Master Thesis

Master in Neuroengineering and Rehabilitation

Exploration of peripheral electrical stimulation adapted as a modulation tool for reciprocal inhibition through the activation of afferent fibers during gait

REPORT

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Abstract

The most accessible manner to perform physical activity and allow locomotion in human beings is walking. This activity is allowed thanks to reciprocal Ia inhibition mechanism, controlled by the spinal and supraspinal inhibitory circuits. The idea of this mechanism is to deactivate the antagonist muscle while the agonist is being contracted, allowing the proper muscle coordination necessary to walk.

The interruption of spinal fibers produced after Spinal Cord Injury, disrupt this control on reciprocal Ia inhibition. The result of this lack of control is a co-activation of antagonist muscles generating spasticity of lower limbs which induce walking impairments. The importance of walking recovery for the independence and society re-integration of patient, raise the quantity of emerging walking rehabilitation therapies. One of these therapies, the application of peripheral nerve stimulation, has demonstrated promising results although more studies are necessary.

This theory is the base of this Master Thesis which aim is to develop and validate a gait neuromodulation platform that induce neuroplasticity of spinal circuits, improving reciprocal Ia inhibition. The idea of the platform is to deliver afferent stimulation into the Common Peroneal Nerve innervating Tibialis Anterior muscle, to induce reciprocal Ia inhibition onto the antagonist Soleus muscle.

This platform has been validated in 20 healthy volunteers in order to assess its effectiveness. The first part of the experimental protocol is an off-line analysis of Gait Cycle to evaluate the activation of muscles during the different phases of this cycle. Then, there is an assessment of the activity of antagonist muscle previous to the stimulation intervention by using the analysis of soleus H-reflex. Posteriorly, the afferent stimulation is applied during a 10 minutes treadmill training using three different strategies depending on patient: In-phase stimulation during swing phase, Out-of-phase stimulation during stance phase, and Control strategy to check if stimulation has a real effect. The final processes of experimental protocol are two different assessments of the soleus activity, one immediately after the intervention and other 30 minutes after to evaluate the duration of effects.

The results obtained demonstrate that afferent electrical stimulation has a real effect on modulation of reciprocal Ia inhibition. On the one hand, when electrical stimulation is applied during the swing phase, there is an improvement of reciprocal Ia inhibition. On the other hand, when stimulation is delivered during the stance phase, there is a worsening of reciprocal Ia inhibition.

These results conclude that afferent electrical stimulation, applied at the swing phase of gait cycle, is a promising strategy to induce reciprocal Ia inhibition in Spinal Cord Injury patients. The induction of this inhibitory circuit will lead to the proper activation of muscles during walking, recovering impaired walking.

Resumen

La forma más accesible de locomoción y actividad física en los seres humanos es caminar. Esta actividad se realiza gracias al mecanismo de inhibición recíproca, controlado por los circuitos inhibitorios espinales y supraespinales. La idea de este mecanismo es desactivar el músculo antagonista mientras se contrae el agonista, permitiendo la adecuada coordinación muscular durante la marcha.

La interrupción de las fibras espinales tras una Lesión de la Médula Espinal desajusta el control de la inhibition reciprocal. El resultado de esta falta de control es una co-activación de los músculos antagonistas generando espasticidad en las extremidades inferiores, lo que genera alteraciones en la marcha. La importancia de la recuperación de la marcha para lograr la independencia y la reintegración del paciente en la sociedad, ha incrementado el número de terapias emergentes en rehabilitación de la marcha. Una de estas terapias, la estimulación del nervio periférico, ha demostrado resultados prometedores.

Esta teoría es la base de esta Tesis de Máster cuyo objetivo es desarrollar y validar una plataforma de neuromodulación de la marcha que induzca la neuroplasticidad de los circuitos espinales, mejorando los valores de inhibición recíproca. La idea es aplicar estimulación aferente en el Nervio Peroneo Común que inerva el músculo Tibial Anterior para inducir la inhibición recíproca en su músculo antagonista Soleo.

Esta plataforma ha sido validada en 20 voluntarios sanos con el fin de evaluar su eficacia. La primera parte del protocolo experimental es un análisis del ciclo de la marcha para evaluar la activación de cada músculo durante las diferentes fases de este ciclo. Luego, previo a la intervención de estimulación, hay una evaluación de la actividad del músculo antagonista analizando el reflejo H del soleo. La intervención de estimulación aferente se aplica durante un entrenamiento de marcha con una duración de 10 minutos, utilizando tres estrategias diferentes dependiendo del paciente: estimulación 'In-phase' durante la fase de oscilación, estimulación 'Out-of-phase' durante la fase de postura, y 'Control' para comprobar si la estimulación tiene un efecto real. Los procesos finales del protocolo son dos evaluaciones de la actividad del soleo, una inmediatamente después de la intervención y otra 30 minutos después para evaluar la duración de los efectos.

Los resultados obtenidos demuestran que la estimulación eléctrica aferente tiene un efecto real en la modulación de la inhibición recíproca. Por un lado, cuando la estimulación eléctrica se aplica durante la fase de oscilación, hay una mejora de la inhibición recíproca. Por otro lado, cuando la estimulación se administra durante la fase de postura, hay un empeoramiento de la inhibición recíproca.

Estos resultados concluyen que la estimulación eléctrica aferente, administrada en la fase de oscilación del ciclo de la marcha, es una estrategia prometedora para inducir la inhibición recíproca en pacientes con Lesión de la Médula Espinal. La inducción de este circuito inhibidor generará la adecuada activación de los músculos durante la marcha, recuperando el ciclo de marcha normal.

Resum

La manera més accessible de locomoció i activitat física en els éssers humans és caminar. Aquesta activitat es realitza gràcies al mecanisme d'inhibició recíproca, controlat pels circuits inhibitoris espinals i supraespinals. La idea d'aquest mecanisme és desactivar el múscul antagonista mentre es contrau l'agonista, permetent la coordinació muscular adequada durant la marxa.

La interrupció de les fibres espinals després d'una lesió medul·lar desajusta el control de la inhibició reciprocal. El resultat d'aquesta manca de control és una coactivació dels músculs antagonistes generant espasticitat a les extremitats inferiors, cosa que genera alteracions a la marxa. La importància de la recuperació de la marxa per a la independència i la reintegració del pacient a la societat, ha incrementat el nombre de teràpies emergents de rehabilitació de la marxa. Una daquestes teràpies, lestimulació del nervi perifèric, ha demostrat resultats prometedors.

Aquesta teoria és la base d´aquesta Tesi de Màster que té com a objectiu desenvolupar una plataforma de neuromodulació de la marxa que indueixi la neuroplasticitat dels circuits espinals, millorant els valors de inhibició recíproca. La idea és aplicar una estimulació aferent al Nervi Peroneal Comú que inerva el múscul Tibial Anterior per induir la inhibició recíproca al múscul antagonista Soli.

Aquesta plataforma ha estat validada en 20 voluntaris sans per avaluar-ne l'eficàcia. La primera part del protocol experimental és una anàlisi del cicle de marxa per avaluar l'activació de cada múscul durant les diferents fases del cicle de la marxa. Després, amb la intervenció d'estimulació prèvia, hi ha una avaluació de l'activitat del múscul antagonista analitzant el reflex H del soli. La intervenció d'estimulació aferent s'aplica durant un entrenament de marxa amb una durada de 10 minuts, utilitzant tres estratègies diferents depenent del pacient: estimulació 'In-phase' durant la fase d'oscil·lació, estimulació 'Out-of-phase' durant la fase de postura, i 'Control' per comprovar si la estimulació té un efecte real. Els processos finals del protocol són dues avaluacions de l'activitat de soli, una immediatament després de la intervenció i una altra 30 minuts després per avaluar la durada dels efectes.

Els resultats obtinguts demostren que l'estimulació elèctrica aferent té un efecte real en la modulació de la inhibició recíproca. D'una banda, quan s'aplica l'estimulació elèctrica durant la fase d'oscil·lació, hi ha una millora de la inhibició recíproca. D'altra banda, quan s'administra l'estimulació durant la fase de postura, hi ha un empitjorament de la inhibició recíproca.

Aquests resultats conclouen que l'estimulació elèctrica aferent, a la fase d'oscil·lació del cicle de la marxa, és una estratègia prometedora per induir la inhibició recíproca en pacients amb lesió medul·lar. La inducció d'aquest circuit inhibidor generarà a l'activació adequada dels músculs durant la marxa, recuperant el cicle de marxa normal.

Acknowledgments

During the last two years of my life, in which I have been deepening my knowledge in the world of neuroengineering and rehabilitation, it has given me time to understand that our role as professionals in this field has an unattainable value. All of us, as social beings, give special importance to our health and that of all individuals in our life. Therefore, the creation of algorithms, equipment, or therapies that improve health provide a physical and emotional benefit for individuals. This is undoubtedly the reason that has motivated me the most to continue with my studies and to imagine my future working on ideas and knowledge in favor of human welfare.

In particular, this Master Thesis has been a personal as well as academic challenge for me. Being able to develop and validate a platform that could contribute and be key to the future rehabilitation of Spinal Cord Injury (SCI) patients, has made me give my best.

First of all, I would like to thank the director and supervisor of the project from the Universitat Politècnica de Catalunya (UPC), Josep Maria Font Llagunes and Massimo Cenciarini. Secondly, I would like to thank enormously the role of Neural Rehabilitation Group (NRG) from Consejo Superior de Investigaciones Cientificas (CSIC) in this project, especially the indispensable role of Juan Camilo Moreno Sastoque and Filipe André Oliveira Barroso, who presented me the idea of this project that motivated me from the beginning. Their involvement and their technical and scientific co-management have made it possible for the work to have become so satisfactory. Thanks for the patience, the help, the clear and precise explanations, and the flexibility that were given to me at every moment during this project. The help of the rest of workers and students of this group, especially Cristina Montero Pardo and Javier Gil Castillo, has also been essential for the development of the project. Additionally, I would like to thank the professionals for Hospital Nacional de Parapléjicos (HNP), for their willingness to resolve the doubts and help me improving the experimental protocol.

These months of work and effort have involved many hours of anguish and tiredness, not only for me, but for my inner circle that has been by my side day and night supporting me. For this reason, I thank my parents for their effort and sacrifice that has allowed me to study which motivates and inspires me, my brother for always making me smile at the worst moments, and Pedro for accompanying and giving me his hand during all these years. Thanks also to the rest of my family for understand my processes and encourage me in my personal and academic life.

Finally, thanks to my friends, those of all life and those of the UPC, who appeared two years ago to stay forever. Thanks to them day by day became much easier, hours in university more pleasant and anxiety before each test less suffocating.

Thank you very much.

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Abbreviations

ABCs Airway, Breathing, and Circulation.

ASIA American Spinal Injury Association.

ATLS Advanced Trauma Life Support.

CNS Central Nervous System.

CPGs Central Pattern Generators.

CPN Common Peroneal Nerve.

CREB Centre de Recerca en Enginyeria Biomedica.

CSIC Consejo Superior de Investigaciones Cientificas.

CVDs Cadiovascular Diseases.

DBS Deep Brain Stimulation.

EMG Electromyography.

FES Functional Electrical Stimulation.

FSR Force Sensitive Resistor.

GC Gait Cycle.

GUI Graphical User Interface.

HNP Hospital Nacional de Parapléjicos.

ICU Intensive Care Unit.

IMUs Inertial Measurement Units.

ISI Interstimulus Interval.

ISNCSCI International Standards for Neurological Classification of Spinal Cord Injury.

NIH National Instituto of Health.

NRG Neural Rehabilitation Group.

NSCISC National Spinal Cord Injury Statistical Center.

PTN Posterior Tibial Nerve.

SCI Spinal Cord Injury.

SCIM Spinal Cord Independence Measure.

sEMG Surface Electromyography.

SENIAM Surface ElectroMyoGraphy for the Non-Invasive Assessment of Muscles.

SF-36 Short-Form Health Survey.

SOL Soleus.

TA Tibialis Anterior.

tDCS Transcraneal Direct Current Stimulation.

TENS Transcutaneous Electrical Nerve Stimulation.

tES Transcraneal Electrical Stimulation.

TMS Transcraneal Magnetic Stimulation.

UPC Universitat Politècnica de Catalunya.

WHO World Health Organization.

WISCI II Walking Index for Spinal Cord Injury I.

1 Preface

1.1 Origin of the project

This project *'Exploration of peripheral electrical stimulation adapted as a modulation tool for reciprocal inhibition through the activation of afferent fibers during gait'* arises from the need to carry out a Master's Thesis.

After completing the theoretical subjects of the *Neuroengineering and Rehabilitation* Master taught by the Universitat Politècnica de Catalunya (UPC), it was necessary to carry out an external internship that helps me to perform my Master Thesis. The Centre de Recerca en Enginyeria Biomedica (CREB) belonging to the UPC offered me the possibility of joining a group completely related to my academic career, the Neural Rehabilitation Group (NRG). This group belonging to the Instituto Cajal, a research center of the Consejo Superior de Investigaciones Cientificas (CSIC), would help me to perform this project thanks to its facilities and professionals.

The NRG concentrates its primary research efforts on the creation of technologies and methodologies that could aid in comprehending and managing human biological systems and their interactions with the environment. To improve the quality of care and quality of life for individuals with disabilities, an interdisciplinary research program is specifically designed with the goal of pursuing the development of novel techniques, therapies, and assistive technologies cooperatively.

Its primary objectives are on the examination and assessment of the neuromusculoskeletal and cognitive systems that enable people to move and make decisions in a variety of unstructured contexts. It actively pursues cutting-edge and significant research to create techniques that better the functional or health outcomes of people with physical disabilities, including but not limited to people with cerebral palsy, Spinal Cord Injury (SCI), or stroke. It also has an interest in ways to artificially or naturally improve human physical and cognitive abilities.

