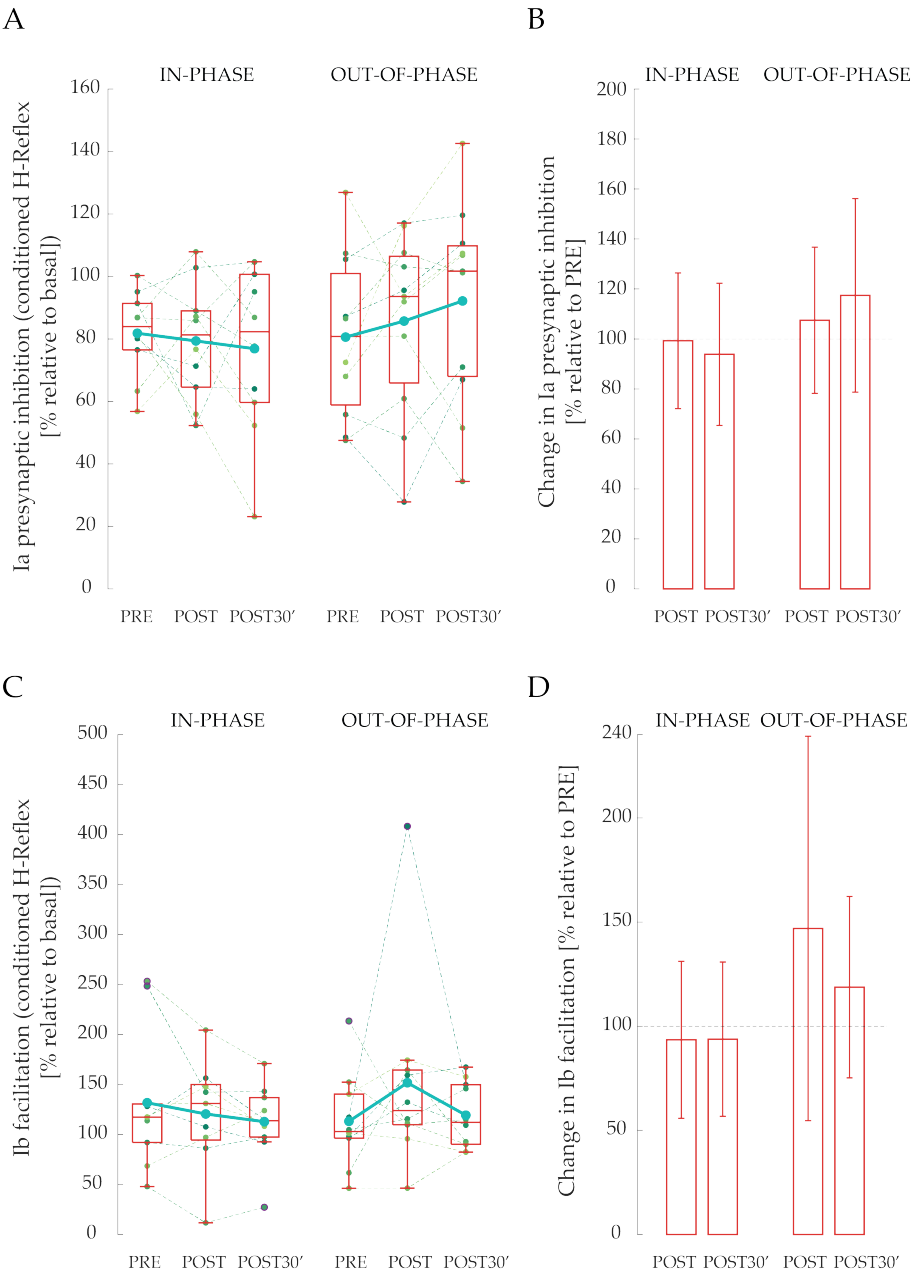

FIGURE 4.6: A, C. boxplots representing Ia presynaptic inhibition and Ib facilitation across time assessments and stimulation conditions, respectively. Small dots and dashed lines represent individual subject data; solid green lines represent averaged values across subjects; red horizontal lines within the boxes represent the median values. B, D. average change and standard deviation in Ia presynaptic inhibition and Ib facilitation at the POST and POST30′ assessments compared to the PRE assessment, respectively.

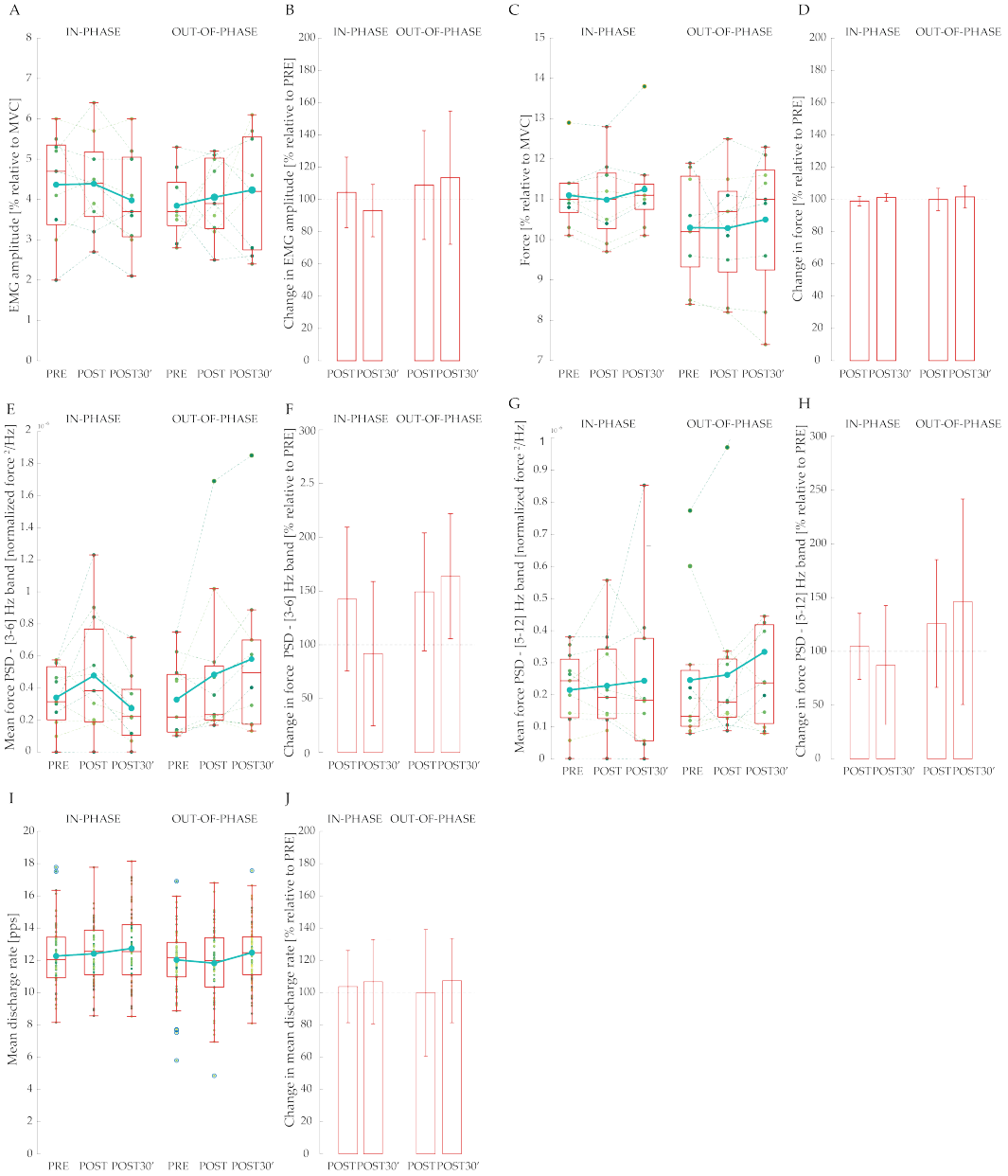

FIGURE 4.7: Neurophysiological assessments. A, C, E, G, I. Boxplots representing EMG amplitude, level of force produced, power of the force produced in the [3-6] Hz and [5-12] Hz bands, and MUs mean discharge rates across time assessments and stimulation conditions, respectively. A, C, E, G. Small dots and dashed lines represent individual subject data; solid green lines represent averaged values across subjects; red horizontal lines within the boxes represent the median values. I. Small dots represent single identified MUs; solid green lines represent averaged values across subjects; red horizontal lines within the boxes represent the median values. A, C, E, G, I. solid green lines represent averaged values across subjects. B, D, F, H, J. Average change and standard deviation in EMG amplitude, level of force produced, power of the force produced in the [3-6] Hz and [5-12] Hz bands, and MUs mean discharge rates at the POST and POST30' assessments compared to the PRE assessment, respectively.

and extensor groups as antagonist muscles, and the rapid movements with an average frequency around 4 Hz increased the amount of trains of electrical stimuli paired with the muscle activity, favoring the induction of plasticity mechanisms based on Hebbian principles (Song et al., 2000). The mean delay between the stimulation artefacts and the mimicked tremor muscle activity was also investigated to test the accuracy of the SATS strategy. Results showed that the subjects were capable of performing the alternating contractions at the required frequency and following a FCR-ECR activation nearly similar to the out-of-phase pattern described for patients exhibiting pathological tremor (Gallego et al., 2015b). Likewise, the analysis showed that the SATS strategy locked the stimulation to the phase of the muscle activity with an average delay below 17 ms. Considering that the RMS windows used to detect real-time muscle activity were set to 15 ms, the SATS strategy provided accurate stimulation synchronously with the muscle activity.

4.6.1 Identification of the adapted neural mechanisms

Previous studies in the lower limb showed that the repetitive delivery of electrical stimuli to the common peroneal nerve induced an increase in the Ia reciprocal inhibition for the soleus muscle if the stimulation was synchronized with the swing phase of gait, while the opposite effect was induced when the stimulation was applied during the stance phase (Obata et al., 2018). This study expands those findings to the upper-limb and the disynaptic Group I inhibition circuit at the wrist. Modulation of the wrist Group I inhibitory interneurons was dependent on the phase of stimulation. The tested protocol induced neuromodulation of the aforementioned circuits. However, they were not effectively maintained 30 minutes after the application, similarly to other studies applying stimulation protocols paired with physiological activity in the lower limb (Perez et al., 2003; Khaslavskaia et al., 2002). Nevertheless, it is noteworthy that in ten out of eleven subjects the disynaptic Group I inhibition values remained reduced compared to the baseline levels 30 minutes after the stimulation with the SATS-INP protocol. While changes in reciprocal inhibition mentioned in other studies have been attributed to modulation of Ia fibers or Ia interneurons for the ankle (Obata et al., 2018), the absence (or difficulty to demonstrate) true Ia reciprocal inhibition for the wrist muscles prevents us from assuming that similar changes occurred in one or more sites along the spinal circuit in this study (Wargon et al., 2006). Additional measurements of Ia presynaptic inhibition assessment did not reveal significant modulation of this mechanism, although descriptive statistics showed a similar trend to the disynaptic Group I inhibition, with an increase of Ia presynaptic inhibition after the stimulation was delivered in-phase with ECR activity. The presented evidence is not sufficient to determine the short-term modulation of Ia fibers, either at presynaptic or postsynaptic level into the disynaptic Group I interneurons. Regarding the excitability of FCR motoneurons and Ia fibers, no significant alterations of the basal FCR H-reflex were found after the interventions, which suggests that excitability of Ia fibers and motoneurons from FCR were affected by neither the phase of the stimulation nor the task performed.

Disynaptic Group I inhibition is mediated by multiple afferent fibers arising from homonymous, heteronymous and antagonist muscles forearm muscles, including Ib fibers, as well as Ia and possibly other group II afferents (Wargon et al., 2006). Hence, excitability of Ib fibers was non-invasively assessed to describe the contribution of these afferents to the disynaptic Group I inhibition modulation (Aguiar et al., 2018). According to these results, excitability of Ib fibers was not adapted after the stimulation interventions, althoughresults should carefully interpreted due to the high

variability reported. The compilation of neurophysiological assessments supports the hypothesis that short-term modulation induced after SATS interventions occurs at the disynaptic group I inhibitory interneurons.

Complementary assessments of the neural drive while the subjects performed submaximal isometric flexion of the wrist sought to determine whether the neural adaptations were limited to the targeted spinal circuit or could be expanded to other behaviors of motor control. Neither the force level produced nor the neural drive estimated through normalized EMG amplitude were altered after the interventions. When analyzing the neural drive estimation by means of differences in MU discharge rate, results showed that MU discharge rate behavior remained consistent after applying the two interventions (SATS-OOF or SATS-INP), suggesting that none of the stimulation patterns induced significant effects on the MU discharge rate.

Particularly, power spectral analysis of the force produced during the submaximal wrist flexion revealed a potential increase of the power in the frequency band associated with the stimulation after both phase-locked interventions. These changes could be attributed to an oscillatory disruption in the stable motoneuron recruitment elicited by the stimulation delivered at a given frequency, which was proven to alter spinal circuits through feedback inputs from afferent fibers. Since the changes were reported after both interventions using opposite phase-locked stimulation, the execution of the rapid flexion-extension at the same frequency could also be responsible for the neural adaptations specific to the frequency band.

Overall, the timely recruitment of the afferent fibers has been proved to be determinant to induce specific neural adaptations at the spinal cord. Particularly, Ia or Ib fibers were not found to be conclusively modulated by the stimulation. Instead, disynaptic Group I interneurons are proposed as the site where short-term modulation may occur. However, these phase-specific neural adaptations located at the spinal cord cannot be attributed to be exclusively mediated by local spinal modulation mechanisms: other supraspinal pathways may instead be involved in the neuromodulation (Chen et al., 2017; Grosprêtre et al., 2020). The changes in disynaptic Group I inhibition were assessed through the FCR H-reflex conditioning paradigm, and therefore those alterations could represent the global excitability of the CNS (Meunier et al., 2007). On the other hand, the outcomes from the neural drive assessments support the evidence that short-term neuromodulation could be specific to the wrist flexion-extension task performed and no major alterations of the CNS were elicited (Giboin et al., 2020). Then, it remains unresolved whether other mechanisms such as the propriospinal system along with other supraspinal circuits are adapted after phase-locked stimulation.