Neuroscience, Physical Therapy, Biomechanics, Control, Robotics, Modeling, Machine Learning, and others areas of knowledge are combined in this multidisciplinary group.

Once I contacted one of the main researchers of the NRG expressing my enormous interest in carrying out a project in their department, they assigned me this project, which ended up being my Master's Thesis. The experimental protocol of the project has been carried out with healthy subjects in the facilities of the NRG while future experimentation with pathological subjects will be carried out in Hospital Nacional de Parapléjicos (HNP). In this last institution, professionals have also helped me in order to improve the signal acquisition necessary during this project.

Therefore, the complete project has been carried out as a collaboration between several educational entities: UPC, CREB, CSIC, Instituto Cajal, and NRG. In addition, I have been supported by the HNP to solve both theoretical and technical doubts.

Figure 1: Entities collaborating in this project.

1.2 Prerequisites

The idea of this Master Thesis is to apply all the knowledge acquired during the master and the previous knowledge that should have been acquired during the degree.

The project is divided into three different parts that include analysis of Gait Cycle (GC) using footswitch sensors and Electromyography (EMG) recording, the application of afferent electrical stimulation, and finally the evaluation of this stimulation intervention by using again EMG and electrical stimulation. These processes will be explained deeply in 2.4 and 4.1 sections.

Obviously, as this experiment has been conducted into healthy subjects, it is necessary a really strong background into medical topics such as anatomy, physiology and pathology, among others. The Biomedical Engineering degree offered me a very strong background in these aspects that was later reinforced thanks to the Neuroengineering and Rehabilitation Master.

The analysis of GC during this project has been easily performed thanks to the high experience that I acquired during the Biomechanical courses both in the degree and the Master. During these courses I performed different mechanical projects where I was asked to analyse the kinematic and the dynamic of certain performed movement. In this thesis, the performed movement is walking, which is one of the easiest movements to analyse, biomechanically speaking. In addition, the huge literature related to the analysis of this movement has also helped me in order to compare my results with the literature. Regarding the materials used for this analysis, although I had never used footswitches, I had a lot of experience using EMG. This device has been used in several subjects during my student period, especially in subjects such as Biomedical Signals and Biomedical Equipment where I learnt to localize muscles, position properly the electrodes and acquired a signal as clean and reliable as possible, besides learning to process all these signals acquired.

In terms of applying afferent electrical stimulation I did not have much experience because students are not allowed to stimulate patients themselves. They have to be always under the supervision of a professional. During the Master, it was impossible to do that due to the big amount of students we were. This is the reason why my experience with electrical stimulation was only theoretical and visual when it was performed by a professional. However, this exterior knowledge about stimulation has helped me to learn faster how to stimulate by myself. Besides, the Neuroengineering and Rehabilitation Master provided by the UPC was also in collaboration with the Institut Guttmann, one of the best neurorehabilitation hospital of this country. This helped us, the students, to visualize how therapies such as EMG recording, afferent stimulation, or analysis of reciprocal inhibition, among others, were performed by professionals.

All the parts of this thesis also require an important knowledge in terms of electronics and programming as all the materials are programmed in a determined way just to perform in the desire time and in an established manner.

Finally, it is important to mention two general requisites (not related to Biomedical Engineering) that are extremely important to perform this Master Thesis. On the one hand, it is essential to know English. The reason is that both the drafting and presentation of this project will be in English; in addition to the fact that almost all the literature is presented in this language. On the other hand, it is very important to know how to work in a group and especially an interdisciplinary group. The difficulty of this thesis makes it necessary to be in close contact with other professionals such as doctors, physiotherapists or electrical engineers, among others.

2 Introduction

2.1 Motivation

One of the fundamental physiological principles that allows the execution of movements such as walking is reciprocal inhibition. This physiological phenomenon allows the contraction of the muscle agonist of movement while inhibiting the contraction of its antagonist muscle, which is essential for movements such as walking.

After SCI, there is a interruption of fibers communicating brain with spinal cord producing an affectation of inhibitory spinal circuits such as reciprocal inhibition. This damage of reciprocal inhibition produces a co-contraction of antagonist muscles, preventing subjects from having a coordinated movement [1] which results in walking impairments.

World Health Organization (WHO) estimates that every year, around the world, there are between 250,000 and 500,000 people suffering a SCI. It also estimates that annual global incidence is 40-50 cases per million population [2]. However, global estimations are not reliable because quantification of worldwide prevalence and incidence is very difficult and usually, prevalence and incidence data are provided in terms of countries or regions. Regarding **incidence**, or number of new SCI cases during a specific period of time, the developed countries have a really high incidence ratio [3]. North America is the region with the highest incidence of traumatic SCI, with 54 cases per million according to the National Spinal Cord Injury Statistical Center (NSCISC) [4]. In terms of **prevalence**, or quantity of population who have traumatic SCI at a specific period of time including both new and preexisting cases, Unites States of America is the country with the highest prevalence being 721 cases per million, followed by Australia with 681 cases per million and Iceland with 526 cases per million [5]. The proportion of non-traumatic cases has also being growing during the last decades, specially in Canada, where its prevalence is 1,227 cases per million [6] [3]. Some of the studies that describe incidence and prevalence in terms of regions and type of SCI are the study [5] performed by Anoushka Singh et al (2014) and the study [3] performed by Cripps et at (2010). In Figure 2 there is a representation of the annual incidence in countries (red color scheme) and in regions (blue color scheme) worldwide.

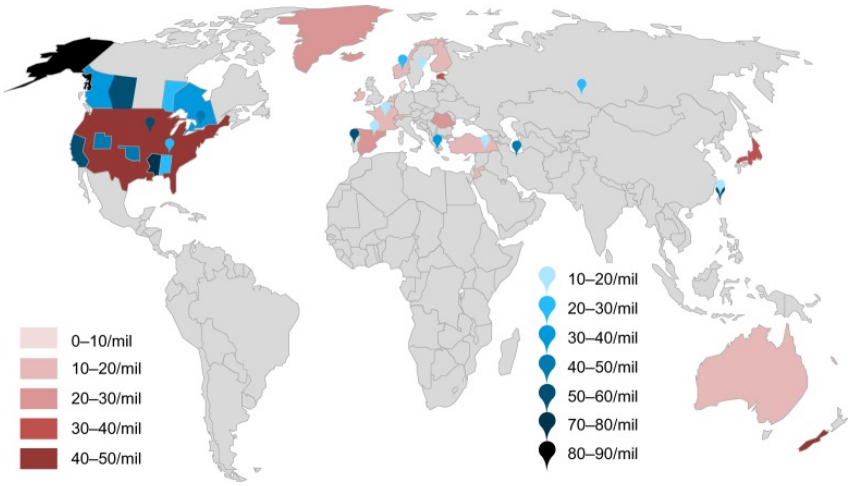

Figure 2: Relative annual incidences of SCI per million individuals of countries represented by red scheme, and of states/provinces/regions represented by blue scheme. Retrieved from [5].

Considering the incidence of SCI in Spain, there has been an increment in the last decades. Spain has always been one of the countries with the lowest incidences, with 8.1 cases per million individuals estimated in the 1980s. During this period, the area of Spain with the highest incidence was Aragon

with 12.1 cases per million. However, the comparison between these numbers estimated in the 1980s and the numbers estimated during the 2000s, demonstrate a drastic increase for 8.1 to 23.5 cases per million between 2000 and 2009 [5]. This dramatic increment makes Spain the third country of Europe with the highest SCI incidence, after Estonia (39.7 cases per million) and Romania (28.5 cases per million), as it can be seen in Table 1.

Table 1: Annual Incidence of SCI in determined countries. Adapted from [5].

Country	Incidence (per million)	Male:Female ratio	Peak age
USA (1970-1977)	40.1	2.25:1	Overall: 15–24
Estonia (1997-2007)	39.7	5.5:1	M: 20–29, F: 30–39
Romania (1975-1993)	28.5	3.35:1	Overall: 51–60
Spain (2000-2009)	23.5	NotSpecified	NotSpeficied
Iceland (1975-2009)	22.6	2.6:1	Overall: ≤ 30

This affliction is associated with the development of secondary conditions that can cause higher weakness or higher mortality probability such as deep vein thrombosis, urinary tract infections, muscle spasms, osteoporosis, pressure ulcers, chronic pain, and respiratory complications [2]. The SCI itself and its associated complications render a person dependent on caregivers. This dramatically reduces the independence of patients and also their rates of school, work and social inclusion. The drastic change in patients life due to this lack of independence and inclusion on society, generates that more than 20-30% of people with SCI suffer signs of depression, reducing even more the quality of life of patient and their relatives.

This increment of SCI incidence is dramatic because of its association to higher mortality rates, SCI patients are two to five times more likely to die than healthy subjects. The highest mortality rate, from 4% to 17%, occurs during the acute phase of disease. Although, this mortality rate decreases after hospital discharge, complications associated to the disease produce a 3.8% of patients dying during the first year after injury, which progressively decrease during the following years. These mortality rates are higher for non-traumatic (27.7%) than for traumatic (14.8%) injuries due to the increased number of complications in other important systems associated with the main disease [7] [2].

All these factors related to SCI produce a really elevated economic impact, producing substantial individual and societal costs. However, although all SCI have economical impact, those injuries in the upper part of spinal cord induce higher costs. Regarding the directs costs; those that are related to the management, therapy and rehabilitation of injury; these are higher during the first year after SCI onset and then they are reduced during the next years. These costs are related to direct medical costs as the necessity of patients and their relatives of health care, professionals, approved therapies, experimental therapies, accessible infrastructures and chronic disability support. However; indirect costs that are the side-effects of SCI such as unemployment; are constant several years after the injury and often exceed the direct costs. In conclusion, costs associated to SCI are higher than those of other neurological conditions such as dementia, cerebral palsy or multiple sclerosis [2].

Due to all these factors, to enhance patient independence and social inclusion, it is necessary the application of neurorehabilitation to generate a functional recovery. In the case of functional gait recovery after a SCI, the induction and guidance of activity-dependent plasticity is essential.

2.2 Research in context

Several previous studies have tried to determine which is the best way to induce neuroplasticity of reciprocal Ia inhibition to recover walking ability. These are some of the studies that confirm short-term effects of electrical stimulation improving spinal reciprocal inhibition.

The study conducted by Thierry Paillard (2021) [8] analysed the effects of sensory electrical stimulation, below the motor threshold intensity, on the restoration of postural balance in healthy and SCI patients. The results concluded that sensorial stimulation is effective reinforcing or restoring the postural balance in pathological patients.

Other studies demonstrated the hypothesis that repetitive stimulation, as well as activity and patterned based stimulation produce a potentiation of reciprocal inhibition. A study performed by Monica Perez et al (2004) [9] demonstrated when repetitive afferent inputs were applied in a pattern that mimicked the sensory feedback during walking, there was a potentiation of spinal plasticity. This increase of plasticity resulted in a strength of reciprocal Ia inhibition between ankle flexor and extensor muscles of healthy and pathological subjects. Other study performed by Obata et al (2018) [10] demonstrated this hypothesis obtaining better short-term effects in the improvement of reciprocal Ia inhibition after the application of afferent electrical stimulation in combination with cyclic movements such as pedaling or walking during the swing phase of GC. Another study performed by Monica A.Perez et al (2003) [11] highlighted the importance of combining electrical stimulation therapies with sensory feedback provided by the limb movement of treadmill training, to produce spinal circuit plasticity in use-dependent manner, and therefore enhance more effectively reciprocal Ia inhibition. The study conducted by Clarissa Crone et al (1986) [12] illustrated the effects of delivering afferent electrical stimulation during dorsiflexion, activation of Tibialis Anterior (TA), on the phase-dependent modulation of neuroplasticity resulting in an increase of reciprocal Ia inhibition between ankle flexors and extensors in healthy subjects. Another study performed by Maria Knikou et al (2006) [13] confirmed this phase-dependent modulation of reciprocal inhibition improvement when afferent electrical was delivered during hip flexion, it means during swing phase of GC.

All these previous studies analysed the effects of repetitive, activity and phase-based afferent electrical stimulation. However, other types of electrical stimulation has also been tested. This is the case of Yukihiro Hara (2008) [14] who confirmed that when Functional Electrical Stimulation (FES) was initiated triggered by EMG patterns, the resultant motor function was improved. Therefore, FES was more effective to recover motion in hemiparetic upper extremity in stoke patients when there was activity-dependent stimulation.

However, all these studies analyzed the short-terms effects of electrical nerve stimulation. To induce long-term effects, other studies have concluded that it is necessary a combination of brain stimulation such as Transcraneal Direct Current Stimulation (tDCS) or Transcraneal Magnetic Stimulation (TMS) with patterned electrical stimulation. The study performed by Tomofumi Yamaguchi et al (2016) [15] concluded that combination of these therapies activated the spinal inhibitory interneurons, inducing plasticity in reciprocal Ia inhibition and therefore, producing a better motor recovery in healthy and SCI patients. The study conducted by Berthe Hanna-Boutros et al (2015) [16] demonstrated that combination of both stimulation therapies during stance, late stance, and swing; increased task-dependent neuroplasticity of reciprocal inhibition.

These findings demonstrate the presence of short-term plasticity within spinal inhibitory circuits when applying repetitive, activity and phase-based electrical nerve stimulation. This activation generates reciprocal Ia inhibition improving walking ability. The generation of this plasticity of reciprocal inhibition is key to develop rehabilitation strategies that allow walking recovery after SCI.