4.6.2 Limitations of the study

Despite the single-blinded paired randomized study design, the relatively reduced sample size (eleven subjects) limits the generalization of the results. Further studies with larger sample sizes can add more evidence and enlighten the source and duration of the short-term neural adaptations. The average duration of each experimental session was approximately 3 hours, from which all the neurophysiological assessments were performed following the same order. The assessment of disynaptic Group I inhibition was performed in the first place to prioritize these outcomes. It cannot be ruled out that the different assessments, such as the Ib fibers excitability, might be affected by the fatigue induced as a result of the length of the experiment, as well as by the total amount of test and conditioning electrical stimuli applied over the radial and median nerves during the FCR H-reflex assessment trials.

The study was designed to characterize the neural adaptations of FCR motor control elicited after a PES-based intervention. Alterations of the ECR derived from the stimulation of the ECR were not tracked during the experiments. However, information about the excitability of the ECR motoneurons, as well as the Ia and Ib fibers arising from the ECR would support the identification of the neural mechanisms modulated based on the stimulation phase. Moreover, addition of measurements aiming at identifying the potential alterations on brain circuits and their projections into motoneurons and spinal interneurons will be necessary to fully clarify the extent of the neuromodulatory effects across the CNS.

4.6.3 Implications for neural rehabilitation and tremor reduction

This is the first study exploring the short-term neuromodulatory effects on the FCR after an intervention using activity phase-locked PES of afferent pathways. The outcomes of this study with healthy subjects will contribute not only to a better understanding of the physiology of the spinal circuits controlling the wrist, but also to describe some of the neural adaptations elicited after PES interventions used as a neural rehabilitation tool in different motor disorders such as pathological tremor or spasticity. In the case of ET, alternating activation of agonist and antagonist muscles of the wrist and the contribution of Ia fibers have been hypothesized to favor pathological tremor through a mechanism similar to Ia reciprocal inhibition (Gallego et al., 2015a). Additional studies in favor of the implication of afferent fibers in pathological tremor suggest that cutaneous and Ib fibers are involved in both tremor and rigidity in Parkinson's disease through the propriospinal system (Delwaide, 2001; Hao et al., 2013). On the other hand, dysregulation of spinal pathways such as the stretch reflex, or the Ia reciprocal and presynaptic inhibitions, among others, might play a major role in spasticity (Boorman et al., 1996; Morita et al., 2001).

In those scenarios where the spinal cord receives aberrant input from supraspinal pathological circuits, which are then amplified through afferent loops, the timing of PES could be used to specifically modulate spinal pathways by shifting the net excitatory output projected to the muscles towards functional states. Moreover, there is evidence of other forms of sensory PES targeting and modulating brain structures with functional implications (Pascual-Valdunciel et al., 2022b). Thus, the phase-locked strategies explored here could presumably induce other activity-dependent plastic mechanisms in multiple sites along the CNS.

Chapter 5

SATS of afferent pathways as a tremor reduction strategy

The contents from this chapter have been previously published in the following conference article, in which the author of this PhD dissertation contributed as first author:

Pascual-Valdunciel, A., González-Sánchez, M., Muceli, S., Adán-Barrientos, B., Escobar-Segura, V., Pérez-Sánchez, J. R., Jung, M. K., Schneider, A., Hoffmann, K. P., Moreno, J. C., Grandas, F., Farina, D., Pons, J. L., Barroso, F. O. (2021). Intramuscular Stimulation of Muscle Afferents Attains Prolonged Tremor Reduction in Essential Tremor Patients. IEEE transactions on bio-medical engineering, 68(6), 1768–1776.

5.1 Abstract

This study proposes and clinically tests intramuscular electrical stimulation below motor threshold to achieve acute and short-term (lasting) reduction of wrist flexion/extension tremor in ET patients. The developed system consisted of a customized embedded processing unit that included an EMG amplifier and a voltage-controlled electrical stimulator and a control algorithm for the timing of intramuscular or surface stimulation based on EMG (SATS closed-loop stimulation). Data were recorded from nine ET patients presenting wrist flexion/extension tremor. Patients participated in two experimental sessions comprising: 1) afferent stimulation of wrist flexors and extensors via thin-film multichannel intramuscular electrodes; and 2) afferent stimulation with surface electrodes of the nerves innervating the same target muscles. For each session, four of these patients underwent random 60 s trials of two stimulation strategies: 1) SATS based on EMG of the antagonist muscle; and 2) continuous stimulation (CON) of the target muscles. Two patients underwent SATS stimulation trials alone while the other three underwent CON stimulation trials alone in each session. Kinematics of wrist, elbow, and shoulder, together with clinical scales, were used to assess tremor before, right after, and 24 h after each session. Intramuscular SATS achieved, on average, 32% acute (during stimulation) tremor reduction in each trial, while continuous stimulation augmented tremorgenic activity. Furthermore, tremor reduction was significantly higher using intramuscular rather than surface stimulation. Short-term tremor reduction (after the stimulation ceased) was observed for all patients receiving intramuscular SATS strategy, and prolonged reduction of tremor (24 h after the experiment) was observed in four patients. These results showed acute and short-term (up to 24 h) tremor reduction using a minimally invasive neurostimulation technology based on SATS of primary

sensory afferents of wrist muscles. This strategy might open the possibility of an alternative therapeutic approach for ET patients.

5.2 Goals

The main objective of this study was to test the hypothesis that intramuscular stimulation of wrist flexor and extensor muscles delivered through the SATS strategy applied to the antagonist tremorgenic muscle can achieve increased acute and short-term levels of tremor reduction in ET patients compared to a continuous (open-loop) stimulation strategy. The SATS strategy proposed in Chapter 4 would be ultimately validated as an effective tool to induce acute and short-term tremor reduction in ET patients, providing additional evidence on the impact of stimulation synchronization with physiological activity to modulate the CNS and reduce tremor. Additionally, the results obtained here would add evidence about the safety and clinical efficacy of intramuscular thin-film electrodes as neural interfaces.

5.3 Introduction

Pathological tremor is the most prevalent movement disorder globally and the fundamental clinical sign of ET. Chapter 1 provided a general description of the pathophysiology of the disease and Chapter 2 provided a detailed review of the state of the art on solutions to manage pathological tremor. In short, the main tremor management approach in ET is pharmacotherapy, although the average tremor reduction using these medications is only approximately 50%, and one in three patients ends up discontinuing treatment (Shanker, 2019; Louis et al., 2010). For the drug-resistant cases, neurosurgical therapies, such as DBS or HIFU are second line treatments with high efficacy in tremor reduction (Ondo, 2020). However, both interventions are still highly expensive and cannot be performed in all patients due to the risks and implications related to neurosurgery. Thereby, the potential development of less invasive therapies is deemed very appealing, and PES has been explored in the last years as an alternative solution.

PES using current amplitudes above motor threshold (MT) to elicit muscle contractions (FES) and counteract tremorgenic forces were firstly explored. Nonetheless, fatigue and interference with voluntary movements prevent the use of stimulation above MT as a therapeutic approach for patients with tremor in the long-term. Recent studies have shown that stimulation of afferent fibers below MT induce acute and short-term tremor reduction without the main drawbacks of stimulation above MT (Dosen et al., 2015; Barroso et al., 2019b). Through Chapter 3 evidence was generated about the potential of PES of afferent pathways below MT to inhibit voluntary muscle activity through activation of spinal reflexes. These acute effects of PES were extended with the results showed and discussed in Chapter 4. The SATS strategy was developed in order to provide precise recruitment of afferent pathways synchronously with the physiological activity detected through EMG. This strategy does not rely on prediction of the next tremorgenic cycles, instead, the EMG is measured and analyzed in real-time to provide stimulation on the antagonist muscle group if tremorgenic activity is detected. Indeed, SATS strategy was proved to induce short-term activity-dependent plasticity of the spinal circuits in healthy subjects (Chapter 4).

Overall, most of studies have not provided evidence about the physiological mechanisms underlying tremor reduction during or after PES of afferent pathways; or robust rationale about the utilization of a stimulation strategy or protocol to reduce tremor. For instance, a wrist-worn peripheral nerve stimulator using a calibrated open-loop stimulation strategy locked to the patient tremor frequency showed its potential to reduce tremor right after stimulation compared to sham stimulation (Lin et al., 2018; Pahwa et al., 2019). Nevertheless, open-loop stimulation strategies do not allow timely activation of the afferent circuits, thus, tremor reduction could be optimized accordingly to the hypotheses based on specific neuromodulation and activity-dependent plasticity induction. Conversely, closed-loop strategies such as the out-of-phase implementation would allow recruitment of afferent pathways synchronously with the tremorgenic activity (Dosen et al., 2015). If stimulation is applied on the antagonist muscle timed with the tremorgenic activity, the spinal circuit would, in theory, inhibit the involuntary tremor coming from supraspinal inputs at the motoneuron synapse, and therefore it would reduce the tremorgenic activation of the muscle. Consequently, mechanical tremor would be mitigated. Dideriksen et al., 2017 assessed the potential of modulating this disynaptic Group I inhibition of wrist flexors and extensors by means of applying intramuscular stimulation following an out-of-phase pattern in two ET patients. Intramuscular stimulation was hypothesized to selectively recruit Ia afferent fibers. However, results were not conclusive due to the limited sample size.

Important insights on how to improve PES of afferent pathways protocols have been given by the comprehensive review of the existing PES solutions, as well as from physiological and simulation studies aiming at identifying the neural mechanisms involved in tremor generation and transmission (Chapter 2). Firstly, Ia afferents might provide significant common tremor input due to passive stretch, which highlights the interplay between supraspinal input and spinal afferents in tremor generation mechanisms (Gallego et al., 2015b). Secondly, tremorgenic patterns in ET patients are not constant over time since a pair of antagonist wrist muscles can be in-phase and out-of-phase activated based on the motor task (Puttaraksa et al., 2019). Thirdly, PES of afferent pathways applied as a tremor reduction strategy is not only limited to acute tremor reduction, since short-term tremor reduction effects have been reported in ET patients (Heo et al., 2015; Isaacson et al., 2020). Finally, PES of afferent pathways has been proven to neuromodulate spinal circuits in healthy subjects (Chapter 3), and particularly, the phase of the SATS strategy is a fundamental feature to induce such neuromodulatory effects in the antagonist muscle (Chapter 4).

Thereby, throughout this study four combinations of PES of afferent pathways protocols comprised of two stimulation strategies (EMG-based closed-loop vs open-loop) and two stimulation electrodes (intramuscular vs surface) were tested in eleven ET patients in order to determine the effect of the stimulation strategy in the acute and short-term tremor reduction. Additionally, the short-term (up to 24 h) tremor reduction effect after a single session of PES of afferent pathways was explored as a preliminary step to characterize the capabilities of this intervention as a therapeutic approach.

5.4 Methods

5.4.1 Participants

Eleven ET patients were recruited from the Movement Disorders Clinic of Gregorio Marañón Hospital (Madrid, Spain) between April 2019 and January 2020, and

were clinically examined by movement disorders specialists of the Neurological Department. Inclusion criteria included diagnosis of ET according to Tremor Research Investigation Group criteria (Bhatia et al., 2018), presenting clinically postural tremor; aged between 18-80 years; tremor affecting at least one of the upper limbs, with prominent wrist flexion-extension; absence of another neurological or musculoskeletal pathology; ability to understand the procedure and sign the informed consent. Exclusion criteria included coexistence of other diseases that distort movement; mixed or complex tremors, with involvement of multiple muscles and concomitant important medical pathology; and anticoagulant treatment. Two patients were excluded from data analysis because tremor was mostly absent on the first experimental session. Table 5.1 reports individual description of the nine patients included in the analyses.

TABLE 5.1: Patients sample description

P	GENDER	AGE	DD	TREAT	FTM S (MAA) [0-12]	FTM SMT (MAA) [0-20]	FD [0-32]	FTM TOTAL SCORE [0-156]	SD (%)	CGI-S [0-7]	ST	IntraStim	SurfStim
1	F	72	7	PNL	4	10	12	39	40	5	Left	SATS CON	SATS CON
2	F	61	15	ZNS, APZ, GBP	7	9	8	37	60	4	Right	SATS CON	SATS CON
3	M	70	40	PMD	5	17	13	55	60	5	Right	SATS CON	SATS CON
4	M	73	18	ZNS, APZ	5	12	15	46	50	5	Left	SATS CON	SATS CON
5	M	63	50	PNL	5	7	13	36	45	4	Right	SATS	SATS
6	M	67	20	PNL, PMD	6	7	9	29	70	4	Right	SATS	SATS
7	F	78	40	APZ, GBP	5	17	19	62	90	5	Right	CON	CON
8	M	72	30	PMD	5	11	14	49	90	5	Right	CON	SATS
9	F	77	8	PNL	7	8	13	49	70	5	Left	CON	SATS CON
MEAN		70.3	25.3		5.4	10.8	12.8	44.6	63.8	4.6			

Individual ET patients' description. All patients assessed in the study were under their current medical treatment. Propranolol and primidone were the most common employed drugs. They exhibited postural and kinetic tremor in the upper limbs with marked activation of flexor-extensor wrist muscles. P: Patient; M: Male; F: Female, DD: Disease Duration; TREAT: Treatment; PNL: Propranolol; ZNS: Zonisamide; APZ: Alprazolam; GBP: Gabapentin; PMD: Primidone; FTM: Fahn-Tolosa-Marín; S: Severity; SMT: Specific Motor Tasks; MAA: Most Affected Arm; FD: Functional Disability; SD: Subjective Disability; CGI-S: Clinical Global Impression of Severity; ST: Stimulated Side; IntraStim: Intramuscular Stimulation; SurfStim: Surface Stimulation; CON: Continuous Stimulation; SATS: Selective and Adaptive Timely Stimulation; SATS CON: Both stimulation strategies.

All patients volunteered to participate in this study, were informed about the procedures and possible adverse effects, and signed the informed consent to participate. All procedures were conducted in accordance with the Declaration of Helsinki and approved by a local ethics committee, as well as by the Spanish Agency of Medicines and Medical Devices - record 714/18/EC. Adverse effects were monitored during the experimental session, 30 minutes after, 24 h after, and one week after each session.

5.4.2 Stimulation strategies

Two different stimulation strategies were tested in this study: SATS and continuous open-loop stimulation (CON) . The fundamentals of SATS strategy were conceived and described in Chapter 4, where the stimulation was applied exclusively to one muscle or nerve (radial nerve) based on the activity of the extensor carpi radialis (ECR, in-phase) or the flexor carpi radialis (FCR, out-of-phase). Here, the stimulation strategy was configured to detect the tremorgenic activity in both ECR and FCR muscles and to deliver stimulation pulses to the antagonist muscle if tremorgenic activity overcomes an adaptive threshold (Figure 5.1-A). Sequential operations (recording and stimulation windows) were used to avoid the contamination of the EMG with stimulation artifacts. Particularly, the implementation for this experiment was the following: EMG data from FCR and ECR were recorded during 1 s recording windows, and both signals were demodulated to extract the main tremor frequency using the approach similar to the one described by Dideriksen et al., 2011. If the main frequency component fell in the range (3-12 Hz), tremor would be detected, an adaptive threshold would be updated from the RMS value computed during the recording window, and the stimulation window would be enabled for the next 2 s. Within each 2 s stimulation window, the RMS of consecutive 10 ms EMG epochs was computed for both muscles and, if any of the RMS values exceeded its adaptive threshold, a stimulation train bursts with a duration of 40% of the tremor period would be delivered to the antagonist muscle (Figure 5.1-B), which allowed quasi-synchronous stimulation. Thus, SATS implementation allowed not only out-of-phase stimulation of FCR/ECR but also simultaneous stimulation of both muscles if they were activated in-phase. The cycle repeats again starting with another 1 s recording window.

CON stimulation strategy consisted on an open-loop control algorithm in which consecutive 1 s recording EMG window were followed by 2 s simultaneous stimulation of both muscles. During the recording window, no analysis of the EMG signals was performed and stimulation was applied unconditionally, since the goal was to mimic the recording window used for SATS strategy so the amount of stimulation time would be similar.

5.4.3 Stimulation sessions

Each patient underwent two different sessions, which took place at least one week apart and in randomized order: 1) intramuscular stimulation (IntraStim); and 2) surface stimulation (SurfStim) (Figure 5.2). At the beginning of each session, a neurologist assessed the basal condition of each patient by means of the Fahn-Tolosa-Marín (FTM) (Fahn et al., 1988) tremor rating scale (specific and motor tasks) and the Clinical Global Impression of Severity (CGI-S) / of Change (CGI-C) (Guy, 1976) (pre-CLINIC assessment). Depending on the posture that would elicit increased wrist flexion/extension tremor amplitude, subjects were asked to hold their arms pronated and outstretched, or to hold their elbows flexed and pronated facing both hands, for all trials in the same session. Afterwards, patients were instrumented.

For the IntraStim session, two intramuscular electrodes (Fraunhofer Institute, Germany) were inserted in the muscle belly of FCR and ECR of the side most affected by tremor using a hypodermic needle (25G, B. Braun AG, Germany), under ultrasonography guidance (Appendix B-Supplementary Video 1). The needle was removed after insertion, leaving the electrode within the muscle. These double-sided multichannel electrodes are built on a thin-film substrate of polyimide and embed twelve contacts for EMG recording and three contacts for electrical stimulation

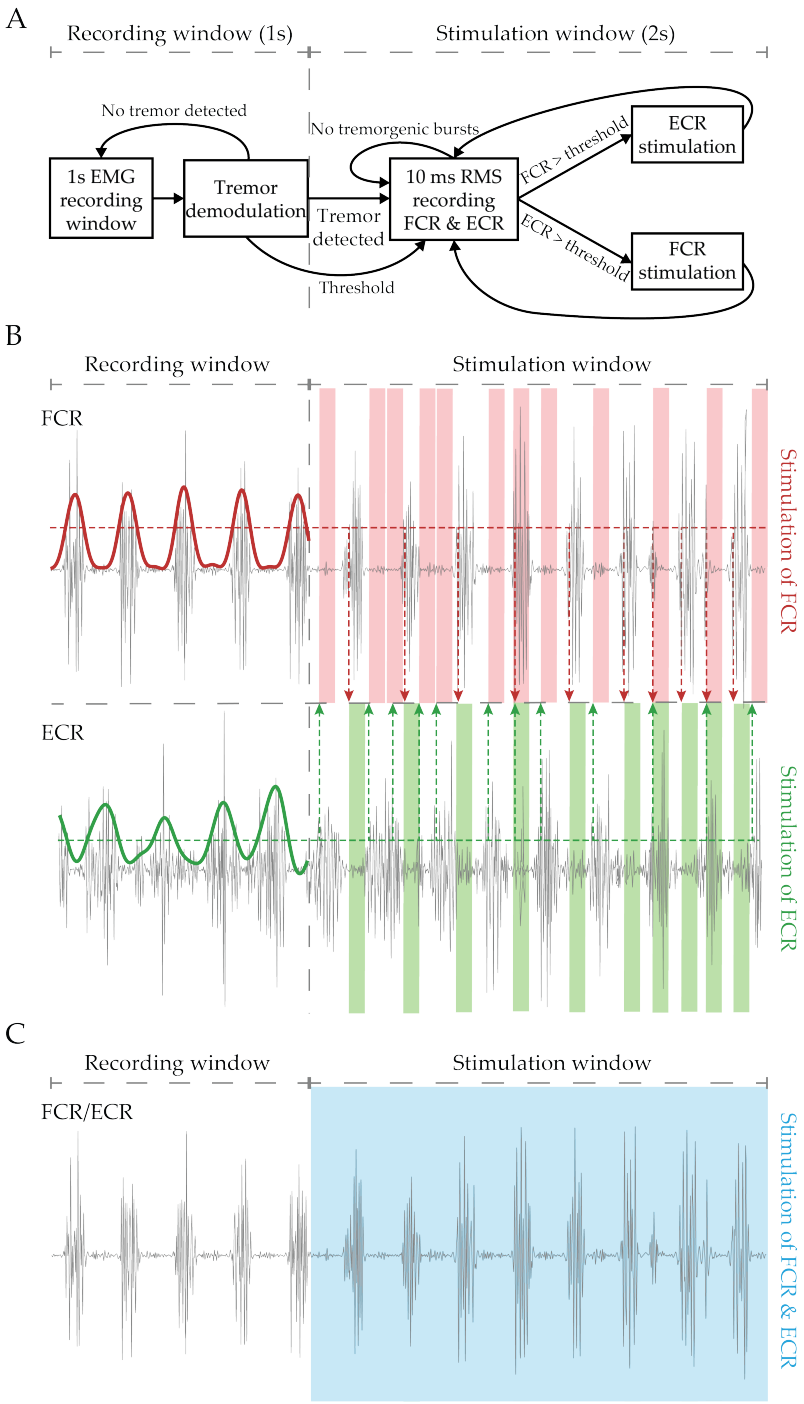

FIGURE 5.1: A. Control flow diagram of SATS strategy. B. Illustration of SATS strategy for a pair of tremorgenic muscles (FCR and ECR). When tremor is detected in the 1s recording window, the stimulation is activated for the next 2s. If tremor is not detected, the recording window restarts. Note that SATS allows simultaneous stimulation of both muscles or consecutive stimulation of the antagonist muscle depending on whether the muscles activated in phase or out-of-phase pattern, respectively. C. Continous stimulation strategy (open-loop). The recording and stimulation windows duration are similar to the SATS strategy: EMG recording during 1 second (no EMG processing is performed), followed by a 2 seconds stimulation window.

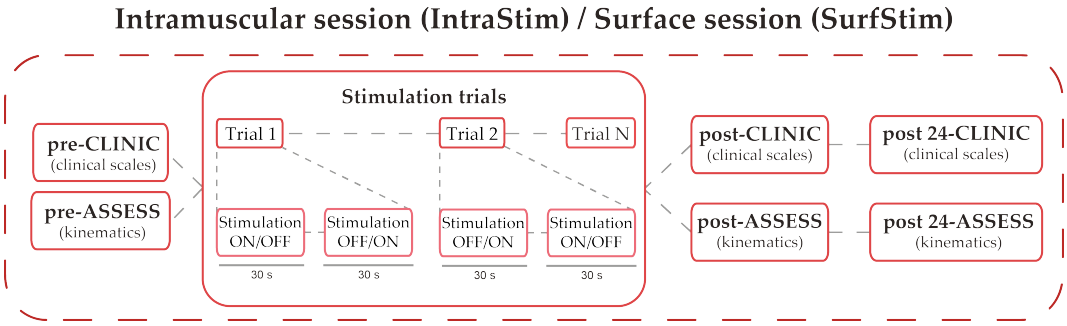

FIGURE 5.2: Schematics of one experimental session for surface (SurfStim) or intramuscular (IntraStim) stimulation. Each stimulation trial lasted 60 s and was divided in two consecutive 30 s windows, in which the stimulation system was randomly turned OFF and then ON, or vice-versa. The number of trials completed by each patient (N) depended on the comfort and fatigue perception, ensuring the completion of at least six stimulation trials. Patients were allowed to rest the time they requested between trials.

(Figure 5.3) (Muceli et al., 2019). EMG data from each of the twelve recording points were visually inspected and the channel presenting qualitatively better signal-to-noise ratio was selected as input for SATS. A customized embedded processing unit that included an EMG amplifier and a voltage-controlled electrical stimulator (OT Bioelettronica, Italy) acquired intramuscular signals at 1 kHz. Wet wristbands were used as EMG reference. Additionally, seven inertial measurements units (IMUs) (Technaid S.L., Spain) were placed on each subject: one IMU over the dorsal side of each hand and forearm, one over the lateral side of each arm, and one over the chest. Raw quaternions data from IMUs were sampled at 50 Hz. Finally, a surface electrode (5x5 cm, ValuTrode Cloth, Axelgaard Manufacturing, Denmark) was placed over the olecranon to act as ground for the stimulation.

For the SurfStim session, two round adhesive electrodes (3.2 cm diameter, ValuTrode Cloth, Axelgaard Manufacturing, Denmark) were placed over the median and radial nerves at the humeral region (Pascual-Valdunciel et al., 2019). A surface ground electrode was also placed over the olecranon. Bipolar surface electromyography (sEMG) electrodes were placed over the muscle belly of FCR (between the medial epicondyle of the humerus and the palmar surface of the base of the second metacarpal) and ECR (between the common head of the lateral epicondyle of the humerus and the base of the third metacarpal), after cleaning the skin with alcohol (Eleanor, 2017). Surface EMG signals were acquired at 1 KHz. The ground electrodes for EMG recording and stimulation, as well as the acquisition of the IMU data were unvaried with respect to the IntraStim session.

After instrumenting patients, basal tremor was quantitatively assessed (pre-ASSESS trial): each participant was asked to hold their upper limbs in the same posture as in the pre-CLINIC trial, for 60 s, while IMUs data were recorded. For each session, data from the customized EMG-stimulation unit were synchronized with IMUs data. Raw data were stored and analyzed offline.

After assessing basal condition of tremor, two stimulation parameters were calibrated for each patient and muscle: the perception threshold (PT), which is the current at which the patient started to feel the stimulation, and the motor threshold (MT), defined as the minimum amount of current that elicited motor response. For the IntraStim session, the stimulation amplitude was increased gradually until either the MT or the safety limit of intramuscular electrodes (2.4 mA) was reached.

The deepest of the three stimulation contact points was initially selected for stimulation. When no sensation was evoked through the stimulation of this channel, then the other two channels were used. For the SurfStim session, PT and MT were also identified, and the maximum current delivered never exceeded 5 mA (technical limitation of the stimulation unit). For both types of stimulation, the maximum current was always below MT and below the safety limit. Stimulation frequency was set at 100 Hz (Dideriksen et al., 2015; Dideriksen et al., 2017), with biphasic pulse width of 200 μs and maximum stimulation amplitude and 2.4 mA for IntraStim (Muceli et al., 2019), and 400 μs and 5 mA for SurfStim (Dosen et al., 2015), respectively.

After calibrating the stimulation parameters, each session proceeded with several stimulation trials, each one consisting of two consecutive 30 s windows (Figure 5.2). For each trial, subjects were asked to hold their arms in the same posture as in pre-ASSESS. In one of the two 30 s windows, the stimulation unit was turned OFF, whereas SATS or CON stimulation were delivered in the other 30 s windows (ON). The order of ON and OFF windows for each trial was randomly assigned. Each patient was blinded to the stimulation strategy applied (SATS or CON). The number of trials completed by each patient depended on the comfort and fatigue perception, ensuring the completion of at least six stimulation trials. Patients were allowed to rest the time they requested between trials.

The first four patients enrolled for the study received SATS intermingled with CON stimulation trials, in randomized order, for both sessions. Two other patients underwent SATS stimulation trials alone whereas the other three underwent CON

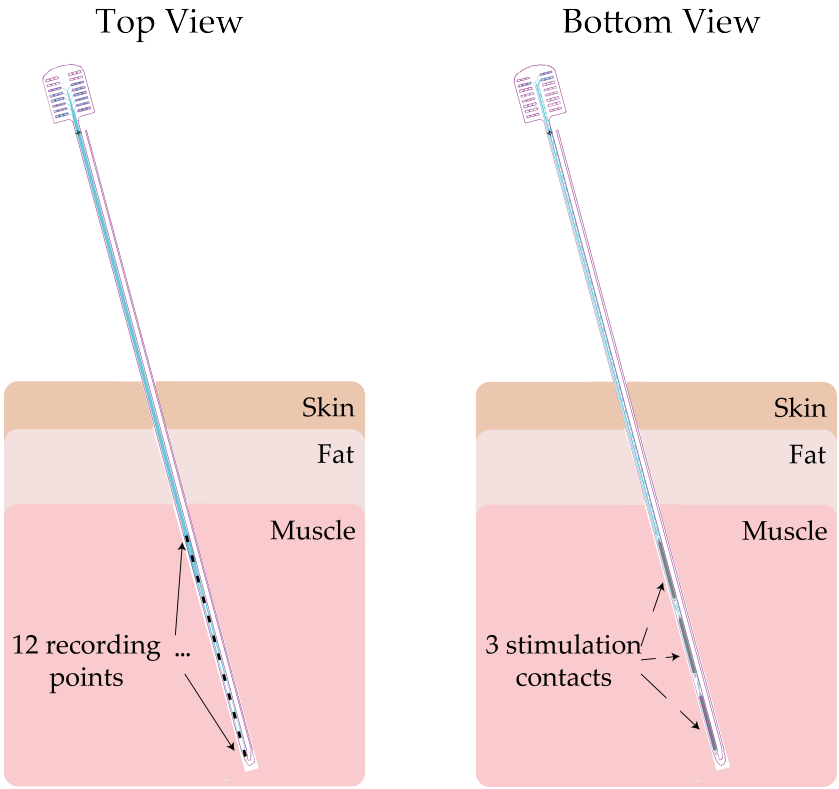

FIGURE 5.3: The thin-film multichannel electrode developed by Fraunhofer Institute is made of a polyimide substrate and embeds 12 EMG recording points and 3 stimulation contacts on the two sides of the substrate.

stimulation trials alone in each session, with the main goal of investigating the independent effect of each stimulation strategy. At the end of each session, the effect of stimulation trials was assessed through kinematics (post-ASSESS) and clinical scales (post-CLINIC) (Figure 5.2). A final assessment (post24-ASSESS and post24-CLINIC) was also performed 24h after each session to assess possible prolonged effects on tremor reduction.