2.3 Objectives and hypotheses of the project

The studies explained in Section 2.2 demonstrate that one of the ways to induce plasticity in the spinal circuits by improving reciprocal inhibition, is to use sensory input pathways. These studies have attempted to create the best strategy to recover walking ability by combining the application of electrical stimulation with cyclic activities such as walking or cycling. Therefore, one of the eminent fields in the rehabilitation of SCI subjects, is the therapy of afferent electrical stimulation. This technique uses low-intensity electrical impulses, below motor threshold, to excite only sensory nerve endings providing only sensory stimulus. The result is a stimulation of muscles without causing their contraction, inducing the plasticity of the spinal cord by improving reciprocal inhibition. This improvement might result in an increase in the ability to perform daily activities such as walking or the use of upper limbs for grooming or dressing.

For all these reasons the goal and purpose of this thesis, that is the exploration of peripheral electrical stimulation on Common Peroneal Nerve (CPN) as a modulation tool for reciprocal inhibition through the activation of afferent fibers during gait, is more than justified.

The hypothesis of the studies presented in Section 2.2 and this thesis is that neuroplasticity induced by electrical stimulation might help spinal cord of patients to re-learn or re-activate certain spinal circuits that result in muscle coordination necessary for walking. When this electrical stimulation is delivered using activity-based strategies such as treadmill training, there is an addition sensory feedback produced by the leg movement that is necessary to induce locomotor spinal circuits' plasticity in an use-dependent manner. In addition, the more the strategy is performed, repetitive Ia afferent inputs improve the effectiveness of spinal circuits controlling human locomotion [9]. Finally, some of the later studies have concluded that these stimulation therapies are more effective when applied in a pattern-based modality. The combination of these three points: activity-based, phase-based and repetitive strategies can lead to produce a higher and stronger induction of neuroplasticity that results in a more effective improvement of reciprocal Ia inhibition, therefore improving abnormal gait [11].

The main objective of this thesis is the exploration of peripheral electrical stimulation adapted as a modulation tool for reciprocal inhibition through the activation of afferent fibers of CPN during gait of healthy subjects. The idea is to create and validate a gait neuromodulation platform that is able to analyse the GC, generate phase-dependent afferent stimulation and assess spinal excitability. Then, this platform is tested and optimized in different pilot studies with healthy subjects. Finally, the platform is validated in one study involving 20 healthy volunteer subjects. In this phase, the reciprocal Ia inhibition improvements after stimulation intervention are assessed. The ideal results are a maximum as possible improvement of reciprocal inhibition.

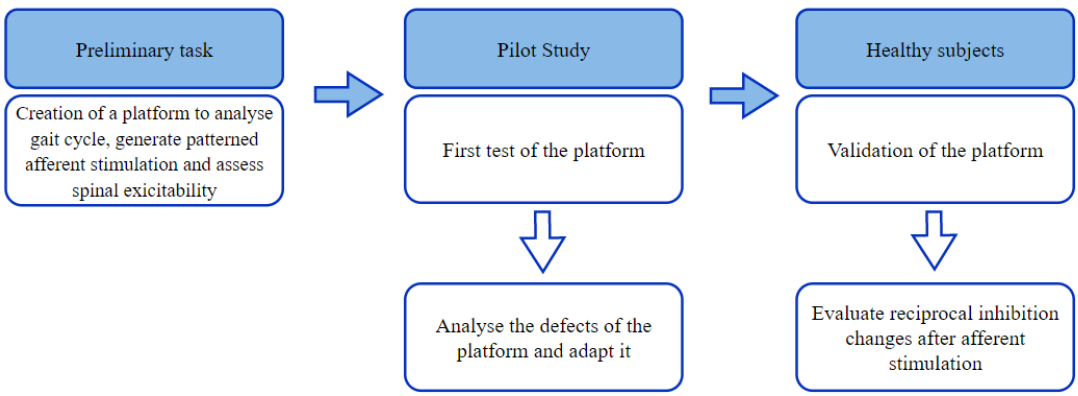

Figure 3: Objectives of the thesis.

It can be concluded that primary objectives of this thesis are:

- Automation of the detection of 'swing' and 'stance' phases.
- Study of muscle activation dependent on the GC phase.
- Location of the CPN and application of afferent stimulation during treadmill training.
- Study of the effectiveness of short-term plasticity in the spinal circuits, by analysis of the Soleus (SOL) H-reflex prior to and post afferent stimulation intervention.
- Visualization of the H-reflex before afferent stimulation to find the ideal stimulation intensity for each subject.
- Application of all the above processes in 20 healthy subjects (8 subjects with In-phase stimulation, other 8 subjects with Out-phase stimulation and 4 subjects with Control stimulation). The reason of performing these three strategies is; first of all, confirm that stimulation has any real effect on subjects; and secondly if the literature results regarding the effectiveness of stimulation in swing phase instead of stance phase are correct.
- Main and final objective: To verify the feasibility of induction of plasticity in neural circuits to increase reciprocal inhibition.

This platform will help us to evaluate the process of induced plasticity thanks to afferent stimulation of CPN during different phases of walking, to establish a starting point for a potential intervention for gait in SCI subjects.

Our long-term hypothesis is that during In-phase stimulation, there are changes in plasticity improving the reciprocal inhibition. If this hypothesis is confirmed in healthy subjects, the experiment could be implemented in a future with SCI subjects. If there is also an improvement in reciprocal inhibition of pathological patients, afferent stimulation intervention during swing-phase of GC could be consolidated as a reliable therapy for patients suffering from a neurological injury. This would be a great advantage for patients who return to daily life after injury in terms of independence and quality of life improvements.

2.4 Structure of the thesis

This thesis is divided in different sections that cover all the necessary points. This division into sections make the thesis lecture easier and more pleasant. There is a previous section or Preface were the origin of the project as well as the pre-requisites for its elaboration are deeply explained.

The second section is composed by the introduction of the thesis where the motivation, objectives, and structure of the thesis are described. In this section, the previous related studied are introduced and the need of new therapies that provides a better locomotor recovery in SCI patients is highlighted.

The third section presents the State of Art on walking activity and the physiological mechanisms and characteristic necessary to perform this activity in healthy subjects. Posteriorly in this section, it is explained how these walking mechanisms are affected after SCI and how proper walking ability can be recovered and rehabilitated. In addition to walking impairment, the rest of SCI complications are also explained, as well as their rehabilitation treatments. The final part of this section explains the walking rehabilitation therapies used nowadays such as electrical stimulation, and also introduces the future therapies needed to achieve a better recovery of gait pattern.

The forth section describes the materials and experimental methods used in this thesis, the creation and development of the whole experimental protocol considering equipment, platform characteristics, and stimulation strategies. The later part of this section explains the validation of this protocol with healthy subjects.

The fifth section illustrates the results obtained in the experiments with healthy subjects in terms of performance, usability and comfort of the developed experimental platform, and its effects on spinal plasticity.

The sixth section provides a discussion of the obtained results presented in the previous section, as well as, a description of the limitations found in the project.

The seventh section describes the final conclusions regarding the experiments, explaining its potential applications and the future work necessary to obtain better results.

Final section present the budget and project timeline of the thesis, describing the regulatory and socioeconomic framework, and the Gantt diagram describing the timeline of the thesis.

References of the literature consulted to perform the thesis and annexes such as consent forms, information sheets, experimental protocols, and data collection sheets can be found at the end of this thesis document.

3 State of the art

Cambridge Dictionary defines the act of Walking as the human capacity of going for a walk, especially for three different objectives: for pleasure, to achieve a goal, or as physical exercise. In humans, it is the most efficient and natural mode of locomotion to travel independently because it requires really low energy consumption even when performing long distances walk. Besides, when performed by healthy subjects, it does not require concentration because it is a more than internalized process. This fact leads to the confusion that it is a simple human process when it is actually extremely complex, one of the most complex in terms of mechanisms involved.

Explaining it in biological terms, this method of human locomotion use both legs in an alternating way in order to enhance support (keep stance stable) and propulsion (move the body ahead). This pattern of limbs motion is repeated all the time during walking, this is the reason why Walking is composed by identical and repetitive series, each one known as GC. It is important not to confuse GC and gait. As already explained, GC is the individual series of limb motion during walking while Gait is the way in which subject performs walking process, it can be normal or pathological.

It is also important to understand the difference between walking and running that is that during walking, before the posterior foot is lifted, the one in front must be always in touch with the ground. This means, Walking always needs at least one foot being in contact with the ground.

The execution of Walking is controlled by internal components such as supraspinal control, Central Pattern Generators (CPGs) in the spinal cord, and sensory feedback. Sensory feedback analyses the environment and sends inputs to the brain regarding the type of locomotion to perform and the necessary adaptations to the environment, then supraspinal circuits analyse this information and sends orders to CPGs. These self-organizing neural circuits in charge of generating rhythmic outputs (movements and contractions) needed to enable proper walking [17].

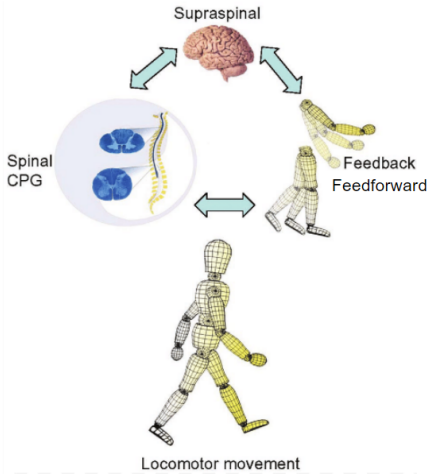

Figure 4: Internal controls of Walking. Retrieved from [17].

As stated before, thanks to sensory feedback and feedforward, these outputs produced by CPGs are adapted to different environmental circumstances. Feedback is a slow control that analyses the erroneous aspects of current gait and tried to correct it for future gait. However, feedforward control is a faster control that intends to anticipate different situations in order to choose which performance is the best in a determined situation.

Keeping the body active is very necessary to improve both mental and physical health and to prevent diseases both in the present and in the future. That is the reason why the WHO gives some recommendation regarding physical activity necessary in healthy adults. These recommendations state that the ideal is to, every week, perform 150 minutes of moderate aerobic activities such as cycling or walking, or 75 minutes of any vigorous activity such as basketball or tennis [18] [19] [20].

Therefore, it can be concluded that Walking is the most accessible form of performing physical activity being a crucial element for the **prevention** of illnesses such as strokes, mental illness, and Cadiovascular Diseases (CVDs). In addition to the prevention of diseases, many research studies highlight the walking **benefits** on human physical and mental health such as the improvement of sleeping, memory, concentration, stress and so on. Another important Walking contribution, according the National Instituto of Health (NIH), is its essential role during the **neurorehabilitation** programs.

Nowadays, sedentary lifestyle is predominant due to the jobs characteristics and the huge quantity of indoor activities performed such as playing computer games, watching Television or accessing computers. All these activities force us to be sited during long periods of time, generating numerous negative consequences for our bodies and health. The most common consequences of inactivity are the deterioration of musculoskeletal endurance [21] and the damage of circulatory system. These consequences influence both physical maladies such as overweight, bones and muscle fractures, high cholesterol levels, CVDs, metabolic disorders, or cancers, and mental maladies such as anxiety, stress or depression [22]. Focusing on CVDs, several research studies [7] [23] identified physical inactivity as one of the most important risk factors to develop stroke, accounting for more than 80% of stroke incidences rates worldwide.

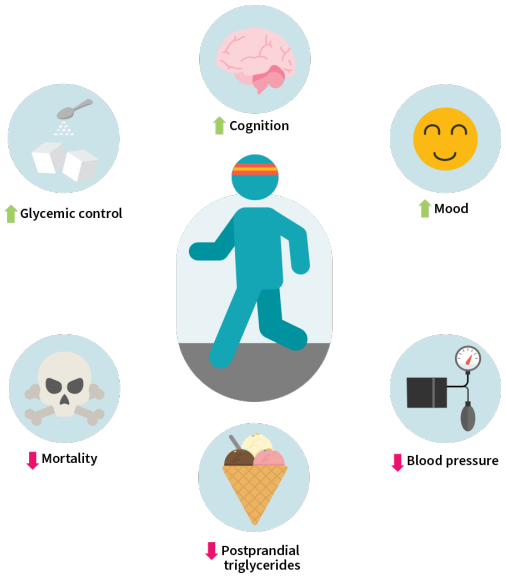

Figure 5: Physical and Mental benefits of Walking. Retrieved from [24].

The studies that highlight the importance of physical activity for the human health [25] [26], analyse the difference of biomarkers amounts pre and post walking. On the one hand, the biomarkers that determine the decreased chance of developing strokes after performing routine walking are C-reactive protein, P-selectin protein and homocysteine. On the other hand, the parameters that determine improvements in patients with mental illnesses after performing routine walking are the Perceived Stress Score and Epworth Sleepiness Score. In addition, other parameters that are useful for determining both the physical and mental improvements of patients after routine walking are the three major monoamine neurotransmitters: dopamine, noradrenaline and serotonin. These neurotransmitters modulate activities such as movement, memory, motivation, behaviour, cognition, attention, sleep, mood, learning, mood, digestion, nausea, wound healing, blood clotting and sexual desire.

The results obtained are that level of biomarkers determining the increase on CVDs prevention improves after routine walking, reducing the chances of having a stroke by 30% or more. In addition, the parameters or scores that determine mental improvements are also improved by 10% or more on average, performing a reduction in stress and sleeping problems. In the case of the three neurotransmitters, the imbalance of neurotransmitters produced during a physical or mental illness is fixed after introducing walking in the daily life of patients, producing an improvement of these illnesses.

These outcomes are the empirical probe that changing the lifestyle and adding walking in the routine life, improves the recovery from many physical and mental illness as well as reducing the likelihood of developing some diseases such as strokes. For this reason, Walking is highly promoted as essential factors in the prevention and treatment programs of several and different diseases.

3.1 Muscle coordination during walking

During the process of walking, the support is provided by one of the limbs while the other limb projects forward allowing movement to a desired location. This sequence of events is periodically repeated by alternating each of the limbs until reaching the desired location. The whole walking process is generated by repetition of individual series known as GC [27].

A GC is considered a single sequence of these events in which one limb performs support and posteriorly projection. It is also defined as the time interval between the appearance of one of the events of walking until the same event is performed by the same foot again. It means, the time interval between two successive occurrences of the same events of walking performed by the same foot. By consensus, the event considered as the start of a GC is the corresponding to the first step of right limb balance, the **right heel strike**. This is the instant at which right foot contacts the ground. Considering this assumption, it can be assumed that GC starts when heel of the right foot initiates floor contact, and then it continues until the same foot contacts the ground again. Obviously, the left foot performs exactly the same process, but in this case, start of left cycle is half a cycle displaced in time [28].

The GC is divided into two different periods or gait phases, stance and swing. One the one hand, Stance phase is referred as the total period during which the right foot is contact with the ground and it is initialized by the heel initial contact or heel strike. On the other hand, Swing phase refers to the period of time the right foot is in the air with the function of projecting forward to produce limb advancement and it is started as soon as the right foot is raised off the ground, called toe-off.

Besides these two main phases, GC can also be analysed in terms of seven different major events as illustrated in Figure 6. Considering the principal limb as the right represented in figure with gray color and the opposite limb as the left represented in figure with blue color, the principal events during GC are [27]: initial contact of right foot, toe off of left foot, heel rise of right foot, initial contact of left foot, toe off of right foot, feet adjacent, and finally tibia vertical of right foot. The next event corresponds to the first event of the next GC, initial contact of right foot again. Based on these events, on the one hand, stance phase also called support or contact phase is initialized with initial contact of right foot and ends with toe off of right foot. While on the other hand, swing phase starts with toe off of right foot and ends with the initial contact of right foot corresponding to the next GC.

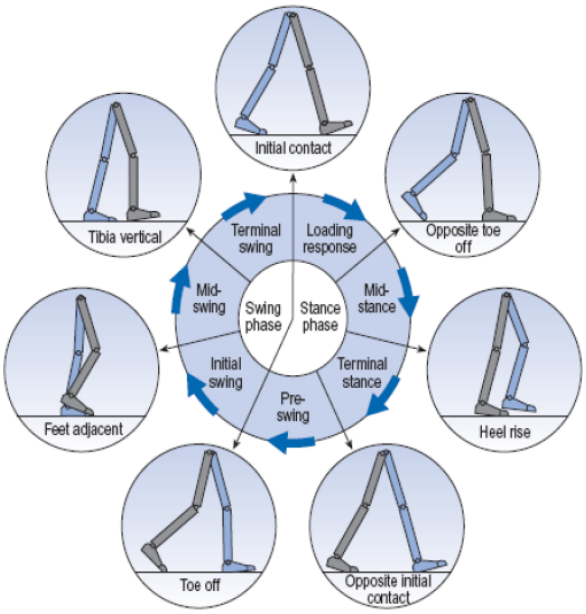

Figure 6: Major events and periods during the GC. Retrieved from [27].

These principal events subdivide GC into eight different periods [27]: Heel strike or Initial contact, Loading response, Mid-stance, Terminal stance, Pre-swing, Initial swing or Toe-off that is the phase of acceleration, Mid-swing, and Terminal swing that is the phase of deceleration. The first five periods presented in this list belongs to the stance phase, while the last three periods belongs to the swing phase.

The GC time starts, as already explained, with the right heel strike or right initial contact when the left foot is still on the ground. This is the reason why GC starts with a brief period of **double support** or

double limb stance. During the next step, the left side is completely in swing and only right foot is on ground, providing a **right single support** or single limb stance period. This period finishes when left foot produced the initial contact, it means that at this time there is another period of **double support** that ends with toe off of right foot. Posteriorly, there is a period of **left single support** when right limb is at swing phase. This period ends when right limb performs again the initial contact, finishing the GC. Each of these processes allows the realization of three functional tasks at the lower limbs: weight acceptance, single-limb support and limb advancement [28] [29].

Summarizing all the periods explained before, it is clear that each GC is composed by, in this order, a period of double support, right single support, double support, left single support. Each period of double support account accounts for 10% of GC while each period of single support accounts for 40%. As during a full GC there are two double support periods, they account for 20% of complete cycle and similarly for the two single support periods, that account for 80% of complete cycle. Dividing these support events into the two different gait phases; stance includes double support, right single support and another double support period while swing phase only includes left single support phase. This is the reason why stance phase lasts about 60% of the whole GC while the swing phase only 40%. This explanation is properly understood thanks to the Figure 7 presented below.

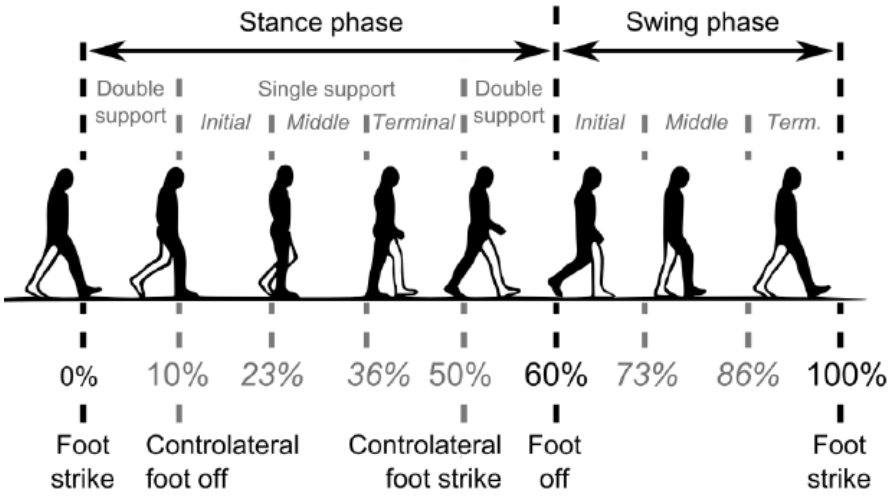

Figure 7: Percentage of different support periods during GC. Retrieved from [28].

Gait analysis performed on healthy subjects is essential in order to generate a standard against which posteriorly compare pathological gait pattern. The gait pattern of each individual can be analysed through different methodologies. Kinematics, for example, is the study of bodies in motion without contemplating the internal or external forces that cause the body movement. This study gives results regarding positions, angles, velocities and accelerations of body segments and joints. On the other hand, Kinetics is the study of the factors that propel bodies into motion, that describe the forces acting on the body and the body segments. Finally, another very important aspect of GC that can be analysed is muscle activity of lower and upper limbs during GC through EMG records [30].

The natural and repetitive movements performed at GC are controlled thanks to the coordination and activation of different muscles. Thus, the muscles play a specific and essential function during the GC, acting as gait's motors. An electric signal is produced when muscles are effectively contracted under neural control, and EMG can capture this signal allowing us to analyse the activation of each muscle at the different phases of GC. Therefore, the activation patterns of the different muscles involved in walking depends on the GC periods and can be analysed by visualizing the Figure 8.

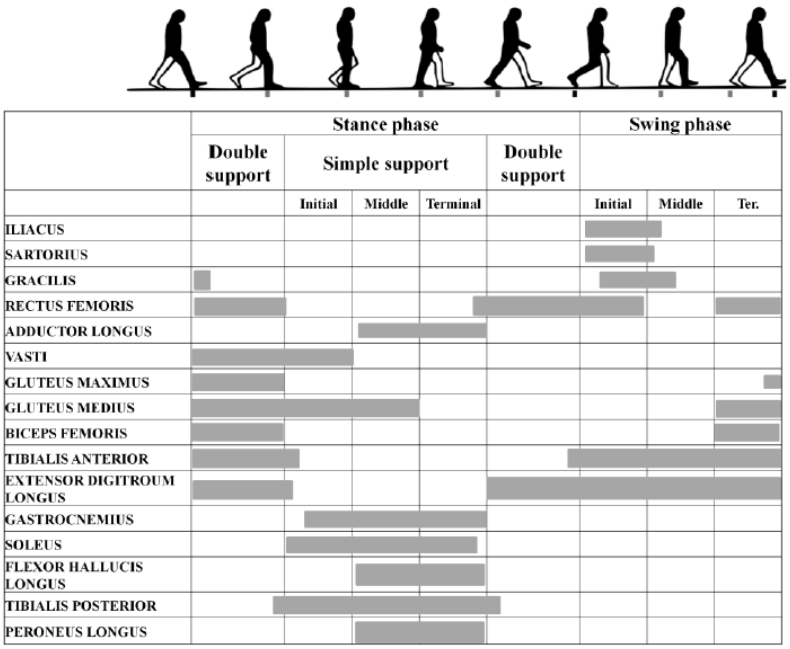

Figure 8: The activation patterns of different muscles during GC. Retrieved from [28].

First of all, the muscles that activate during the first event of the stance phase, right initial contact with the ground, are the gluteus maximus and biceps femoris that control hip flexion movement whereas the TA controls and slows down the foot movement. Then, at the stance single support phase, the objective is to maintain the balance while allowing forward progression of the opposite limb. Several muscles such as Adductor longus, Gluteus medius, Gastrocnemius, SOL, Flexor Hallucis longus, Tibialis Posterior and Peroneus Longus activate during this phase where others such as Rectus Femoris, Vasti, Gluteus Maximus, Biceps Femoris, TA and Extensor Digitroum Longus deactivate. Then, at the end of this stance phase, TA starts its preparatory activity for swing phase.

At initial swing phase three main muscle groups activate to advance the thigh and flex the knee: hip flexor muscles, the biceps femoris, and finally the TA and extensor digitorum longus muscles. Then, at mid swing phase, the muscle activity is limited and a big quantity of muscles cease their activity. However, at this period, TA supports and maintains the ankle position, while the contralateral gluteus medius supports pelvis position. Finally, at terminal swing phase there are three main muscles in action: hamstring muscle that slow down the forward movement of the leg, the rectus femoris muscle that extends the knee and the TA that positions the ankle joint to prepare for the next ground contact [28].

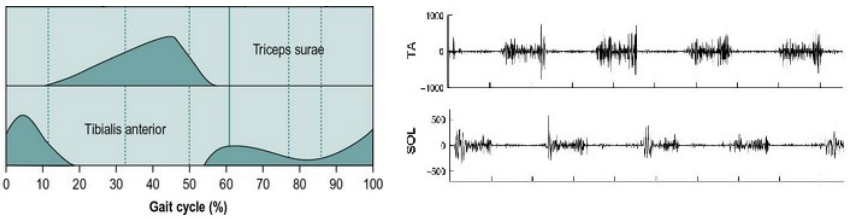

Figure 9: The activation patterns of TA and SOL during GC. Retrieved from [31] [30].

Focusing on the TA and SOL muscles that are the ones analysed during the project, it can be seen on Figure 9 how there is a coordination between them during the GC. This synchronization is produced thanks to the reciprocal Ia inhibition pathway explained in the following section.

3.1.1 Spinal inhibitory circuits and neuronal pathways: Reciprocal inhibition

Healthy subjects perform what is stipulated as normal gait thanks to several forms of interaction within the spinal cord. These interactions are presynaptic, reciprocal, and non-reciprocal Ib inhibition and they act in synchronization during the movement [32] [33] [34]. These spinal inhibitory circuits and neuronal pathways can be indirectly assessed in humans by means of H-reflex, which will be explained in depth in Section 3.1.2.

Monosynaptic Ia exitation and Homosynaptic depression: Monosynaptic Ia excitation is the normal neural process that occurs after activating Ia afferents fibers, which produces the proper contraction of the muscles. Homosynaptic or post-activation depression is caused by the continuous activation of these Ia afferents that produces habituation resulting in a lack of contraction of the muscles. When successive electrical stimulus are triggered with brief inter-stimulus intervals, habituation to the stimulus occurs and the homosynaptic depression is dramatic. However, when this inter-stimulus increases, homosynaptic depression decreases progressively. This phenomenon of habituation occurs at the presynaptic terminal, at the synapse between the SOL Ia afferents and alpha-motoneurons. In spinal cord or supraspinal neural injuries, the reduced homosynaptic depression may be the cause of muscular rigidity and spasticity. In these cases, successive stimulation does not produce habituation and muscles contract more than in healthy subjects.

Presynaptic Inhibition of Ia afferents: This presynaptic inhibition process is also defined as the gating of reflexes. This inhibitory circuit is based on the idea that sensory feedback send by afferent inputs from different parts of the body to the spinal cord, needs to be controlled for the proper motor task to be executed. This sensory feedback is controlled at the presynaptic inhibitory synapses of afferent terminals on alpha-motoneurons. At this location, there are a group of interneurons that are the ones in charge of mediating this presynaptic inhibition. These interneurons are activated by the group I afferents, inhibited by flexor reflex afferent and controlled by descending tracts. In this way, interneurons decide and control which afferents inputs need to be executed and which one are cancelled by inhibition or dis-facilitation, as represented in Figure 11. This gating process is also a way to control the monosynaptic reflex amplitude before signals arrive to the motoneurons. This gating of the Ia afferents is done based on predictions about the gait state, which enables presynaptic inhibition as a type of feedforward control. Due to this function, presynaptic inhibition constitutes an inhibitory mechanism that modulates monosynaptic reflexes, such as H-reflex.

Summarizing all this information, presynaptic inhibition contributes significantly to neural regulation of movement by gating sensory afferent feedback to the spinal cord and facilitating the efficient performance of a movement or motor task. In this way, several contraction or relaxation processes could be modulated by adjusting the presynaptic inhibition of Ia afferents to motoneurons producing muscle synergy or movement patterns appropriate for the motor task [35].