5.4.4 Quantification of tremor

Stored data were analyzed offline using custom software in MATLAB (Mathworks, Natick, MA). Raw data from quaternions were converted into Euler angles and filtered in the tremor band (3-12 Hz) (Elble et al., 2006), which allowed computation of the angle displacement of wrist, elbow, and shoulder joints. The power spectral density (PSD) was calculated (2 s Hamming window and 50% segment overlap) and then integrated in the typical range of tremor frequencies (3–9 Hz) (Dideriksen et al., 2017) to quantify the tremor power in the assessment and stimulation trials. For the wrist joints, we calculated the power for the flexion-extension angle, whereas for the elbow and shoulder joints, we calculated the average power for the 2 and 3 degrees of freedom of these joints, respectively. Power values were used to compute different tremor scores, depending on the conditions being compared, according to equation (1):

$$TremorScore = 0.5 + 0.5 \cdot \frac{(conditionA - conditionB)}{max(conditionA; conditionB)}$$

where conditions A and B represent the tremor power or clinical score of two conditions compared through kinematics or clinical scales, such as different time assessments (e.g. PRE-ASSESS and POST-ASSESS), or OFF-ON windows (referred as acute tremor score), among others. According to (1), tremor score equal to 1 would correspond to 100% tremor reduction in condition B with respect to condition A; tremor score equal to 0.5 would correspond to unchanged tremor amplitude; and tremor score equal to 0 would correspond to 100% tremor aggravation. Some trials when patients presented very low tremor (power < 0.2 deg^2/Hz) were excluded from the analysis as done in similar studies (Dosen et al., 2015). Additionally, the trials when patients did not receive SATS stimulation for at least 50% of the time they should (essentially due to low tremor amplitude) were also discarded from the analysis. Linear relationships between the tremor scores estimated via kinematics and via clinical scales were computed. This analysis was meant to contribute to validate kinematics (objective measurements) by comparing them with standard clinical tremor rating scales such as FTM scale.

5.4.5 Statistical analysis

One-sample t-tests were used to compare the effects of each combination of stimulation strategy and electrodes on acute tremor reduction against the tremor score value 0.5, which corresponds to unchanged tremor amplitude. Independent sample t-tests were applied to compare differences on acute tremor scores between stimulation conditions (SATS IntraStim vs SATS SurfStim, SATS IntraStim vs CON IntraStim, SATS SurfStim vs CON SurfStim) for each joint. Normality tests were applied to check the normal distribution of the data. Statistical significance was set to p-value < 0.05. Descriptive statistics were used to analyze the short-term effects after the stimulation (post-ASSESS, post-CLINIC, post24-ASSESS, and post24-CLINIC) due

to the limited sample size of participants receiving one single stimulation strategy along the same experimental session.

5.5 Results

The number of trials completed and stimulation parameters applied on each patient and session are shown in Table A.3. In some cases, patients did not feel any sensation when stimulation was delivered. For FCR, stimulation amplitude was on average 1.7 ± 0.8 mA and 4.8 ± 0.4 mA for IntraStim and SurfStim, respectively. For ECR, stimulation amplitude was on average 1.4 ± 0.9 mA and 4.6 ± 0.8 mA for IntraStim and SurfStim, respectively. On average, 28% of the completed trials were excluded from the analysis due to very low tremor power at the stimulated wrist. Information on adverse effects is detailed in Table A.4. Experiments were well tolerated and none of the stimulation sessions caused important adverse events. Seven patients reported transient mild to moderate pain when intramuscular electrodes were inserted or removed. Two patients felt mild transient paresthesias. A patient exhibited moderate fatigue during IntraStim session.

5.5.1 Acute tremor reduction

Figure 5.4-A represents wrist flexion-extension angles of a representative patient during a IntraStim session, including data segments from the kinematic assessments as well as a SATS IntraStim trial. Qualitatively, tremor amplitude decreased in post-ASSESS and even more in post24-ASSESS compared to pre-ASSESS, for this patient. Frequency analysis presented in Figure 5.4-B quantitatively confirmed this observation. Figure 5.4-C presents mean acute tremor scores for all joints and both sides across all subjects and stimulation conditions. These were calculated according to Equation (1), considering as conditions A and B tremor power during the 30 s OFF windows and the 30 s ON windows, respectively, for each trial and patient. SATS IntraStim was the only condition achieving significant acute tremor reduction (0.63±0.03; $p<0.01$; equivalent to 26% tremor reduction) on the stimulated wrist, which was significantly higher compared to the other 3 conditions (SATS SurfStim, CON IntraStim, and CON SurfStim; $p<0.05$ for the three comparisons). Tremor score was significantly higher ($p<0.01$) for SATS SurfStim (0.53±0.08) than CON SurfStim strategy (0.35±0.07). In fact, CON stimulation strategy not only did not acutely reduce tremor amplitude at the stimulated wrist, but it even aggravated ($p<0.05$) tremor during SurfStim. Although SATS IntraStim achieved, on average, acute tremor reduction on the ipsilateral (stimulated) elbow (0.60±0.10) and shoulder (0.58±0.08) (Figure 5.4-C), differences were not significantly different from the periods without stimulation ($p = 0.06$). Moreover, there was no significant acute tremor reduction on any of the contralateral (non-stimulated side) joints.

Figure 5.4-D represents the tremor score as a function of the 2 s stimulation windows (Figure 5.1-B), with condition A from equation (1) representing 30 s OFF windows and condition B from (1) representing 2 s stimulation intervals inside the 30 s ON windows from each trial, respectively. In this case, acute tremor reduction on the stimulated wrist was significant for SATS IntraStim strategy (0.66±0.09; $p<0.02$; equivalent to 32% tremor reduction), and higher than SATS SurfStim (0.53±0.08, $p<0.05$) and CON IntraStim (0.26±0.18, $p<0.05$) conditions. SATS SurfStim also achieved isolated acute tremor reduction on the contralateral (non-stimulated) wrist

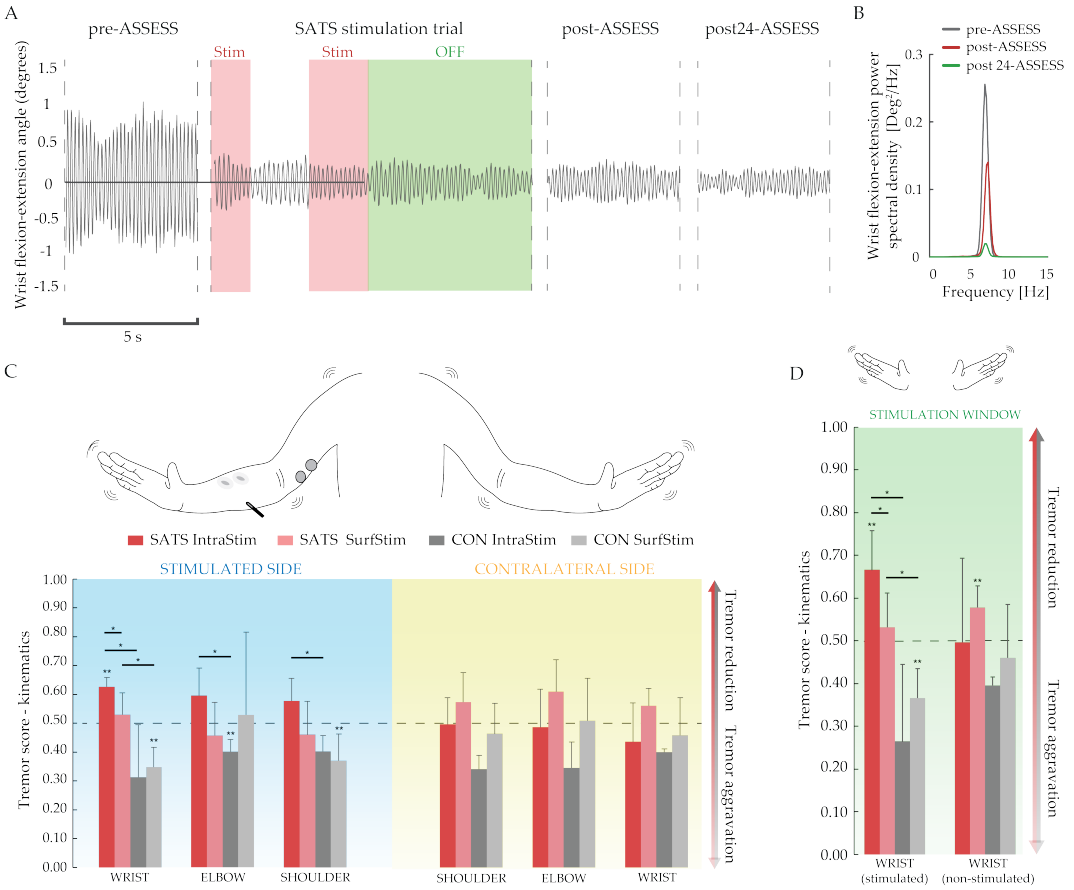

FIGURE 5.4: Acute tremor reduction results. A. Wrist flexion-extension tremor data segments recorded during the IntraStim session of P04. B. PSD of the flexion-extension angle of the stimulated wrist for pre-ASSESS, post-ASSESS and post 24-ASSESS for P04 IntraStim session. C,D. Acute tremor scores on kinematics for all patients. Results represent mean ± standard deviation across subjects and trials. C. Tremor scores between the 30 s OFF period and 30 s ON period for each assessed joint. D. Tremor scores between the 30 s OFF period and the 2 s stimulation windows segmented from the ON period. Note that tremor score equal to 1 corresponds to 100% tremor reduction, tremor score equal to 0.5 corresponds to unchanged tremor amplitude, and tremor score equal to 0 corresponds to 100% tremor aggravation. *p<0.05 (independent samples t-test); **p<0.05 (one-sample t-test).

(0.58±0.05, p<0.05), although differences were not significantly different from the SATS IntraStim condition (0.50±0.20).

5.5.2 Short-term reduction

Four patients (P01 - P04) underwent random trials of SATS and CON stimulation for both stimulation sessions (IntraStim and SurfStim). Post-ASSESS showed a short-term tremor reduction effect on the stimulated wrist (Figure 5.5-A). To calculate short-term effects, conditions A and B from (1) were pre-ASSESS and post-ASSESS, respectively. Despite the reduced sample size (n=4) and variability of tremor, mean reduction of tremor on the stimulated wrist was higher after IntraStim sessions (0.82±0.20) compared to after SurfStim sessions (0.57±0.39). Similarly, variability

in short-term tremor reduction was reduced for the sample of patients receiving IntraStim stimulation. Nonetheless, tremor score was on average higher than 0.5 at the end of both IntraStim and SurfStim sessions (post-ASSESS) and all assessed joints (Figure 5.5-A).

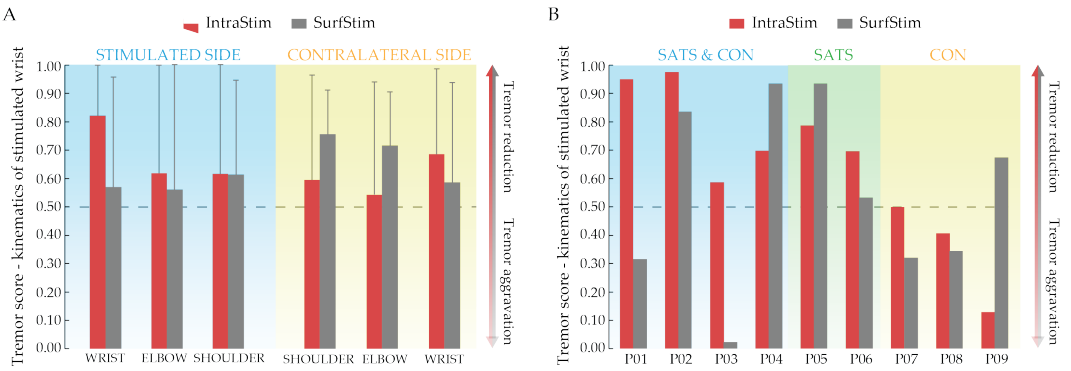

FIGURE 5.5: Short-term effects of electrical stimulation on kinematics tremor scores. A. Tremor scores for all the assessed joints during the post-ASSESS for the first 4 ET patients assessed in the study, who received trials with both SATS and CON strategies. Results represent mean ± standard deviation of tremor score. B. Individual tremor scores for the wrist kinematics obtained during the post-ASSESS (short-term effects) assessment.

After reporting short-term tremor reduction effects for the first sample of patients receiving both SATS and CON strategies, the following subset of patients underwent IntraStim or SurfStim sessions in which only one of the two stimulation strategies (SATS or CON) was applied in order to determine what strategy could be responsible for the short-term effects. Short-term tremor reduction at the stimulated wrist was reported for the two patients (P05 and P06) who only received SATS during the IntraStim session (average tremor score 0.76±0.07, Figure 5.5-B), whereas the three patients receiving CON during IntraStim session showed tremor aggravation (average 0.35±0.2, Figure 5.5-B). All patients (six out of six) who received IntraStim stimulation combined with SATS strategy in any of the trials attained tremor reduction after the intervention. The short-term effects of the stimulation were additionally assessed through clinical scales. Figure 5.6 shows spiral and line drawings performed by two patients corresponding to the specific motor task of the FTM clinical scale. The trace of the drawings performed by P06 (Figure 5.6-A) was noticeably less affected by tremor in the post-CLINIC and post24-CLINIC assessments. The tremor scores (post-CLINIC and post24-CLINIC) computed with the FTM severity scores were positively and significantly correlated (ρ=0.56, p<0.001, Figure 5.7-A) with the tremor scores computed with the kinematics measurements (post-ASSESS and post24-ASSESS). Regarding the CGI-S/CGI-C scale, the tremor score values were likewise correlated with the kinematic measurements (ρ=0.34, p<0.05, Figure 5.7-B). However, correlation between the tremor scores computed with the FTM specific motor tasks scores and the tremor scores computed through the kinematics measurements was weaker and statically non-significant (ρ=0.22, p=0.2, Figure 5.7-C).