Reciprocal Ia inhibition: Reciprocal Ia inhibition is one of the main and most important functionalities of Ia afferents. During human movement, they take part in neuronal pathways that inhibit the antagonist alpha-motoneurons, by means of Ia inhibitory interneurons, generating a pattern of reciprocal activation between the agonist and corresponding antagonist muscles. This pattern generates a protective mechanism because when a muscle contracts/stretch, the inhibition of the antagonist muscle prevents muscles from working against each other during muscle stretching responses, sending an inhibitory response to its antagonist. Therefore, it can be concluded that reciprocal inhibition is a phenomenon of simultaneous "tension-distension" complementarity occurring between two physiological components, agonist and antagonist muscle.

It is essential to highlight that this inhibition of alpha-motoneurons of the antagonist muscle occur by means of the interneurons, never directly between motoneurons. The reason of interneurons intervention is that, in some cases, the simultaneous contraction of opposing muscles is necessary. For

this reason, direct connection between motoneurons must be reserved to activities such as catching a heavy object at the air.

This inhibitory neural circuit follows a specific pathway represented in Figure 10 and Figure 11: the agonist muscle stretches or contract and its stretch receptors activate. These receptors present afferent fibers that transport the information until the Spinal Cord as represented in the figure by the green lines. In the Spinal Cord some of these afferent fibers project to efferent fibers by producing the contraction of the stretched or agonist muscle as represented by the red lines on the right part of figure, while other fibers project to Ia interneurons represented by black lines. These interneurons inhibit motoneurons efferent fibers of the antagonist muscle, red lines on the left part of figure, by releasing inhibitory neurotransmitters such as GABA or glycine. Experimentally, this inhibition has been proved numerous times seeing how, when TA is contracting, the SOL H-reflex decreases meaning a decrease of its activation.

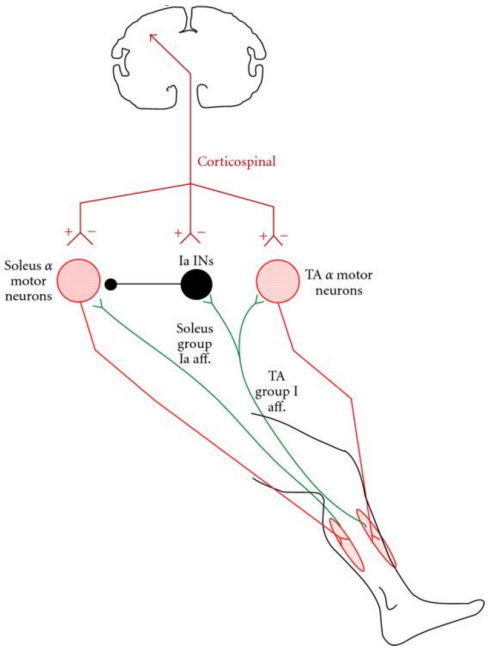

Figure 10: Spinal pathway during reciprocal Ia inhibition. Retrieved from [33].

The previous explained pathway is only based on the segmental control of reciprocal Ia inhibition, and more concretely of Ia inhibitory interneurons. However, they are influenced by segmental interneuronal circuits, afferents, and supraspinal inputs. The corticospinal descending inputs from the brain also control this inhibition by converging on Ia inhibitory interneurons that, as already explained, project to motoneurons of the antagonist. When voluntary movements are required, these inputs from the brain connect to both simultaneously, alpha-motoneurons and Ia inhibitory interneurons, generating a coordinated contraction and relaxation of antagonist muscles. This is an example of the mechanism followed when walking is required, there is segmental but also supraspinal control [12][16].

This inhibition is one of the most important keys for normal gait during walking, being the major segmental reflex circuit contributing to the phase-dependent modulation of muscle activation.During walking, each muscle is activated or deactivated at different phases of GC due to the successive induction of reciprocal inhibition. This successive control also belongs to one of the feedforward controls in locomotion [12].

Non-reciprocal (or Ib) inhibition: Golgi tendon organs are force-sensitive receptors, located at the muscle-tendinous junction, that react to both active and inactive muscle force. The Ib afferents of these receptors are excited by muscular loads, generating a neuronal pathways that on the one hand inhibit motoneurons from projecting to synergistic muscles (autogenetic inhibition) and on the other hand facilitate motoneurons from projecting to antagonists. This produces a regulation of muscle stiffness by deactivating synergistic muscles and activating antagonist muscles.

Summarizing, this neuronal mechanism is essential to regulate muscle stiffness because it allows nervous system to be informed about the force generated by each muscle. This means that Golgi tendon organs provide the information necessary to the rest of the nervous system to maintain constant muscle stiffness during several movements. During walking, this inhibition affects the ankle extensors providing the necessary timing of each phase in the GC, for example reinforcing weight bearing during the stance phase.

Recurrent inhibition: Recurrent inhibition is also based on the fact that movement is supported by neuronal circuits composed by inhibitory neurons. One of these neurons are the Renshaw cells that are rhythmically active and modulated by ipsilateral and contralateral locomotor networks as well as by motoneurons. These cells are the ones in charge of providing recurrent inhibition to the alpha-motoneurons that target the synergistic muscles. In addition to alpha-motoneurons, they also project to gamma-motoneurons, interneurons that mediate reciprocal Ia inhibition, and other Renshaw cells. The wide number of segmental reflex pathways in this inhibition, as well as its wide convergence, declare the versatile local feedback regulation of recurrent inhibition.

Regarding the function of recurrent inhibition, it can be said that it is a stabilizing or limiting feedback mechanism as represented in Figure 11. It synchronizes alpha-motoneuron discharge patterns and it inhibits motoneurons to slow contracting muscle fibers during rapid contractions. On the one hand, during a weak tonic voluntary contraction, there is Renshaw facilitation meaning that recurrent inhibition increases. On the other hand, during strong contraction, there is Renshaw inhibition meaning that recurrent inhibition decreases. This versatility suggests that recurrent inhibition is a mechanism that work as gain regulator of motor output. Given all these points about its functionality, it is apparent that recurrent inhibition is involved in motor tasks in situations where equilibrium is compromised by selecting the correct muscle synergy patterns at each time. It means, it plays a essential role in neural control of movement.

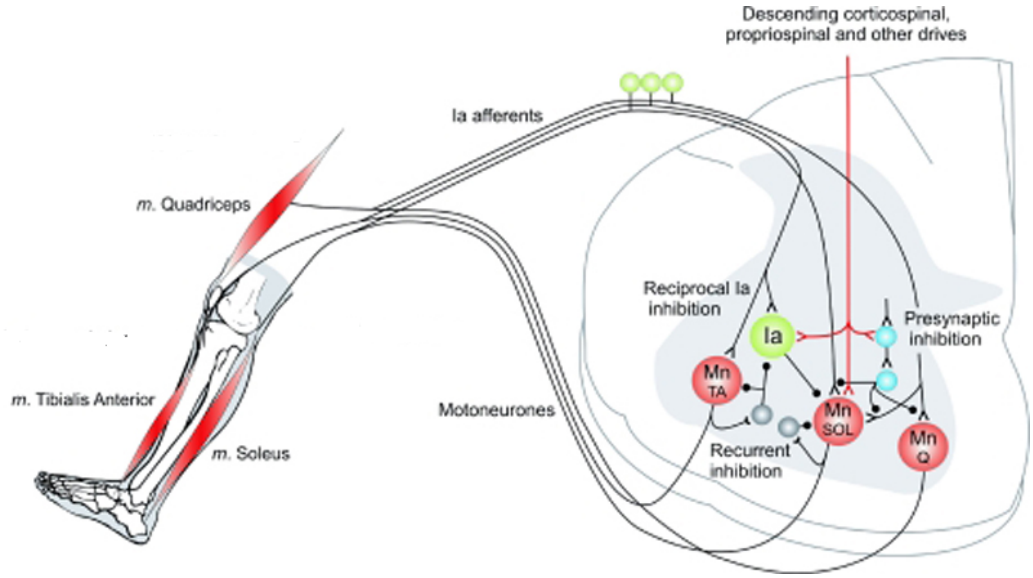

Figure 11: Spinal pathways involved during all the spinal inhibitory circuits. Retrieved from [34].

When a subject suffer a lesion in the Spinal Cord, all of the previous spinal inhibitory circuits are damaged producing an abnormal activation of muscles. This is the reason why spinal inhibitory circuits defects are correlated with spasticity or lack in muscle coordination during walking in SCI subjects.

3.1.2 Stretch reflex and H-reflex as a probe to study spinal excitability

Most of the human actions are controlled by the brain that decide which movement or action has to be performed. Once this decision is taken, nerve signals must travel from the brain through the spinal cord to the target receiver. Finally, once action is performed impulse goes back by the reverse pathway to inform the brain that the action is completed. These actions are brain-controlled and conscious.

Other body actions, however, can be carried out without direct thought. These actions are called autonomic processes and they are increased, decreased, or maintained by the body using external or internal signals. A very specific automatic response performed by nervous system is the reflex

[36]. The performance of this reflex is that, on the basis of a particular stimulus, there is an automatic and repeatable subconscious motor response provided by the body. It is important to note that reflex patterns can not only be triggered by peripheral stimuli, but also by internal ones. The most common reflex is the stretch reflex, also known as myotatic reflex.

The stretch reflex is a pre-programmed response to a stimulus, usually passive stretching, that causes a muscle to stretch or contract. When a muscle spindle is stretched, the spinal nerve receives an impulse that triggers a contraction of the muscle. It is a very quick impulse (1 or 2 ms) because it only needs to travel to the spinal cord and return to the effector, the brain does not participate [36].

Regarding this pathway, it can be observed how the first component is the muscle spindles found inside the muscle belly, between and parallel to muscle fibers. Its ability to detect passive stretching is possible by its composition based on intrafusal fibers and nerve endings. Its excitation caused by the stretching of the muscles generates the stretch reflex. This reflex generates impulses, called muscle spindle afferents, that make a continuous loop from the muscles to the spinal nerve and back again to the muscles. When stretch impulse is received at anterior horn of spinal cord, the alpha motorneuron activity is increased, which causes the muscle fibers to contract and obstruct the muscle lengthening [36]. In addition, through the mechanism of reciprocal inhibition explained in 3.1.1, a different subset of neurons instruct the stretched muscle and its synergistic muscles (agonists) to contract and the antagonistic muscles to relax. Thanks to activation of agonists muscles and deactivation of antagonists, the reflex process maintains the muscle at a constant length preventing from over-extension or muscle torn. During this simple path, the gamma efferent cells also have a crucial function as they keep the muscles (both agonist and antagonist) ready for the stretch reflex, compensating the agonist with stronger contraction while protecting the antagonists from excessive stretching.

As the body is constantly under different forces or stretch on muscles, this stretch reflex is crucial in helping to maintain proper posture and balance. When this reflex presents delays in or absence, it is a sign of possible neurological or neuromuscular disorder.

The electrical induced reflex analogous to the mechanically induced Stretch reflex is the Hoffmann reflex, known as H-reflex [37]. The main difference between Stretch and Hoffmann reflexes is that instead of a mechanical stretch of the muscle spindle, H-reflex is evoked by low-intensity electrical stimulation of the afferent peripheral nerve. In addition, H-reflex presents even a simpler reflex circuit because stimulation bypasses the muscle spindle, it simply generates a monosynaptic excitation of alpha-motoneurons which elicits the H-reflex.

The simplicity of its circuit is the reason why H-reflex is an useful tool in the non-invasively evaluation of the modulation of spinal inhibitory circuits and neuronal pathways explained before, analysing Central Nervous System (CNS) sensorimotor integration and plasticity [38]. In this way, H-reflex can be used to evaluate the response of nervous system to different neurologic or musculoskeletal conditions or therapeutic modalities, because it measures the efficacy of synaptic transmission.

The neurophysiological characteristics of H-reflex varies depending on the electrical intensity applied to the peripheral nerve [37], as illustrated in the Figure 12. When a low-intensity electric stimulus is delivered, the action potential travels selectively in sensory Ia afferents (2 in figure), because they are the thicker and larger fibers. Theoretically, when axon is larger, it is easier to stimulate that neuron. When potential arrives to spinal cord, it synapses on alpha motoneuron of the corresponding muscle, generating excitatory postsynaptic potentials. These action potentials generated by alpha motoneurons travels along efferent fibers (3 in figure), until reaching the neuromuscular junction where it produces a twitch response in the muscles, a synchronized contraction. This contraction produces a determined wave in the EMG, the H-reflex. This circuit generated at low electrical intensities is known as evoked reflex response and it will be modified by increasing the electrical intensity.

If the intensity of stimulation starts to increase, but not reaching the motor threshold to stimulate directly efferent fibers, there is a recruitment of more Ia afferent fibers and consequently more motoneurons are activated. This results in the increment of the H-reflex amplitude.

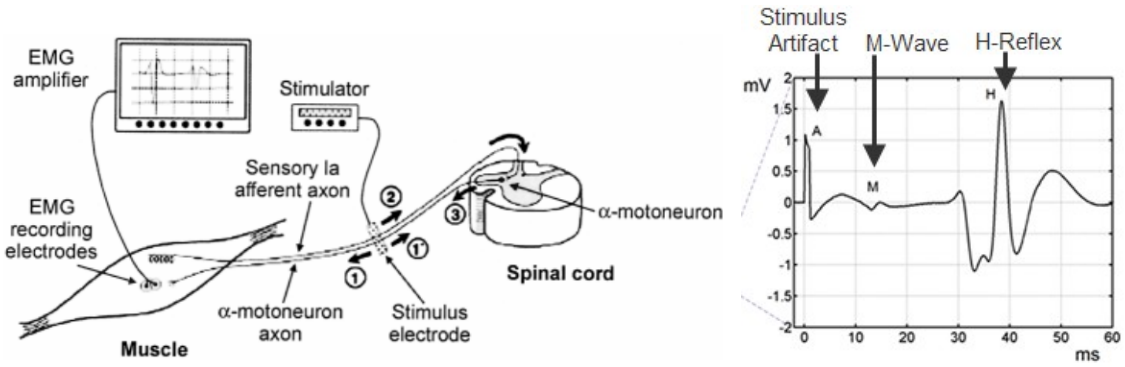

Figure 12: Pathways related to H-reflex depending on applied intensity and the corresponding EMG signal. Retrieved from [37].

Due to the short length of motor axons, in order to activate them, it is necessary to increase the stimulus intensity. For that reason, when intensity of stimulation is increased above a motor threshold, in addition to the previously explained circuit, there is also a direct activation of efferent/motoneuron fibers. In this case, action potentials appear directly in the thinner axons of the alpha motoneurons that send action potential directly from point of stimulation to the muscle. It means, it is an orthodromic motor volley (left 1 in figure) because action potential travel to the muscle, in the physiologic direction. This circuit produce a different response in the EMG, the muscle response or M-wave. Simultaneously, there are also action potentials in the thinner axons that propagate toward the spinal cord. It means, it is an antidromic motor volley (right 1 in figure) because action potential travel to the spinal cord, opposite the physiologic direction. These antidromic motor volley collide with orthodromic afferent volley provided by the evoked reflex response, cancelling partially the H-reflex response. In conclusion, when intensity above motor threshold is provided, the homonymous muscle produces two responses, M-wave and a continuously decreasing H-reflex.

Finally, at the maximum stimulus intensity, all motoneuron axons of the efferent fibers present orthodromic and antidromic action potentials. As imagined, orthodromic motor volley generates the maximum M-wave while the antidromic motor volley collide with orthodromic afferent volley of the evoked reflex response. If the antidromic motor volley is smaller than the orthodromic afferent volley, the afferent volley is reduced but continues to the muscle, as happens before. This generated the decrease of H-reflex but not its complete absence. However, in this case, as the antidromic motor volley is larger than the orthodromic afferent volley, the H-reflex is completely cancelled.

Recruitment curve is a simultaneous representation of both curves, H-reflex and M-wave, depending on the stimulus intensity provided [38]. Visualizing this curve in Figure 13, it is clearly seen how H-reflex appears at low intensities. However, when stimulation intensity starts to increase and motor fibers are activated, there is a simultaneously appearance of the H-reflex and the M-wave that starts to appear. At a certain stimulus intensity, H-reflex reaches its maximum and then starts to decrease, while M-wave continues increasing until reaching its maximum and maintains stable. In conclusion, this recruitment curve shows how gradually increasing the stimulus intensity (x-axis), the amplitudes of both curves changes (y-axis).

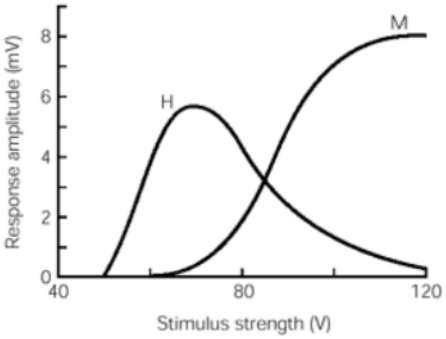

Figure 13: Recruitment curve.

The ascending slope of the H-reflex curve is important because it contains information regarding the reflex gain. However, during the descending part of the H-reflex curve, the motoneurons are influenced by Ib and recurrent inhibition pathways. To avoid this factor and acquire proper results, by consensus it has been decided that control H-reflex has to be elicited by an intensity corresponding to the ascending slope of the H-reflex curve. The recruitment curves are normalized by eliciting the H-reflex as a percentage of Mmax. So proper results will be obtained if intensity selected corresponds to a point of the curve where H-reflex represents 25-45% of the Mmax and where the M-wave amplitude is minimal and stable [38]. However, as in many experiments is difficult to reach the Mmax, it is also possible to evoke a control H-reflex at 50-60% of the Hmax, with M-wave being as minimal as possible.

This analysis of H-reflex and M-wave can be elicited in almost all the nerves of the human body; however, the most extensively used has been the SOL H-reflex. The reason is that, due to its location in lower limbs and its easy detection, it allows the study of movement neural basis and spasticity in people with and without neurological injury. This SOL H-reflex is elicited by applying stimulation to the Posterior Tibial Nerve (PTN) represented in Figure 14 that innervates the SOL muscle.

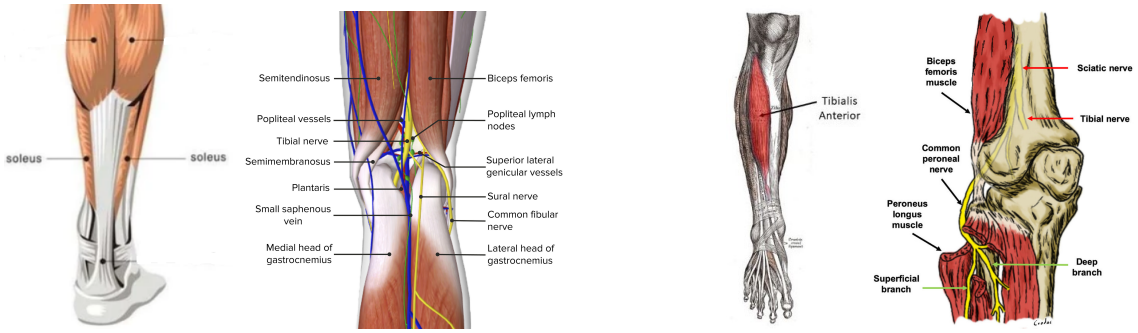

Figure 14: Left image: Posterior leg representing the anatomy of PTN innervating the SOL muscle. Right image: Lateral leg representing the anatomy of CPN innervating the TA muscle. Retrieved from Google Images.

It is important to note that depending on subject limb length and on how close the nerve is to the spinal cord, the length of H-reflex pathway differs resulting in different times between the application of the stimulus the the H-reflex appearance on the EMG, it means different H-reflex latencies. In the case of SOL H-reflex, the latency is approximately 30 milliseconds. In the case of the latency of M-wave, it is essential to remember that it is elicited by the direct stimulation of motor axons. Due to the fact that the path from point to stimulation to directly the muscles is shorter, the SOL M-wave appears at a shorter latency than SOL H-reflex, at 6-9 milliseconds [38].

Nowadays, there is not even clear which is the optimal subject positioning for recording the H-reflex. However, something that is clear is that H-reflex is affected by some factors such as head position, eye closure, joint angle, and concentration level. The most important point when performing H-reflex analysis is to reduce as much as possible the variability and improve the subject comfort. For this reason, it is important to establish a exact position for all the H-reflex analysis.

There is also controversy regarding the necessity of muscle contraction during performing H-reflex analysis. On the one hand, some studies claim that H-reflex recording should be conducted during voluntary, sustained, homonymous muscle contractions in order to stabilize motoneuron excitability and minimize post-synaptic effects. However, when performed in this way, the amplitude of H-reflex may be affected by changes of alpha-motoneuron excitability. On the other hand, other studies affirm that when reflex recordings are conducted at rest, H-reflex amplitude is more stable representing the reality more faithfully and other neuronal systems are less active reducing the interference between systems [12]. For this reason, this study, that obtain such a good result using resting conditions, recommends performing H-reflex at rest. In addition, other studies such as [38], recommend to perform

H-reflex test with the subject sat in a chair in straight position with the foot under examination fixed to a static footplate. In this position, the knee is at 160-170º and the ankle is in small plantarflexion of 110º [38].

Finally, regarding the usability of H-reflex, it can be concluded that it is a very useful tool to study the performance of the different spinal neuronal pathways such as reciprocal Ia inhibition. As explained before, during this circuit Ia afferents activates Ia inhibitory interneurons that inhibit the antagonist alpha motoneurons allowing proper movement in humans. The theory proposed by Hoffmann states that, when the antagonist muscle (TA) is contracting, the SOL H-reflex decreases.

Based on this theory, the study of reciprocal Ia inhibition is done by computing the percentage of SOL H-reflex difference between the test SOL H-reflex amplitude and the conditioning H-reflex amplitude, as represented in Figure 15. On the one hand, the test stimulus is the activation of PTN innervating the SOL muscle. On the other hand, the conditioning stimulus is the activation of the agonist peripheral nerve, in this case CPN innervating the TA muscle as represented in Figure 14, followed by and activation of PTN. The intensity provided at the test stimulation is the one that corresponds to 15-25% of the Mmax while the intensity of the conditioning stimulation is the one corresponding to the motor threshold. In the case of the reciprocal Ia inhibition, the stimulus to the CPN is performed at 2,3, or 4 ms before test stimulus to the PTN. This means that the conditioning-test intervals or Interstimulus Interval (ISI) during the conditioned stimulus are 2,3, or 4 ms. Regarding these short intervals, it can be concluded that time interval between agonist activation and antagonist deactivation in reciprocal inhibition is really short. This intervals vary depending on the inhibitory circuit under study [13].

Considering test H-reflex peak-to-peak amplitude as the standard, conditioned H-reflexes are normalized to this standard. In this way, reciprocal inhibition is evaluated as the difference between conditioned and mean control H-reflex. If reciprocal inhibition is working properly, as in Figure 15, the amplitude of the test SOL H-reflex has to be higher than the amplitude of conditioning SOL H-reflex because of the activation of reciprocal Ia inhibition after the CPN stimulation. This confirm the idea that when a muscle is stimulated (TA), its antagonist muscle (SOL) reduces its activity. On the other way, if the conditioning SOL H-reflex has higher amplitude than the control one, facilitation of the antagonist muscle is being performing as a result of the improper reciprocal inhibition functioning [12] [16].

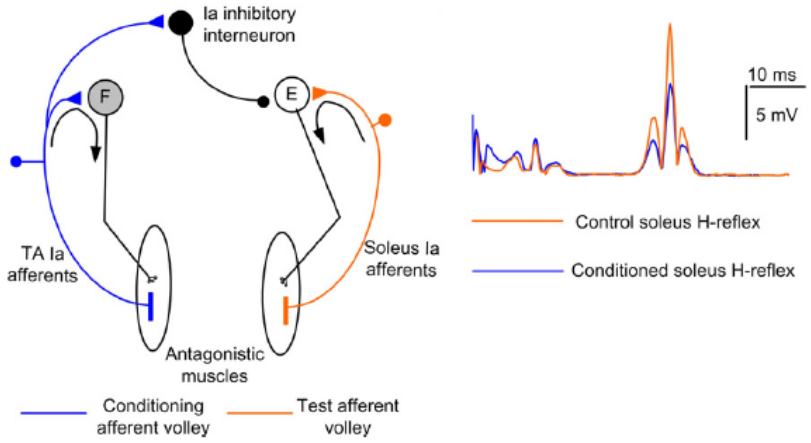

Figure 15: Conditioning-test paradigm to analyze reciprocal Ia inhibition. Retrieved from [32].

The conclusion is that, due to its ability to analyse modulation of spinal inhibitory interneuronal circuits, H-reflex can be used as a tool for the analysis of short and long term plasticity of the neural system during neurologic conditions. Clinically, this reflex has been already used to evaluate several diseases such as musculoskeletal injuries, and to assess the effects of therapeutic modalities.

3.2 Spinal Cord Injury. Causes and pathophysiology

Physiologically, the Spinal Cord is a collection of nerve cells (neurons and glia), nerve fibers and blood vessels that is cylindrically disposed inside the spine. The nerve cells participate in the coordination of complex patterns of movements essential in human life such as walking or breathing, the nerve fibers connect the brain with all the different parts of the body and vice-verse, and the blood vessels supply blood to the spinal cord itself, through the spinal arteries and distribute the blood supplied by the heart to the rest of the body. The extension of spinal cord is, from the lower portion of the brain until the lower back.

Theoretically, the Spinal Cord is composed by to different tracts [39]. The ascending tract transmit sensory information from receptors to the brain while descending tract communicate motor orders from the brain to the effector. The following pathway represents the one involved in the neural transmission of information:

Peripheral or internal receptors transport the sensory information until the spinal cord by introducing their spinal nerve roots into the sensory or dorsal root of the spinal cord. This sensory nerve signal is send to the brain through the afferent fibers, the brain analyses and generates a response that will be conducted by efferent fibers until the spinal cord again. At this point, motor information lefts the spinal cord through the motor or ventral root and is transported until the effector organs.

Therefore, due to the importance of spinal cord, it must be protected by different structures. It is surrounded by a column of spinal bones known as vertebral column and, in addition, by a substance that acts as shock absorber called cerebrospinal fluid (CSF) [39].

In humans, the protective vertebral column of the spinal cord is divided into different segments depending on the type of vertebrae [39]. Starting from the superior until the posterior part of the column, there are 7 cervical, 12 thoracic, 5 lumbar, and 5 sacral vertebrae. Each of this region contains several nerves of the total 31 pairs of nerves present in the spinal cord, containing thousands of axons. Each of these nerves innervate a specific region of the human body, associating the function of this region with a specific segment of the spinal cord as it can be observed in Figure 16. The cervical spinal nerves, from C1 to C8, are in charge of controlling signals going to the superior part of the body such as posterior part of the head, neck, shoulders, arms, hands and the diaphragm. The thoracic spinal nerves, from T1 to T12, regulate signals going to the middle part of the body such as chest muscles, back muscles, many organ systems and abdomen. The lumbar spinal nerves, from L1 to L5, control signals that are send to the lower part of the middle body such as lower abdomen, lower back, buttocks, some part of external genital organs and some parts of the legs. Finally, sacral spinal nerves, from S1 to S5, regulate signals going to the lowest part of the body such as lower parts of the legs, thighs, feet, external genital organs and anus.