Similar to what was observed through the kinematic assessments, the two patients who received SATS during IntraStim session showed reduced FTM severity scores after the stimulation intervention (0.77±0.03), while there were no changes (0.52±0.04) in those 3 patients that only received CON stimulation during the IntraStim session (Figure 5.8-A). The CGI-S/CGI-C tremor scores showed that, all the

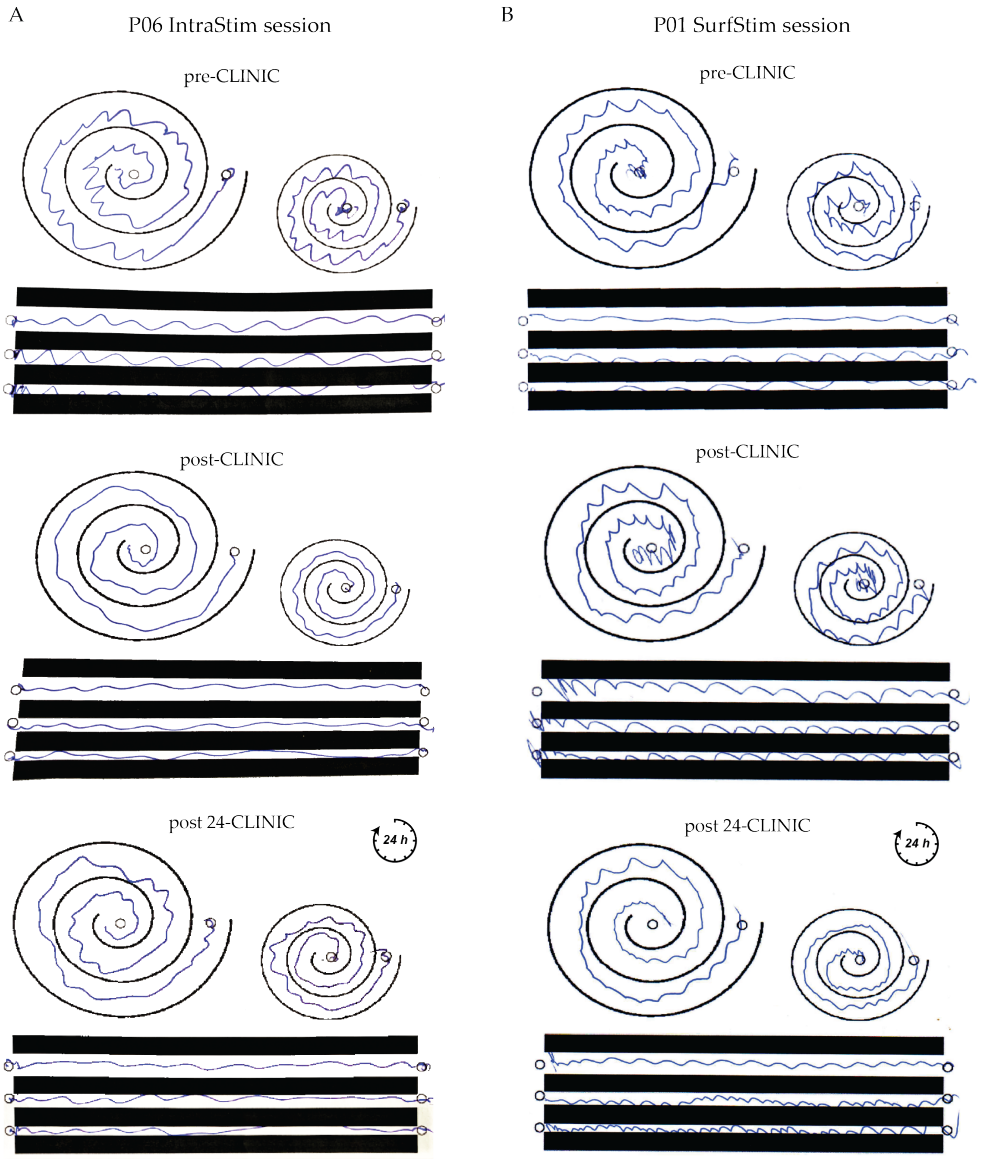

FIGURE 5.6: Spiral drawings performed by two ET patients. Samples of spiral drawings from the FTM specific tasks scale performed by two patients (A. P06, IntraStim session; B. P01, SurfStim session) during the clinical assessments before the stimulation (pre-CLINIC), after the stimulation (post-CLINIC) and 24 h after the stimulation (post24-CLINIC).

patients attained tremor reduction after any of the stimulation sessions (Figure 5.8-B). Particularly, the highest tremor reduction scores were reported for the two patients receiving the IntraStim SATS intervention (0.73±0.03).

5.5.3 Tremor reduction 24 h post stimulation

Tremor reduction at the stimulated wrist (post24-ASSESS) was also observed 24h after experimentation in 2 out of the 3 patients (P02 and P04; assessment for P01 was not performed although the patient reported via phone call to the neurologist that she perceived a tremor reduction effect remaining after 24 h) that underwent random trials of SATS and CON stimulation for both stimulation sessions, with an average

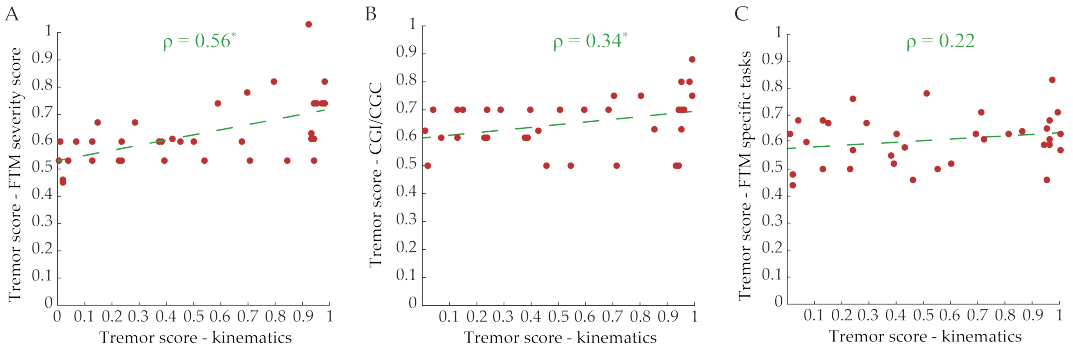

FIGURE 5.7: Relationship between the kinematics assessment and the clinical scales. A. Scatter plot of the tremor score computed through the wrist kinematics against the tremor score computed through the FTM severity scores. B. Scatter plot of the tremor score computed through the wrist kinematics against the tremor score computed through the CGI-S/CGI-C scores. C. Scatter plot of the tremor score computed through the wrist kinematics against the tremor score computed through the FTM specific tasks scores. *p<0.05 (significant correlation).

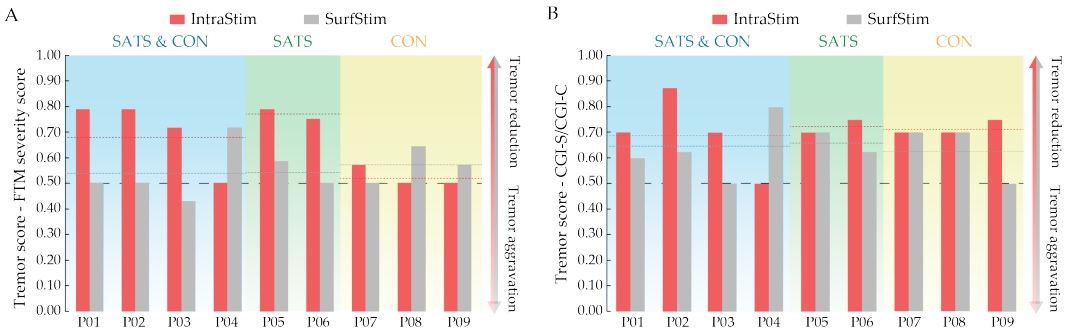

FIGURE 5.8: Short-term effects of electrical stimulation on clinical scales tremor scores. A. Individual tremor scores of the FTM severity scores obtained during the post-CLINIC (short-term) assessment. B. Individual tremor scores of the CGI-S/CGI-C obtained during the post-CLINIC (short-term) assessment.

ipsilateral wrist tremor score of 0.85±0.22 for the IntraStim session and 0.63±0.45 for SurfStim (Figure 5.9; results from P02 on Appendix A-Supplementary Video 2). Additionally, tremor reduction was also observed on the contralateral wrist 24 hours after the stimulation for both IntraStim and SurfStim sessions, with mean tremor scores of 0.80±0.17 and 0.55±0.37, respectively (Figure 5.9-A).

Regarding the other 5 patients that received SATS or CON stimulation alone, except for one of the 2 patients that received SATS SurfStim, all other patients showed aggravated tremor at the wrist 24h after the stimulation sessions for the post24-ASSESS (Figure 5.9-B). It is noteworthy that three patients (P05, P06 and P09) exhibited more prominent tremor on elbow and shoulder joints during the stimulation sessions. Additionally, P05 reported he suffered from stress at work during the day of post24-ASSESS for IntraStim, a condition known to aggravate pathological tremor. Four out of six patients who received any trial of SATS SurfStim showed tremor reduction 24 hours after the intervention. In general, CON strategy alone aggravated tremor compared to SATS strategy 24h after stimulation sessions.

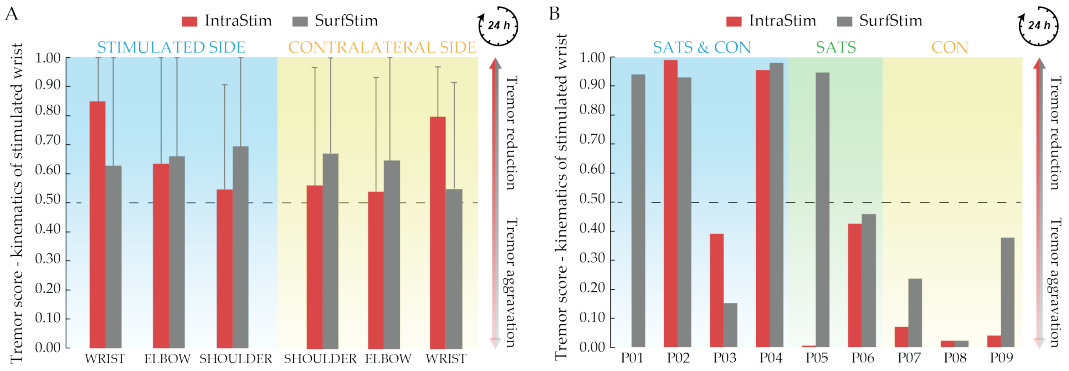

FIGURE 5.9: Prolonged effects of electrical stimulation on kinematics tremor scores. A. Tremor scores for all the assessed joints during the post24-ASSESS for the first 4 ET patients assessed in the study, who received trials with both SATS and CON strategies. Results represent mean ± standard deviation of tremor score. B. Individual tremor scores for the wrist kinematics obtained during the post24-ASSESS (prolonged effects) assessment.

According to the tremor scores computed through clinical assessments (post24-CLINIC), only one patient showed aggravated tremor 24 hours after the CON IntraStim session (Figure 5.10-A). All the patients showed higher tremor reduction scores based on FTM severity scale during the post24-CLINIC assessments. The highest tremor reduction scores were reported for the patients receiving any trial of SATS IntraStim or SATS SurfStim conditions. The tremor scores based on CGI-S/CGI-C scales showed all the patients except one attained tremor reduction 24 hours after the IntraStim session for both SATS and CON strategies (Figure 5.10-B).

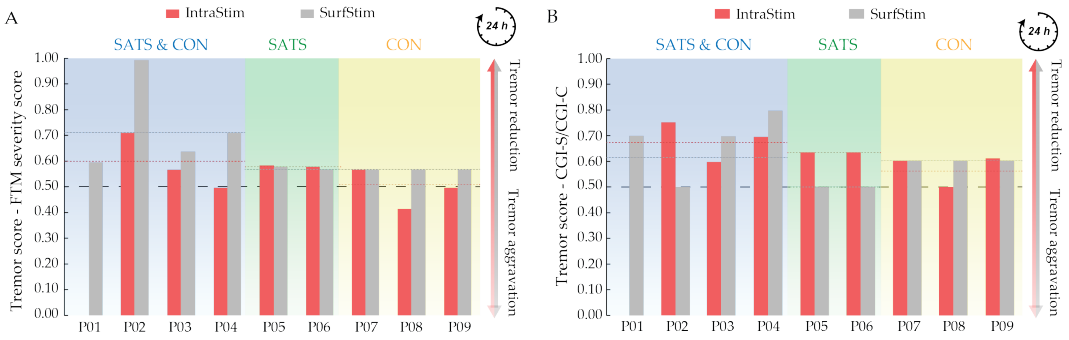

FIGURE 5.10: Prolonged effects of electrical stimulation on clinical scales tremor scores. A. Individual tremor scores of the FTM severity scores obtained during the post24-CLINIC (prolonged effects) assessment. B. Individual tremor scores of the CGI-S/CGI-C obtained during the post24-CLINIC (prolonged effects) assessment.

5.6 Discussion

This study demonstrates the feasibility of PES of afferent pathways through intramuscular (IntraStim) selective and adaptive timely stimulation (SATS) to decrease pathological tremor in ET patients. Qualitative results from clinical scales add more evidence to quantitative tremor scores that SATS IntraStim improved voluntary control of daily living tasks and show that this strategy might open the possibility of an

alternative therapeutic approach for ET patients. Considering the review of the state of the art performed in Chapter 2, this is the first report of short-term and prolonged (24 h) tremor reduction of wrist flexion-extension in ET patients after delivering PES of afferent pathways (below motor threshold) with intramuscular electrodes for no longer than 10 minutes in total.