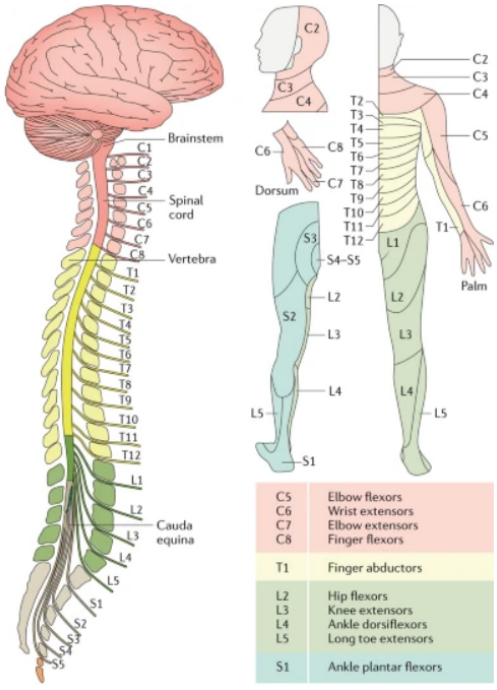

Figure 16: Segments of Spinal Cord and its innervated parts of the body. Retrieved from [39].

This is essential to known because when there is an injury in the spinal cord, the functions of the segments below the level of injury are completely or partially loss.

Considering this definition of Spinal Cord, it can be determined that a SCI is any type of damage to this cylindrical collection of cells, fibers and blood vessels [40]. SCI can be produced in two different way, when there is a problem in the spinal cord itself or when the problem is on the surrounding tissues and bones but it ends affecting also to the spinal cord. After a SCI, depending on its severity, there is a damage or even a loss in the transmission of sensory, motor and autonomic nervous system information. This leads to temporal or permanent changes in movement, sensation and other body functions below the level of the injury. These changes lead to two main characteristics of SCI, an independence loss and a permanent increase of mortality rate, which have catastrophic consequences in the physical, social and vocational life of patients an their relatives.

The severity of the injury and its level at spinal cord are critical points in the disability extension after SCI, and therefore in the recovery and rehabilitation of this injury. When injury affects the cervical segments of the spinal cord, there is usually a paralysis of all the limbs and the trunk, generating tetraplegia or quadriplegia. However, if the segments of the spinal cord affected by the injury are the lowest ones, paralysis affects only the legs and the lower part of the truck, causing paraplegia. In some cases of lower SCI there is not even paralysis, and there is simple a reduction in functionality. The different SCI can be classified based on different parameters:

1) Based on the spinal cord segment affected by the injury:

- Cervical SCI: this injury produces a loss of chest, arms and legs function. As trunk function is also paralysed, breathing, bowel and bladder control is typically affected.

- Thoracic SCI: if injury occurs in the upper part of thoracic segment, there is an affectation of chest and legs causing problems also in the breathing and sometimes in the bowel and bladder control. However, if it occurs in the lower part of this segment there is only chest and legs affectation.

- Lumbar SCI: there is an affectation of the hips and legs, affecting sometimes the bowel and bladder control.

- Sacral SCI: the consequences are similar than the ones on lumbar SCI, affection of hips and legs and in some cases affection in bowel and bladder control.

2) Based on the cause of SCI [41]:

- Traumatic SCI: this injury is elicited when an external physical impacts on the spinal cord producing an acute damage. It is produced by different causes such as an abrupt and traumatic blow which results in a or some vertebrae fracture, dislocation, crushing or compression as occurs in traffic accident or falls, and it can be created by a cut on the spinal cord produced by a gunshot or knife as result of an violent act.

  These injuries occur in a 60% at cervical level, in a 32% at thoracic level and in the remaining 9% at lumbrosacral level.

- Non-traumatic SCI: the spinal injury is elicited by a previous disease such as arthritis, primary or metastatic tumors, blood vessel problems or bleeding such as medullary infarction, compressing myelopathy such as cervical spondylotic myelopathy, neurodegenerative diseases such as motor neuron disease, autoimmune diseases such as multiple sclerosis, inflammation or infections such as epidural abscess, and genetic causes such as spinal muscular atrophy or disk degeneration of the spine. These acute or chronic diseases presented previously start a process in which they generate a primary injury of spinal cord [42].

The most important point studied on epidemiology are the incidence, the causes, and the mortality rates of SCI represented in the Figure 17 [3][43]. The incidence and prevalence have been previously explained in 2.1 concluding that North-American countries have the highest incidences of SCI. In terms of aetiology, regarding traumatic SCI, the first cause are motor vehicle accidents (38%), followed by falls (31%), sports-related injuries and acts of violence such as gunshot wound-related injuries. The causes of non-traumatic SCI are other diseases that damage the spinal cord such as arthritis, inflammation, infections and cancer, or medical and surgical injury. Finally, in terms of mortality rate, although the survival probability of SCI patients has been improving over the years, these patients have still higher mortality rates than those same aged healthy subjects. In the Section 2.1 it has been also explained how highest mortality rate occurs during the acute phase of disease, decreasing progressively once patient obtains the hospital discharge but never disappearing due to complications associated to the disease. Obviously, mortality rate is higher if the injury is more severe, if the level of the injury is on the higher cervical, in older patients and if there is multisystem trauma [41].

There other two factors, gender and age, that are related with the SCI [3][43]. Regarding the gender, there is a male-to-female ratio of 2:1 being the 79.8% of traumatic SCI in males while only the 20.2% remaining in women. In terms of the age, the prevalence of traumatic SCI follows a bimodal distribution. The first peak includes patients between 15 and 30 years and the main cause are high-energy impacts such as traffic accidents, sport-related injuries or gunshot wound-related injuries. The second peak is for patients older than 50 years old and the main cause are low-energy impacts such as falls. In the case of the non-traumatic SCI, it also follows a bimodal distribution in which the first peak accounts for middle-aged adults with inclination to suffer central cord syndrome, disc herniation or SCI caused by infection. The second peak includes the oldest group of patients who suffer predisposition to SCI caused by stenosis, spondylosis with myelopathy, spondylolisthesis, degenerative disc disease, infarcts/ischemia, abdominal aortic aneurysm complications or cancer [41]. In addition, patients that are older are most likely to have higher rates of complications as well as higher mortality rates.

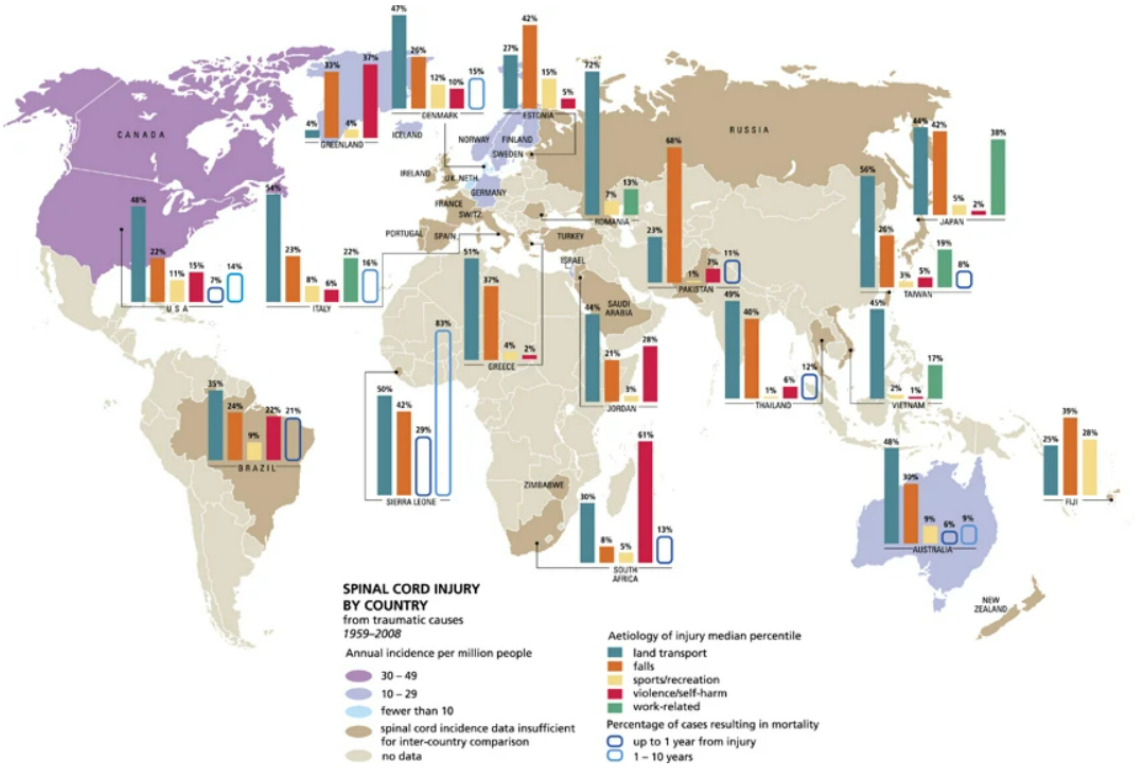

Figure 17: Incidence, aetiology, and mortality representation of SCI worldwide. Incidence is represented by color in the countries, aetiology is represented by colored bars, and mortality is represented by empty bars. Retrieved from [3].

The process of SCI generation can be pathophysiologically divided into primary and secondary injuries [42] [39]:

- The SCI is firstly presented as **Primary Spinal Injury** that is the immediate result of the initial mechanical trauma. This injury permeabilizes and damages the existing neurons and glia cells in a permanent way, initiating the secondary injury that is a very complex cascade process.

- **Secondary injury** appears several days or weeks after the initial injury. It is caused by the accumulation of fluid in the spinal cord and the bleeding, swelling and inflammation produced after primary spinal injury. This is a critical process because it produces a cascade of cell death by necrosis and apoptosis of neurons and glial cells. The cell death, ischaemia and inflammation is posteriorly followed by changes in spinal cord organization and structure.

However, this process can be also temporally divided into acute, subacute, intermediate and chronic phases represented in Figure 18:

- **Acute injury phase:** it occurs during the first 48 hours after the injury. There is a mechanical interruption and luxation of the vertebrae that compress or transect the spinal cord. At this focal region of injury there are primary events such as cell damage, bleeding, and compression that initiate the secondary injury cascade. The events of this cascade are haemorrhage, ischaemia, oedema, and necrosis or apoptosis that adds catastrophic neurological damage and dysfunction due to the damage of nerve fibers and/or neural circuits loss.

- **Subactute injury phase:** it comprises the time between the 48 hours until the 14 days after the injury. There is a continuous ongoing of oedema, vessel thrombosis, vasospasm, and inflammatory cell infiltration that increase even more ischaemia, inflammation, and excitotoxicity. These processes lead to a loss of cellular ionic homeostasis causing the deregulation of a key mediator during cell death such as intracellular calcium, which increase the neuronal and glial death. This death also produces the release of components that activate inflammatory cells propagating inflammation and cell apoptosis, inducing further damage to the spinal cord and a harsh post-injury environment.

- **Intermediate and chronic injury phases:** intermediate phase comprises time between 2 weeks until 6 months after the initial injury while chronic phase occurs 6 months after injury and for the rest of the patients life. The inflammatory response decreases and there is a process of remyelination, vascular reorganization, glial scar, cystic cavitation, and remodeling of neural circuits. Cystic cavitation allows the axonal regrowth and cell migration, glial scar provides local trophic support promoting neovascularization, and remyelination is essential in order to get functional recovery because if fibers do not present myelin the signals cannot travel properly through them.

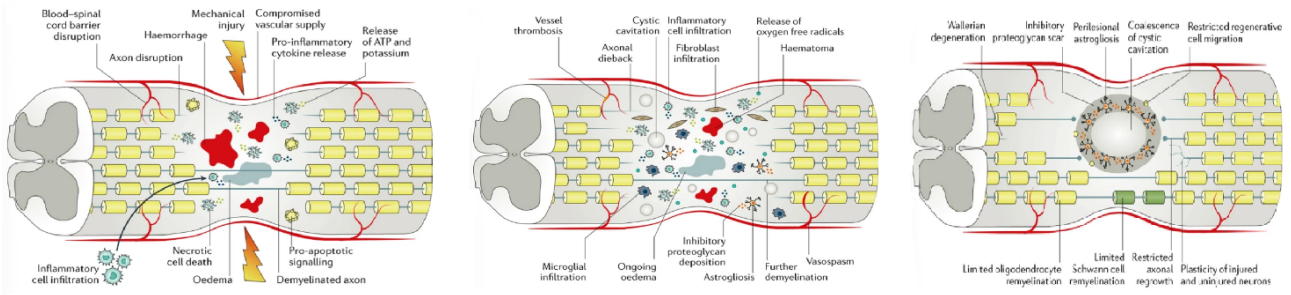

Figure 18: Temporally division of SCI phases: acute phase, subacute phase, and intermediate and chronic phases. Retrieved from [39].

Once SCI is produced is important to follow several management rules at the injury scene, in order to stabilise the patient body and prevent further spinal cord damage [40]. It is essential to perform a baseline assessment based on Advanced Trauma Life Support (ATLS) protocol that determines initial care consisting on a Airway, Breathing, and Circulation (ABCs) evaluation and support, the neck and spine stabilization, blood pressure maintenance by haemodynamic monitoring, multisystem support and sedation. In some life-threatening cases, surgical decompression, blood pressure augmentation and administration of methylprednosolone are also essential.

Once the patient arrive at hospital, it is essential to perform an impeccable diagnosis of clinical manifestations or complications in order to provide the most appropriate treatment [40]. By analysing the clinical manifestations, specialists can induce which are the nerve roots that are affected, being able to recognize which vertebrae of spinal cord is or are damaged. These clinical manifestations vary depending on the type of SCI, the level of injury, the completeness or incompleteness of injury,and the amount of spinal cord tissue preserved after the injury. The problem with some clinical manifestations or complications is that they result in longer hospital stays, isolation of patient from society, reduction of patient self-esteem, psychological distress in patients, worsening of functional recovery, and higher mortality rates [6]. The clinical manifestations and complications after a SCI can be systemic or local [40] [41], being the systemic ones the leading cause of patients early mortality.