This study adds evidence to the relevance of synchronizing the stimulation or recruitment of afferent pathways with the physiological (tremor) activity in order to modulate the CNS towards the desired functional output. The SATS strategy was proved to reduce wrist tremor acutely (while the stimulation was applied), and such tremor reduction was statistically significant and higher when compared to the CON stimulation, an open-loop strategy which not only did not reduce tremor, but aggravated it in some cases. These results are in line with previous studies applying PES of afferent pathways with the out-of-phase strategy in the wrist flexor and extensor muscles (Dosen et al., 2015; Dideriksen et al., 2017). The tremor reduction hypothesis is based on the recruitment of afferent pathways contributing to the inhibition of the antagonist muscle via activation of the wrist disynaptic Group I inhibition circuit at the spinal cord. Moreover, the use of intramuscular electrodes would allow selective recruitment of Ia fibers contributing to such spinal circuit. Although the results from this study support this tremor reduction hypothesis, it is not sufficient to conclude the that the activation of the wrist disynaptic Group I inhibition is the only responsible mechanism for acute tremor reduction. Nevertheless, these results on acute tremor reduction are robust and demonstrate that reduction was achieved by combining modulation of afferent pathways (low-intensity electrical stimulation of primary muscle spindles) and time-selective stimulation.

The short-term tremor reduction results observed after applying SATS strategy with intramuscular electrodes are similar to the results reported by others using surface PES of afferent pathways (Heo et al., 2015; Pahwa et al., 2019; Kim et al., 2020). All patients who received SATS during IntraStim session (six out of six) reduced their tremor during the assessments after the experiment (post-ASSESS and post-CLINIC), and some of them held this reduction even 24 h after the stimulation session (post24-ASSESS and post24-CLINIC). Although prolonged effects are very promising and should be explored in a larger population, this study only allows speculation on the mechanisms underlying these results.

The prolonged effect induced by PES of afferent fibers may be related to induced activity-dependent plasticity at different locations of the spinal cord. In particular, the modulation of the wrist disynaptic Group I interneurons stands as a plausible mechanism responsible for tremor reduction accordingly to the results reported after the application of phase-dependent SATS strategy in healthy subjects (Chapter 4). Those results indicated that out-of-phase stimulation elicits a decrease in the wrist disynaptic Group I inhibition in healthy subjects, a mechanism that if translated to ET patients, could be related to a depression of the spinal circuit which is hypothesized to be involved in the tremor oscillations. Additionally, the tremor generation models in which the Ia fibers play a key role in tremor amplification are also aligned with this hypothesis. Another location where modulation might occur at the spinal cord is the propriospinal system, where supraspinal pathways converge with projections from afferent fibers and has been hypothesized to be involved in the pathological tremor network (Hao et al., 2013; Pierrot-Deseilligny, 1996).

Alternatively, modulation of brain structures involved in the pathogenesis of ET should be considered as well. Some authors have suggested that modulation of afferent pathways may produce an effect in supraspinal centers such as the Ventral Intermediate Nucleus (VIM), which is a brain target for DBS or HIFU for tremor

(Pahwa et al., 2019), or the cerebellum (Barath et al., 2020). Overall, all the hypotheses stated here are non-mutually exclusive and modulation after PES of afferent pathways might occur at different locations of the CNS.

In this study, the quantification of tremor was performed by combining both kinematics and clinical tremor rating scales following the recommendations extracted from the review of the state of the art (Chapter 2). The acute tremor reduction effect was assessed exclusively by means of kinematics, while the short-term tremor reduction effects reported were reinforced by the linear relationship found between the kinematic and clinical scales assessments. Particularly, the highest correlation was found between the flexion-extension angle tremor power, and the severity scores of the FTM scales. Interestingly, both assessments were designed to score postural tremor, a fact that could explain the positive correlation found between the two variables. As discussed in Chapter 2, the combination of both assessments performed in independent trials support the reproducibility of the measurements, which might become convenient due to the implicit intra-patient tremor variability in tremor assessments.

Although the main target of this stimulation protocol was to reduce wrist flexion-extension tremor of the stimulated side, average acute tremor reduction (non-significant) at the ipsilateral elbow and shoulder level was also reported for the SATS IntraStim condition. Additionally, average tremor reduction (non-significant) was also observed on the contralateral wrist 24 h after the stimulation for both IntraStim and SurfStim sessions. Afferent fibers do not only make connections with the homonymous muscle, but also with interneurons connected to agonists muscles from both ipsilateral and contralateral sides. A putative explanation could be again the modulation of oligosynaptic spinal pathways and propriospinal interneurons that project bilaterally (Hanajima et al., 2004; Costa et al., 2008) or the modulation of brain structures involved in the tremor oscillatory network (Barath et al., 2020).

Even though SATS IntraStim achieved higher tremor reduction compared to SATS SurfStim, it should not be concluded that surface stimulation should not be explored as an alternative to reduce tremor. In fact, stimulation conditions did not exactly match between intramuscular and surface stimulation due to differences in pulse width, stimulation intensity and electrode placement. Results reported here for SurfStim should be analyzed carefully due to the low stimulation intensity and capability of the stimulator used, which was limited to 5 V. In any event, this fact should not mask the efficacy of the SATS IntraStim condition, which was proven to induce statistically higher acute tremor reduction compared to the other three conditions explored (CON IntraStim, CON SurfStim and SATS SurfStim). The intramuscular electrodes used in this study are the result of the development of a novel thin-film multichannel electrode within the context of the NEUROTREMOR and EXTEND projects (Muceli et al., 2019). These electrodes allow simultaneous stimulation and recording within the same implant, providing bidirectional interaction with the nervous system. However, the elevated cost of the thin-film multichannel intramuscular electrodes narrowed the number of patients that could be tested at this stage, being the greatest limitation of the presented study. Increasing the sample size of the tested conditions would contribute to generate evidence about the reproducibility of the results and to strengthen the statistical analysis of the different effects reported.

In conclusion, these results show that acute, short-term and prolonged (24 h after stimulation) tremor reduction can be achieved in ET patients using SATS strategy applied with minimally invasive intramuscular electrodes. Overall, these effects are not attributed to a placebo effect due to four main reasons: 1) tremor reduction was significantly higher using intramuscular rather than surface stimulation; 2)

SATS IntraStim was the only condition achieving significant acute tremor reduction; 3) continuous (open-loop) non-specific stimulation usually led to tremor aggravation; 4) patients were blinded to the stimulation strategy applied and, in some cases, they did not feel any sensation when stimulation was delivered. The results of this proof-of-concept study might open the possibility to a new therapeutic approach for tremor patients.

Chapter 6

Summary and conclusions

Essential tremor (ET) is the main neural disease leading to pathological tremor, the most widespread motor disorder worldwide. Currently, there is no cure for ET, a disease with an increasing incidence as people age and which can induce disabling tremors. The quality of life of patients suffering from ET is deteriorated since performing routine tasks such as feeding, typing on a keyboard or writing become a challenge, and for the most severe tremor, an impossible venture. Moreover, available clinical solutions to reduce tremor are neither effective nor eligible for a large population. Thus, a need to develop an effective, accessible and low-risk solution to reduce tremor was identified.

Alternative solutions to mitigate pathological tremor have been researched in the last few decades, ranging from upper-limb exoskeletons, assistive or compensating devices or functional electrical stimulation (FES). Particularly, peripheral electrical stimulation (PES) of afferent pathways has gained interest in the recent years due to its potential to reduce tremor by interfering with the malfunctioning nervous system without significant adverse effects. However, there is a limited number of studies exploring the tremor reduction capabilities of PES of afferent pathways to reduce tremor in ET populations. Despite the fact that PES of afferent pathways is already a technique used in some clinical fields such as sport science, this tool can be applied with a wide range of protocols, and the effects of stimulation on the nervous system have not been fully characterized.

This PhD thesis presents the development of a PES of afferent pathway strategy aiming at reducing pathological tremor in ET patients. Drawing from a detailed review of the published studies using PES to reduce tremor, a stimulation strategy was designed and tested based on delivering timely and precise stimulation of the antagonist muscle to the one activated by the tremorgenic input. The acute and short-term neuromodulatory effects of this strategy were firstly explored in healthy subjects, and the tremor reduction efficacy was later tested in ET patients.

6.1 Conclusions

The main goal motivating this PhD thesis was the development of a solution to manage pathological tremor in ET patients based on the utilization of the peripheral electrical stimulation of afferent pathways. A sequential approach was followed and this primary objective was addressed by means of dividing it into three hypotheses and their subsequent goals.

The initial goal (**Goal 0**) defined for this PhD thesis aimed at providing a comprehensive review about the current status of the PES-based solutions to reduce pathological tremor. Methodological considerations, main results in tremor reduction and the proposed hypotheses behind tremor reduction were revisited for 29

studies meeting the inclusion and exclusion criteria. The outcomes from the review showed that only 13 studies have explored PES of afferent pathways in ET populations. The systematic review also showed heterogeneity in stimulation protocols and results reported, while there was limited evidence about the underlying sources of tremor reduction during or after the application of PES of afferent pathways.

On the basis of the knowledge gathered, some guidelines were proposed to advance in the research of PES of afferent pathways applied to reduce pathological tremor. Achieving a deeper understanding on how the delivery of electrical currents interfaces the neural system was identified as an essential step prior to the development of stimulation protocols applied on ET patients. Particularly, the principle of reciprocal inhibition (known as disynaptic Group I inhibition at the wrist), a motor control mechanism, was identified as a neural pathway which could be targeted for timely stimulation. Therefore, the following stated hypotheses aimed at characterizing the effects of recruiting afferent pathways via PES in healthy subjects, and, finally in ET patients, based on neurophysiological models.

6.1.1 Hypothesis 1

PES of the afferent pathways can acutely inhibit the voluntary muscle activity of the antagonist muscle for the wrist flexor and extensor muscles in healthy participants.

Previous studies had suggested that tremor reduction could be achieved by means of exploiting the reciprocal inhibition spinal circuit with an out-of-phase stimulation strategy (i.e., stimulation of the afferent fibers innervating the antagonist muscle to the one activated by the tremorgenic input, so the tremor activity is inhibited through the spinal loop). In this study, the acute effects of electrical stimulation over the nerves innervating the wrist flexor and extensor muscles were explored in healthy subjects. While the subjects were asked to perform an isometric wrist flexion or extension task, electrical stimuli were delivered to the radial and median nerves respectively, with intensities below and above motor threshold (MT). Previously, the stimulation of the afferent pathways, including the Ia fibers, was assured by means of H-reflex recruitment curves. Results showed that both stimulation below and above MT of the antagonist muscle induced inhibition of the ongoing muscle activity, for both muscles. The latency of the inhibition, which started approximately 20 ms after the delivery of the electrical stimuli and lasted approximately 18 ms, was compatible with the activation of spinal cord mechanisms similar to the classic reciprocal inhibition. Since preceding studies suggested that true reciprocal Ia inhibition is not present at the wrist, it was hypothesized that disynaptic Group I inhibition, probably mediated by Ia fibers, was the main spinal circuit involved in the inhibition. Although the application of stimulation above MT achieved higher mean inhibition of the antagonist muscle due to the recruitment of a large number of afferent fibers, and likely other type of afferents than Group I, this study proved that stimulation below MT is capable of activating afferent fibers and spinal reflex circuits without directly recruiting motor axons. Specific goals were also achieved, since the set of electrophysiological techniques needed was mastered for its application in subsequent studies (**Goal 1.1**); and the outcomes served as basis for the development of tremor reduction stimulation strategies targeting the acute inhibition of the muscle activity by means of recruiting the afferent pathways (**Goal 1.2**).

6.1.2 Hypothesis 2

Selective and adaptive timely stimulation of afferent pathways induces short-term neuromodulatory effects on the spinal circuits controlling the wrist muscles in healthy subjects.

Through the review of the state of the art, several research groups had claimed that tremor reduction could be attained from minutes to hours after the delivery of PES of afferent pathways. Therefore, after proving the acute inhibitory effects of single pulse stimulation, a strategy was designed in order to apply stimulation on the antagonist muscle based on real-time measurements of the muscle activity. The stimulation strategy proposed was named Selective and Adaptive Timely Stimulation (SATS), and a study was conducted to characterize the short-term neuromodulatory effects in healthy subjects. The phase of the stimulation of the radial nerve was locked to the EMG activity of the antagonist muscle (out-of-phase) or the agonist muscle (in-phase) while the subjects mimicked wrist flexion-extension tremor. Results showed that activity-dependent modulation was induced in the disynaptic Group I inhibition circuit at the wrist, and this modulation was opposite if the stimulation was applied in-phase (77±23% compared to baseline; potentiation of the inhibition mechanism), or out-of-phase (125±46%; compared to baseline; depression of the inhibition mechanism). No further spinal mechanisms, including excitability of Ia and Ib fibers, and estimations of the neural drive, were identified to be affected by the stimulation intervention. Thus, the neural adaptations induced after phase-locked PES of afferent pathways might be specific in the targeted neural circuit, while the global motor control remains unaltered. Additionally, analysis of the SATS strategy performance revealed that the stimulation was precisely delivered based on the EMG measurements, with an average synchronization delay below 17 ms, which served as validation of the SATS strategy for further implementation in tremor patients (**Goal 2.1**). Despite these results were limited to 11 healthy subjects, the SATS strategy was proven to be capable of inducing short-term neuromodulatory effects at the spinal cord by exploiting the timely stimulation of the antagonist muscle (**Goal 2.2**).

6.1.3 Hypothesis 3

Selective and adaptive timely stimulation of afferent pathways applied with intramuscular electrodes induces acute and short-term tremor reduction in ET patients.

The SATS strategy was finally tested as solution to reduce tremor in a cohort of 9 ET patients. In order to explore the acute and short-term tremor reduction efficacy elicited exclusively by the delivery of closed-loop stimulation synchronized with the tremorgenic activity of the antagonist muscle, an open-loop (continuous) stimulation strategy was also tested, and both strategies were applied in two experimental sessions with intramuscular or surface electrodes. Measurements of the tremor severity estimated by means of kinematics and clinical scales revealed that the SATS strategy delivered with intramuscular electrodes achieved 32% average acute tremor reduction, a value significantly higher when compared to the open-loop strategy. Furthermore, short-term tremor reduction was reported after the stimulation session for all the patients receiving SATS strategy delivered with intramuscular electrodes. Some of the patients who received SATS strategy attained tremor reduction up to 24 hours after the experimental session. Although this was the first known reporting of tremor reduction prolonged effects, results should be considered thoughtfully

due to the limited sample size and variability. This study did not provide evidence about the physiological mechanisms underlying tremor reduction during or after the application of PES of afferent pathways. Notwithstanding, the experimental design and the results allowed to emphasize about the relevance of recruiting afferent pathways synchronously with the ongoing physiological activity to induce neural adaptations which resulted in functional improvement (**Goal 3.1**). Likewise, intramuscular electrodes allowed robust bidirectional interfacing with the neural system, and specific recruitment of afferent pathways, which led to tremor reduction. Hence, minimally-invasive neuroprostheses in combination with the application of closed-loop PES of afferent pathways could address the shortcomings of existing treatment options and become a solution to reduce chronic tremor in ET patients (**Goal 3.2**).

6.2 Impact and future work

PES of afferent pathways stands as an opportunity to manage pathological tremor in ET populations as an effective, affordable and low-risk therapy. As already stated, approximately 50% of ET population do not receive any effective treatment to mitigate pathological tremor. This ineludible fact, along with the high prevalence of this disease leads to the possibility that millions of patients could potentially benefit from PES-based therapies if they are broadly implemented. The quality of life of the patients would be considerably enhanced as they age, since a reduction of tremor amplitude would allow them to recover autonomy in the execution of ADLs and to improve mental health as they could feel like active members of their social environment. The impact of a broadly accessible solution to manage tremor would also reach the healthcare systems and the economy as a whole by reducing the resources and expenses dedicated through pharmacotherapy and costly neurosurgical interventions, as well as the assistive support services for patients with a high degree of impairment.

Although the benefits of a solution to mitigate pathological tremor are clearly explicit, PES of afferent pathways to reduce tremor is still in a research stage with the exception of a FDA approved wearable device to deliver an open-loop PES of afferent pathways protocol at the wrist level. The review of the state of the art about PES to reduce pathological tremor provided a comprehensive, systematic and updated description of the methodological and efficacy aspects of the existing PES protocols applied to mitigate pathological tremor. As a consequence, the upcoming studies could use the information gathered in this review to identify the methods followed by previous research groups, and follow the recommendations provided to advance in the development of more effective and reproducible protocols. Overall, the main issues which should be addressed to favor the transition from bench to bedside are: 1) increasing the number of patients tested in clinical trials, including control or placebo groups; 2) determining the physiological sources of tremor reduction during and after the delivery of PES of afferent pathways; 3) characterizing the optimal stimulation protocols and parameters, considering the individual features of each patient to deliver personalized treatments.

The first two studies presented in this PhD thesis with healthy subjects sought to deepen into the capabilities of PES of afferent pathways to modulate the CNS and to identify some of the sources for neural adaptations. Firstly, the characterization of the inhibition of muscle activity after the stimulation of the antagonist muscle for the wrist flexors and extensors contributed to the general knowledge in the neurophysiology field. Additionally, the inhibition of the muscle activity can be directly

related with the acute tremor suppression reported in this thesis by means of the SATS strategy and the stimulation of the antagonist muscle to the tremorgenic activity, as it has been hypothesized by other authors previously. In future studies it would be desirable to identify the specific afferent fibers (Ia, Ib, Group II) and interneurons involved in such activation of the spinal circuits, as well as other afferent projections towards supraspinal centers involved in motor control.

The second study conducted in this thesis led to the development of SATS, a PES strategy which was proven to be capable of inducing neuromodulatory changes at the CNS in healthy subjects. The knowledge generated will contribute to a deeper understanding of the motor control of the wrist muscles, and how it is affected by PES of afferent pathways. Activity-dependent modulation was induced, likely at the spinal cord interneurons, but other sources of neural adaptations along the CNS could not be confirmed. Hence, upcoming studies should focus on unveiling the neural adaptations induced at other regions of the spinal cord or at brain centers during or after phase-dependent PES of afferent pathways. Electrophysiological assessments of the corticospinal excitability, such as TMS protocols, or neuroimaging techniques could be useful tools to identify the sources for neuromodulation. Although this study was conducted on healthy subjects, results confirm the potential of SATS of afferent pathways to induce specific neural adaptations. Therefore, it could potentially be applied to other motor disorders such as spasticity, where the misbalanced neural circuits could be shifted back to physiological or functional states by means of inducing the specific modulation.

Ultimately, the final contribution from this PhD thesis was the testing of the SATS strategy in a cohort of ET patients. The tremor reduction results while the stimulation was applied were aligned with previous studies and confirmed the relevance of synchronizing the recruitment of afferent pathways with the neural pathways activated by the tremorgenic inputs. This acute effect would be useful per se, since a wearable device or implant could be used to deliver closed-loop stimulation and reduce tremor while the subject is performing activities of daily living (ADLs) where the tremor is a hampering condition. However, this application would need further validation since this study, as well as the majority of other studies using PES of afferent pathways, have been tested for postural tremor, a constrained condition easily reproducible in research. In addition to the acute effects, outcomes of this study showed for the first time the potential of PES of afferent pathways applied through intramuscular electrodes to induce short-term tremor reduction effects, lasting up to 24 hours for some patients. Furthermore, the relevance of timing the stimulation with the activity of the target neural circuit was a keystone to attain neuromodulation of the tremor network. These results open the possibility to develop therapeutic protocols based on PES of afferent pathways. Traditional pharmacotherapy treatments to reduce ET could be replaced by electroceuticals, a term named by some authors which represents therapeutic solutions based on the delivery of electrical currents (Famm et al., 2013). Thus, ET patients could receive brief interventions of PES of afferent pathways, even while they are performing ADLs, and the tremor reduction effects could last from hours to days. Reproducing the experimental conditions followed in this study and determining the specific spinal and/or brain centers modulated via stimulation would be a necessary step to validate the results presented here and advance towards a clinically implantable solution.

So far, the majority of published studies have targeted the stimulation of a pair of nerves of muscles, typically those controlling the wrist and exhibiting the most prominent tremor, which is the ultimate motor effector in the upper-limb and where

tremor has a greater impact on hampering ADLs. Additionally, ET is a motor disorder which often manifests bilaterally, although there is mild asymmetry and one side typically shows more affectation than the other. Notwithstanding, some studies including the outcomes presented in this PhD thesis suggested that the tremor reduction during or after delivering PES of afferent pathways might be not limited to the stimulated joint, and functional improvement can occur at different ipsilateral and contralateral sites. Hence, future approaches may include PES at multiple locations, including personalized stimulation protocols considering the biomechanical analysis of tremor for each patient. Applying PES on the proximal upper-limb joints is an interesting approach which should be explored, since mechanical tremor frequently propagates from proximal to distal joints (Corie et al., 2019), and as a result, mitigation of tremor in proximal joints could be extended to more distal joints. Some of these alternative stimulation protocols were tested in a pilot study with two ET patients during the development of this PhD thesis (Pascual-Valdunciel et al., 2022a).

The use of transcutaneous or surface electrodes are preferred in research as experimentation, clinical trials or device development remains simpler, with lower risks associated with regards to regularization or commercialization. Nevertheless, neural interfaces based on implants are gaining interest since they might offer a permanent solution with enhanced features compared to external wearable devices and surface solutions. The intramuscular electrodes used in the last study of this PhD thesis offered a minimally invasive solution to extract electrophysiological data and stimulate the nervous system with higher selectivity compared to transcutaneous approaches. The clinical efficacy in tremor reduction and safety data reported in this study should be extended to larger patient populations in order to gain evidence about the benefits of this technology. After improvements in the hardware as well as in the stimulation control algorithms, intramuscular electrodes could be integrated in long-term implantable systems. These systems should be implanted through an ambulatory and minimally-invasive and reversible procedure, overcoming the risks associated to surgical interventions.

In case novel wearable devices or minimally-invasive implants have regulatory approval for marketing as tremor reduction solutions, both the systems and the delivery of PES of afferent pathways should not interfere with the ADLs of the patient, and stimulation should preferentially be administered on demand as tremor is monitored with closed-loop systems. Breakthroughs in real-time signal processing is a necessary step to monitor tremor in real life scenarios where the recording's conditions are not controlled. Machine learning techniques and neuromusculoskeletal models including not only one sensing method (e.g. EMG), but also the combination of several (e.g. EMG and accelerometry) might be a valuable solution to decode and model tremorgenic activity in signals mixed with other voluntary or movement components and lower signal to noise ratio. Moreover, personalized tremor models based on machine learning techniques would allow optimization and automation of the stimulation timing and stimulation parameters which could maximize the tremor reduction outcomes for each individual patient.

6.3 Publications

The work performed along this PhD dissertation led to the development of the several scientific contributions. Some of them were directly related with the studies included in this document and have already been

stated in the respective chapters, while the content from other contributions have not been directly included in the studies here presented:

Pascual-Valdunciel, A., Barroso, F. O., Pons, J. L. (2018). Motor inhibition elicited by electrical stimulation of afferent pathways and its application in tremor suppression. School and Symposium on Advanced Neurorehabilitation (SSNR2018)

Herrero, O., **Pascual-Valdunciel, A.**, Resquín, F., Ibáñez, J., Dimdwayo, I., Brea, M., … Pons, J. L. (2019). Rehabilitation of Reaching Movement After Stroke Using a Hybrid Robotic System and Paired with the Motor Intent. In L. Masia, S. Micera, M. Akay, J. L. Pons (Eds.), Converging Clinical and Engineering Research on Neurorehabilitation III (pp. 483–487). Cham: Springer International Publishing.

Barroso, F. O., **Pascual-Valdunciel, A.**, Pons, J. L. (2019). Review on Tremor Suppression Using Afferent Electrical Stimulation. In Biosystems and Biorobotics (Vol. 21, pp. 1092–1096). https://doi.org/10.1007/978-3-030-01845-$0_2$18

Pascual-Valdunciel, A., Barroso, F. O., Muceli, S., Taylor, J., Farina, D., Pons, J. L. (2019). Modulation of reciprocal inhibition at the wrist as a neurophysiological correlate of tremor suppression: a pilot healthy subject study (pp. 6267–6272). 2019 41st Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC), Berlin, Germany, 2019. https://doi.org/10.1109/embc.2019.8857018

Barroso, F. O., **Pascual-Valdunciel, A.**, Torricelli, D., Moreno, J. C., Del-Ama, A. J., Laczko, J., Rovira, J. L. P. (2019). Noninvasive Modalities Used in Spinal Cord Injury Rehabilitation. https://doi.org/10.5772/intechopen.77698

Gutiérrez Zúñiga, R., Alonso de Leciñana, M., Díez, A., Torres Iglesias, G., **Pascual, A.**, Higashi, A., … Díez Tejedor, E. (2021). A New Software for Quantifying Motor Deficit After Stroke: A Case-Control Feasibility Pilot Study. Frontiers in Neurology, 12, 603619. https://doi.org/10.3389/fneur.2021.603619

Pascual-Valdunciel, A., Hoo, G. W., Avrillon, S., Barroso, F. O., Goldman, J. G., Hernandez-Pavon, J. C., Pons, J. L. (2021). Peripheral electrical stimulation to reduce pathological tremor: a review. Journal of Neuroengineering and Rehabilitation, 18(1), 33. https://doi.org/10.1186/s12984-021-00811-9

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Appendix A

Supplementary Tables

TABLE A.1: Summary of the studies reviewed.

Article	Population	Strategy	Stim. location	Stim. pulse width [us]	Stim. frequency [Hz]	Stimulation protocol	Tremor Assessment	Main Results	Physiological mechanism
Barath et al., 2020	5 ET (mild-severe)	afferent MT: calibrated open-loop	SF: radial and median nerves at wrist	300	150	Clinical-trial: 3 months, 2x40min stim session/day	TETRAS, kinematics at wrist,FDG PET/CT	Median post reduction (kinematics) 72.6%	Cerebellar modulation
Bó et al., 2014	10 ET (moderate-severe)	FES: co-contraction	SF: heterogeneous wrist and fingers muscles	150	40	10-50s/trial, stim ON vs stim OFF, 5-7 trials	Kinematics, tremor power at wrist	Most significant acute tremor attenuation: 37.18%-94.68%	Increasing joint stiffness
Britton et al., 1993	10 ET; 9 PD, 8 HV	Single shock MT	SF: median nerve	500	Single shock	Single shock	sEMG	Significant EMG reduction from 90 to 210ms post stimulus	Afferences reset central tremor oscillators
Dideriksen et al., 2017	4 ET; 5 PD (mild-severe)	afferent MT: out-of-phase EMG based	SF (2 ET, 3PD), IM (2 ET, 2PD): wrist flexors-extensors	400	100	150s/trial, stim ON vs stim OFF, 20% and 40% DC, 10 trials	Kinematics, tremor power at wrist	Average highest acute reduction: 54 ± 20% (IM) and 50 ± 41% (SF)	Ia afferent fibers, reciprocal inhibition
Dosen et al., 2015	2 ET; 4 PD (mild-severe)	FES and afferent MT: out-of-phase EMG based	SF: wrist/finger flexors-extensors	300	100	120s/trial, stim ON vs stim OFF, 5 trials per modality	Kinematics, tremor power at wrist	Average acute reduction: 60 ± 14% (MT) and 42 ± 5% (MT) (p0.05)	Generation of opposite forces to tremor oscillations; Ia afferent fibers, reciprocal inhibition
Gallego et al., 2013	4 ET; 2 PD (mild-severe)	FES: co-contraction	SF: wrist flexors-extensors	250 or 300	30 or 40	30s/trial; stim ON vs stim OFF, 6-12 trials	Kinematics, tremor power at wrist	Average acute reduction: 52.33 ± 25.48% (p0.05)	Increasing joint stiffness
Gillard et al., 1999	3 PD, 3 HV	FES: out-of-phase accelerometer based	SF: wrist/finger flexors-extensors	*	*	30s/trial, stim ON vs stim OFF, 10 trials	Kinematics at finger/wrist	Average acute reduction: 84.50 ± 2.20%	Out-of-phase forces

Grimaldi et al., 2011	1 PD, 1 ET, 1 cerebellar syndrome	FES: co-contraction	SF: wrist/elbow flexors-extensors	100	30	~30s/task, stim ON vs stim OFF, 15 trials (5 tasks)	Kinematics at finger/wrist/elbow; CNF-TES scale	Most significant acute tremor attenuation in one ET patient: ~50%	Increasing joint stiffness
Hao et al., 2017	8 PD (moderate)	afferent MT: continuous (EMG triggered)	SF: radial nerve (dorsal skin of hand)	200	250	15s/trial, stim OFF(5s)-ON(5s)-OFF(5s), 9-13 trials	Kinematics and sEMG at finger/wrist/elbow flexors-extensors	Average acute reduction: 61.56 (kinematics across DOF); 47.97% EMG across DOF)	Cutaneous afferents and propriospinal interneurons
Heo et al., 2015	18 ET (moderate)	afferent MT: continuous	SF: wrist/elbow flexors-extensors	300	100	15s/trial, Pre-Stim ON-Post 5min, 9 trials	Kinematics at finger/forearm/arm	Average acute reduction: 90% (finger), 58% (hand), -50% (forearm); Post 5min: 88% (finger), 61% (hand), 27% (forearm)	Afferences might modulate supraspinal tremor oscillators
Heo et al., 2016	18 ET (mild-severe)	afferent MT: continuous	SF: wrist/elbow flexors-extensors	300	100	Spiral drawing task, Pre-Stim ON-Post 5min, 9 trials	Kinematics at finger/forearm/arm	Acute average reduction: 12%. Significant differences (p0.05) for Pre-Stim and ON-Post 5′	Afferences might modulate supraspinal tremor oscillators
Heo et al., 2018	14 PD (mild-severe)	afferent MT: continuous	SF: wrist flexors-extensors	300	100	15s/trial, Pre-Stim ON-Post 5min, 9 trials	Kinematics at finger/forearm/arm	Reduction in 50-71% of patients. Average acute: 68% (finger), 62% (hand), 53% (forearm); Post 5min: 56% (finger), 59% (hand), 60% (forearm)	Afferences might modulate supraspinal tremor oscillators
Heo et al., 2019	14 PD (same Heo 2018), 9 SWEEDs	afferent MT: continuous	SF: wrist flexors-extensors	300	100	15s/trial, Pre-Stim ON-Post 5min, 9 trials	Kinematics at finger/forearm/arm	No reduction data. Only significant differences (p0.05) for Pre-Stim ON in PD. No reduction for SWEEDs.	Afferences might modulate supraspinal tremor oscillators

Isaacson et al., 2020	263 ET (mild-severe)	afferent MT: out-of-phase kinematics (open-loop)	SF: radial and median nerves at wrist	300	150	Clinical-trial: 3 months, 2x40min stim session/day	TETRAS, select BF-ADL tasks, kinematics at wrist, CGI-I, PGI-I, QUEST	62% (TETRAS) and 68% (BF-ADL) of severe/moderate patients improving to mild/slight	Afferences modulate Ventral Intermediate Nucleus
Javidan et al,. 1992	3 ET, 4 PD, 6 cerebellar tremor	FES: out-of-phase kinematics based	SF: wrist, elbow flexors-extensors	100	30	20min/trial, Stim ON vs Stim OFF, unknown number of trials	Kinematics at wrist	Average acute reduction at wrist: 73 ± 14% (ET), 62 ± 5% (PD), 62 ± 38% (cerebellar tremor)	Generation of opposite forces to tremor oscillations
Jitkritsadakul et al. 2015	34 PD (moderate)	MT: continuous	SF: fingers APB, FDI, SDI	150	50	10s/trial, Pre-Stim ON, 2 trials	Kinematics and sEMG at finger/forearm/ arm; UPDRS	Average acute reduction (tremor power): 49.57 ± 38.89% (p0.05)	Afferences interfere with the cerebello-thalamo-cortical circuit
Jitkritsadakul et al. 2017	30 PD (moderate)	MT: continuous	SF: fingers APB, FDI, SDI	150	50	10s stim, Pre-Stim ON; 30min session; Sham vs Stim	Kinematics at hand (glove); UPDRS; VAS	Average acute reduction (RMS, x-axes): 60.22 ± 38.85% (p0.05); significantly different from sham group	Afferences interfere with the cerebello-thalamo-cortical circuit
Kim et al., 2020	9 ET (moderate-severe)	afferent MT: out-of-phase kinematics based and open-loop	SF: radial nerve at wrist	200	50, 100, 200	20s/trial, stim. OFF (10s)-ON (10s); open-loop, closed-loop; 12.5%, 25% and 37% DC;18 trials	Kinematics at wrist; TETRAS; qualitative assessment	Average acute reduction: 42.17 ± 3.09% (p0.05). No differences open vs closed loop	Not proposed
Lin et al., 2018	23 ET (moderate-severe)	afferent MT: calibrated open-loop	SF: radial and median nerves at wrist	300	150	Pre, 40min stim, Post; Sham vs Stim group	TETRAS (spiral)	Average post reduction (TETRAS) : 60 ± 8.4% (p0.05), significantly different from sham group	Afferences modulate Ventral Intermediate Nucleus
Mones et al., 1969	5 PD	Single shock above MT	SF: ipsilateral and contralateral ulnar nerve at wrist	500	Single shock	Single shock	iEMG	No change in tremor amplitude. Change in tremor frequency after shock	Afferences reset central tremor oscillators

Munhoz et al., 2003	5 ET, 2 peripheral neuropathy	TENS MT: continuous	SF: brachial plexus on neck, C7 spinous process	250	5, 10, 50, 100	Pre, 15min stim, Post	Kinematics at wrist, WHIGET scale	No significant reduction	Wrong afferent fibers targeted or stimulation parameters
Muceli et al. 2019	1 PD	afferent MT: out-of-phase EMG based	SF: wrist/finger flexors-extensors	200	100	60s/trial, stim ON(30s) vs stim OFF(30s), 2 trials	Kinematics, tremor power at wrist	Acute reduction in one patient: 58%	Ia afferent fibers, reciprocal inhibition
Pahwa et al. 2018	77 ET (moderate-severe)	afferent MT: calibrated open-loop	SF: radial and median nerves at wrist	300	150	Pre, 40min stim, Post; Sham vs Stim group	TETRAS, select BF-ADL tasks, CGI-I scale	Average post reduction (task 4 TETRAS) : 46% (stim group) different from 24% (sham group)	Afferences modulate Ventral Intermediate Nucleus
Popovic et al., 2011	3 ET, 4 PD, 5 HV	FES: out-of-phase EMG based	SF: wrist, elbow flexors-extensors	250	40	approx. 60s/trial, stim ON vs stim OFF**	Kinematics at wrist	Average acute reduction: 67 ± 13%	Generation of opposite forces to tremor oscillations
Reis et al., 2020	14 ET	~MT	SF: median nerve at wrist	200	Single shock	Single shock locked to 12 tremor phases	Kinematics at wrist	No significant reduction	Afferences interfere with central tremor oscillators
Spiegel et al., 2002	8 PD	MT	SF: median (IP and CL) and ulnar (IP) nerves at wrist	200	2, 3, 5	approx. 316s/trial, single shock, post 5min, 4 trials without shock-4 trials with shock	sEMG	Tremor amplitude not reported. Change in tremor frequency after stimulation.	Afferences interfere with central tremor oscillators
Widjaja et al., 2011	1 ET	FES: out-of-phase model based (EMG+kinematics)	SF: wrist flexors-extensors	200	25	40s/trial, Stim ON vs Stim OFF, 1 trial	Kinematics at wrist	Individual acute tremor attenuation: 57%	Generation of opposite forces to tremor oscillations
Xu et al., 2016	2 PD (moderate)	afferent MT: continuous (EMG triggered)	SF: radial nerve (dorsal skin of hand)	200	250	15s/trial, stim OFF(5s)-ON(5s)-OFF(5s), 9-13 trials	Kinematics and sEMG at finger/wrist/elbow flexors-extensors	Significant acute reduction compared to OFF (p0.05). No values provided	Cutaneous afferents and propriospinal interneurons

ET: Essential Tremor; PD: Parkinson's disease; HV: healthy volunteer; SF: surface stimulation; IM: intramuscular stimulation; BF-ADL: Bain and Findley ADL; CGI-I, PGI-I: Clinical and Patient Global Impression scores; QUEST: Quality of Life in Essential Tremor; APB: abductor pollicis brevis; FDI: first dorsal interossei; SDI: second dorsal interossei; IP: ipsilateral; CL: contralateral; DC: duty cycle; VAS: visual analog scale; iEMG: intramuscular EMG; *: not described in paper; **: ((3s stim+1s pause)*3+9s pause)*3 reps

TABLE A.2: Electrode types utilized by the studies included in this review.

Article	Electrode
Barath et al., 2020	Self-Adhesive: 2.2x2.2cm
Bó et al., 2014	Self-Adhesive: round ø3.2cm (smaller for forearm)
Britton et al., 1993	*
Dideriksen et al., 2017	Self-Adhesive: round ø3.2cm ; Intramuscular: ø0.5mm pair of Teflon wires
Dosen et al., 2015	Self-Adhesive: 5x5cm (wrist); round ø3.2cm (wrist)
Gallego et al., 2013	Multichannel surface array
Gillard et al., 1999	Self-Adhesive: 4.5x4.5cm
Grimaldi et al., 2011	*
Hao et al., 2017	Self-Adhesive: round ø2.5cm
Heo et al., 2015	Self-Adhesive: 5x5cm
Heo et al., 2016	Self-Adhesive: 5x5cm
Heo et al., 2018	Self-Adhesive: 5x5cm
Heo et al., 2019	Self-Adhesive: 5x5cm
Isaacson et al. 2020	Self-Adhesive: 2.2x2.2cm
Javidan et al., 1992	Self-Adhesive: 2x3cm; Moistened pads
Jitkritsadakul et al. 2015	Self-Adhesive: 5x5cm
Jitkritsadakul et al. 2017	Self-Adhesive: 5x5cm
Kim et al., 2020	Self-Adhesive: round ø2.5cm
Lin et al., 2018	Self-Adhesive
Mones et al., 1969	*
Munhoz et al., 2003	Self-Adhesive
Muceli et al. 2019	Thin-film double-sided intramuscular electrodes
Pahwa et al. 2018	Self-Adhesive: 2.2x2.2cm
Popovic et al., 2011	Self-Adhesive: round ø3.2cm
Reis et al., 2021	Self-Adhesive
Spiegel et al., 2002	*
Widjaja et al., 2011	Self-Adhesive
Xu et al., 2016	Self-Adhesive: round ø2.5cm
Yu et al. 2021	Self-Adhesive: 2.2x2.2cm

*: No description provided about the stimulation electrode.

TABLE A.3: Individual stimulation parameters and number of completed trials for each patient and session.

	IM							SF						
P	**Trials completed**	**Perception threshold FCR [mA]**	**Motor threshold FCR [mA]**	**Stimulation amplitude FCR [mA]**	**Perception threshold ECR [mA]**	**Motor threshold ECR [mA]**	**Stimulation amplitude ECR [mA]**	**Trials completed**	**Perception threshold FCR [mA]**	**Motor threshold FCR [mA]**	**Stimulation amplitude FCR [mA]**	**Perception threshold ECR [mA]**	**Motor threshold ECR [mA]**	**Stimulation amplitude ECR [mA]**
1	16	0.1	0.4	0.3	0.1	0.8	0.7	12	0.1	>4.5	4	0.1	>5.0	5.0
2	10	0.5	0.9	0.5	>2.4	>2.4	1.8	10	1.5	>5.0	4.0	2.0	>5.0	4.0
3	11	2.4	>2.4	1.4	>2.4	>2.4	>2.4	13	0.1	>5.0	5.0	1.0	>5.0	5.0
4	7	1.5	>2.4	2.4	1.3	1.4	1.3	9	2.5	>5.0	5.0	2.0	>5.0	5.0
5	6	>2.4	>2.4	2.4	>2.4	>2.4	2.4	6	4.0	>5.0	5.0	4.0	>5.0	5.0
6	8	>2.4	>2.4	2.4	>2.4	>2.4	2.4	8	3.3	>5.0	5.0	2.6	>5.0	5.0
7	9	1.7	1.7	1.6	0.4	0.4	0.3	9	4.6	>5.0	5.0	4.2	>5.0	5.0
8	8	>2.4	>2.4	2.1	0.4	0.4	0,3	9	1.3	>5.0	5.0	2.7	>5.0	2.7
9	10	1.8	2.2	1.8	0.6	0.7	0.6	8	4.0	>5.0	5.0	4.0	>5.0	5.0

TABLE A.4: Adverse effects of electrode insertion and removal on pain, transient paresthesias and fatigue along an experimental session.

		INTENSITY	VISUAL ANALOGUE SCALE (VAS)	INTRAMUSCULAR ELECTRODES (n = 9)	SURFACE ELECTRODES (n = 9)
Pain	Insertion	Mild	0 - 2	5	0
		Mild-Moderate	3 - 5	2	0
		Moderate-Severe	6 - 8	0	0
		Severe	9 - 10	0	0
	Removal	Mild	0 - 2	2	0
		Mild-Moderate	3 - 5	1	0
		Moderate-Severe	6 - 8	0	0
		Severe	9 - 10	0	0
Transient paresthesias during experiments		Mild	0 - 2	1	1
		Mild-Moderate	3 - 5	0	0
		Moderate-Severe	6 - 8	0	0
		Severe	9 - 10	0	0
Fatigue during experiments		Mild	0 - 2	0	1
		Mild-Moderate	3 - 5	0	0
		Moderate-Severe	6 - 8	1	0
		Severe	9 - 10	0	0

Results represent individual cumulative data. Please note that, if a patient does not report pain, he/she is not assigned a "0", because this classification is assigned to those reporting the less intense pain possible to feel.

Appendix B

Supplementary Videos

Supplementary Video 1. Intramuscular electrode insertion procedure in FCR and ECR muscle bellies guided by ultrasonography.

Supplementary Video 2. Patient P02 holding her arms outstretched during pre-ASSESS, one stimulation trial (SATS condition), post-ASSESS and post24-ASSESS (IntraStim Session).