1) Local SCI clinical manifestations:

- Syringomyelia is a cyst or longitudinal cavity filled with fluid, covering spinal cord segments and producing a gradual myelophathy. It is not common and its treatment is continuous monitoring using MRI or in more severe cases surgical decompression.

- Neuropathic joint arthropathy is a slow gradual joint destruction that generates deformity, ulcerations and life-threatening infections. The treatments are X-ray monitoring, analgesics to treat the symptoms, and a surgical treatment by vertebral fusion.

2) Systemic SCI clinical manifestations:

- Complete or partial loss of sensorimotor function below the injury level.

- Spasticity is the most common SCI manifestation (65-78%) based on a muscle tone increase with excessive deep tendon reflexes produced by the injury disruption in spinal cord fibers. It affects mobilization and daily living activities of patients, promoting other complications such as pressure ulcers, fractures and a severe lack of cardiorespiratory conditioning. Its management is based on physical therapy, pharmacological therapy, intrathecal baclofen pump, injections of botulinum toxin, and tendon release surgery.

- Neurogenic heterotopic ossification is a disorder of ectopic bone formation in the connective tissue around joints. It produces local pain, irritation, fever and an increase of spasticity. The current treatment is based on physical, pharmacological, radiation and ossification surgical removal.

- Impaired respiratory function in cervical and upper thoracic injuries as a result of paralysis of phrenic nerve, intercostal, and abdominal muscles such as the diaphragm. This paralysis reduces the capacity of the lungs, the effectiveness of coughing and, as well, increase the respiratory demand due to an accelerated fatigue. These factors lead to recurrent pneumonia, alveolar collapse, pleural effusion, sleep apnoea, and failure of respiratory system. It is the main cause of mortality in chronic patients and require the temporal or permanent assistance of ventilators or inserted artificial breathing tubes.

- Autonomic dysreflexia is the most life-threatening SCI condition in upper injuries. It is a reflex over-stimulation of sympathetic neurons caused by noxious stimulus such as bladder overdistension, lack of bowel voiding, tight clothes or pressure ulcers below the injury level that results in vasoconstriction and acute hypertension. The body tries to balance this response by activating the parasympathetic outflow above the injury level producing high blood pressure, bradycardia, pounding migraine, sweating, vision changes, and sinus congestion. These complications can produce hypertensive encephalopathy, seizures, cardiac arrest, or even death [42]. The immediate management is essential by correctly positioning the patient, by suppressing the noxious stimulus, and by administering anti-hypertensives drugs.

- Secondary immunodeficiency or immune paralysis in cervical or high-thoracic injuries due to the loss of secondary lymphatic organs innervation increasing the vulnerability of patients to infections, such as pneumonia, urinary, and wound infections. Its accepted management is the monitoring and administration of antibiotic pharmacological therapy. However, the ideal aspect is the prevention of infections.

- Circulatory complications due to the interruption between the brain control and the cardiac nerves which produces a rapid and irregular or a dangerously slow heart beat. In addition, it is also very common the appearance of unstable blood pressure producing hypotension, arrhythmia and blood clots that can result in dizziness, weakness and syncope. The required management is the continuous monitoring, the use of lower limbs compression stockings, and the pharmacological therapy. In the case of blood clots, it is more important its prevention than its treatment because they are so life-threatening that if occurring can be lethal.

- Pressure sores are the break down of skin in body areas where there is a continuous pressure of skin with any surface and a reduced blood flow. This continuous pressure is typically produced by the use of wheelchairs and they stays at bed. These ulcers can be very painful and even life-threatening in cases when it is not properly treated using anti-aseptic therapy or debridement. However, its prevention even more important than treatment only using periodical changes of patient position, daily inspection and cleaning of the skin.

- Neurogenic pain is the temporal or constant pain or burning/stinging sensation, caused by an hypersensitivity. It is really typical in chronic SCI patient and it can be felt even in parts where sensation capability has been lost, because of maladaptive regeneration of spinal cord fibers that produce the inappropriate activation of fibers. The management includes pharmacological therapy using antidepressants or opioids, non-allopathic therapies such as acupuncture or massages, spinal and brain electrical stimulation, and surgery.

- Bladder and bowel complications due to the disruption on innervation of detrusor and sphincters. It produces the inability to empty the bowel and bladder producing acontractile bladder, constipation, urinary incontinence and regular infections. Its current management is the application of a urethral catheter, nutritional changes in diet, urinary stoma surgically or colostomy, digital rectal stimulation or disimpaction, use of suppositories, electrical stimulators, botulinum toxin injections, and pharmacological therapies.

- Sexual dysfunction produced by the interruption between areas of the brain related to sexual function and the sexual effector organs. On the one hand, in upper injuries, there is an affection of psychogenic excitement producing a impairment in brain control of erection and lubrication but a preservation of reflexive excitement allowing genital control of erection, lubrication, and orgasm. On the other hand, in some lower injuries, there is an affection of reflexive excitement but a preservation of psychogenic one. The current management includes pharmacological therapy, electrical stimulation, and surgery.

All these clinical manifestations of SCI produce a lifestyle change in the patient and their relatives. This change is usually difficult to assume generating a depression that has to be treated by means of psychological and/or pharmacological therapy.

The diagnosis of SCI is very difficult as in many cases the clinical manifestations appear gradually when bleeding and swelling start to appear around the spinal cord. As the time between injury and treatment is the most critical factor in the prognosis and recovery of a patient due to the progression in functional neural tissue lost, it is always assumed that patient has SCI until proved otherwise [40]. In order to minimize this loss of tissue, it is essential to perform a rapid diagnose of patients and start applying as soon as possible the treatment. In conclusion, the central concept in the management of SCI is that 'Time is spine'.

The neurological recovery of SCI patients depends on the injury level, the functional impairments, and the rapid treatment delivery. The idea of SCI treatments is that patients could recover as much as possible, the sensory and motor function. In this way, enhancing the quality of life of patients by improving their independence and their integration in the society.

1) Treatment at the accident scene: First, it is necessary to perform a complete cranio-spinal immobilization using rigid C-collar and placing the patient on a backboard. Then, in order to relax and avoid movement in patient, is necessary to administrate sedatives. Finally, when required, there is an insertion of breathing tube to solve breathing problems or impairments [40].

2) Treatment at the Intensive Care Unit (ICU) in hospital that include the management of systemic complications [40]:

- Monitoring of cardiac, haemodynamic and respiratory parameters.
- Haemodynamics treatment supporting the mean arterial pressure to maintain the correct spinal cord perfusion, avoiding systemic hypotension, and maintaining oxygen saturation. It is essential the early administration of prophylaxis to prevent thrombosis.
- Methylprednisolone sodium succinate injection within 8 hours of injury to decrease inflammation, ameliorate blood flow, and decrease damage of nerve cells. There is still controversy because there are not clear evidence of its benefits in relation to its harm.
- Spinal decompression surgery to mitigate pressure exerted by some objects on the spinal column as well as to realign the spinal column. It is an excellent method to restore the spinal cord stability and decompression needed after a continuous compression of spinal cord that result in ischaemia strengthening the secondary injury. The sooner this surgery is performed, the sooner the spinal column will stop suffering providing better clinical outcomes such as higher functional recovery chance, reduction of hospital stay, reduction of complication rate, and reduction on health care expenses [41].

The diagnosis and treatment processes after a SCI have a really strong impact on economics, from its first acute phase to the last chronic phase. This economic impact comes from direct medical costs such as professionals, facilities, treatments, therapies...and from indirect costs such as unemployment and lost of productivity of patients.

The improvement of patient quality-of-life, their inclusion in the society and the reduction of economical impact are the three major reason why patient recovery is essential. Besides, they emphasizes even more the important role of developing effective treatments or therapies for SCI patients that allow them to recover their normal functionality.

3.2.1 Movement alterations after SCI

After a SCI, as explained in Section 3.2, there is an interruption between the brain and spinal nerves generating absence or reduction in many essential functions such as sensation, reflexes and muscle movement. One of the most important consequences of these dysfunctions is the inability to walk in some cases. Due to the importance of walking for patient physical and mental health, as well as, for achieving its independence and the inclusion in the society, it is essential to recovery this human ability. To be able to recover it, is essential to understand the physiological mechanism that produce loss of movement control.

Movement is produced by the activation of efferent motoneurons that innervate muscles producing their contraction, and in this way, their movement. The characteristics of this movement are determined by three essential factors; the way motor pools activated are combined, their recruitment level, and the effective force produced by the corresponding muscles. After SCI the level of movement is decreased because of the inability to activate or recruit ensembles of motoneurons in a efficient way to produce an effective movement. This inability is linked to three different issues or impairments [44] that should be solved using a potential therapy to recover functional locomotion:

- Inadequate muscle recruitment due to the interruption of the motor pools coordination: SCI produces an interruption between brain an spinal nerves producing adaptive reorganization of the spinal circuits that result in the generation of new functional connections between interneurons and motoneurons. The problem of these new connections is their lack of specificity which produces a reduction of reciprocal Ia inhibition. As this inhibition allows normal gait pattern contracting agonist muscle while inhibiting its antagonist muscle, when there is a reduction of this inhibition there is a co-activation of antagonist muscles. These co-activation leads to poor coordination, unintentional movements, and spasticity which produce an increase in muscle tone or stiffness that deteriorate essential functions such as walking [45][44][46].

- Deficient recruitment level of some motor pools in some incomplete patients: The spinal locomotor networks change their excitability threshold generating hyper-excitable and hypo-excitable synapses. On the one hand, the hyper-excitability produces a co-activation of spinal circuits producing spasticity that worsens the walking ability producing a pathological gait pattern. On the other hand, the hypo-excitability produced by high quantities of inhibitory neurotransmitters such as GABAergic and glycinergic [45][44], generates low levels of recruitment preventing the execution of some GC events such as weight-bearing stepping.

- Progressive weakening of muscle function after a chronic SCI: After SCI, walking ability of patients is lost being necessary the use of wheelchair or bed stays. This chronic reduction of muscle activation and loading levels produce a degradation of muscle properties generating atrophy, loss of force, and fatigue. The consequence is that, for accomplishing any task, patient has to recruit a higher number of motor units than a healthy subject [44] generating a higher difficulty to perform walking or any type of movement.

The general idea is that in healthy subjects, spinal and supraspinal control activate the Ia interneurons to activate and deactivate motoneurons in a determined manner, producing reciprocal Ia inhibition. Thanks to this control, muscles that perform a different function during a determined movement are activated in a coordinated manner, producing a patterned movement such as walking. Chemically, this inhibition of antagonist muscles is modulated by the release of inhibitory neurotransmitters (GABA and glycine) from the interneurons to antagonist muscle motoneurons. After a SCI, the absence or decrement of the spinal and supraspinal control produces a complete absent or reduction of reciprocal Ia inhibition. Therefore, the release of inhibitory neurotransmitters is interrupted and there is an activation of antagonist motoneurons generating spasticity. The co-activation of agonist and antagonist muscle generates a pathological gait pattern.

In order to assess the severity and affection of reciprocal Ia inhibition after SCI, it is important to perform an electrophysiological evaluation as illustrated in Figure 19. This evaluation measures the muscle activity of TA and SOL antagonist muscles during walking. In healthy subjects, the muscle activity is coordinated because when TA activates the SOL deactivates and vice-versa. However, in SCI subjects, there is a co-activation of muscle resulting in walking inability. This can be clearly seen in the EMG of the SOL muscle that does not completely deactivate when the TA activates.

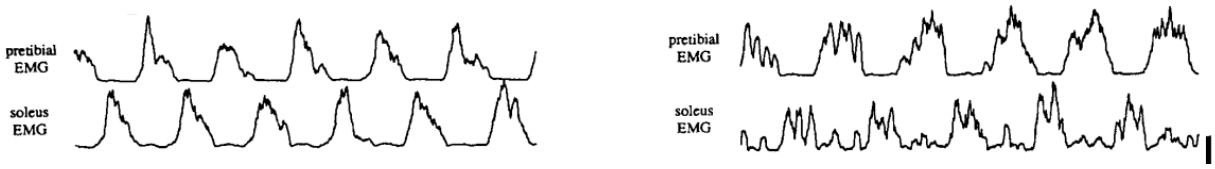

Figure 19: Movement alterations after SCI. Left image represents the EMG of a healthy subject. Right image represents the EMG of a SCI patient. Modified from [38].

Another method to evaluate the affectation of reciprocal Ia inhibition in SCI patients is the analysis of SOL H-reflex depression when its antagonist muscle is activated [45]. As already explained in Section 3.1.2, the test SOL H-reflex is obtained in order to compare it to the conditioned SOL H-reflex obtained by the stimulation of CPN followed by a posterior stimulation of PTN.

In healthy subjects where the reciprocal Ia inhibition is maintained, the conditioned reflex has a smaller amplitude than the test reflex. The reason is that the previous activation of antagonist muscle generates a deactivation of SOL, there is a decrease in reflex stiffness when antagonist muscle is activated. However, in SCI subjects, the amplitude of conditioned and test reflexes is similar [32]. This means that due to spasticity produced during SCI, the natural reciprocal inhibition from pretibial muscles onto the SOL H-reflex is absent or impaired producing a co-activation of agonist-antagonist muscles [45]. This impairment of reciprocal Ia inhibition has functional consequences such as walking inability [32]. Regarding the importance of reciprocal Ia inhibition, its modulation is a promising aspect to treat incomplete SCI patients with spasticity allowing them to recover walking ability.

In terms of rehabilitation, it is important to classify the sensorial and motor impairments because the level of impairment is correlated to the movement expression in a pathology. Therefore, the less severe impairments are, the higher the amount of possible recovery [13]. These functionality and mobility lost can appear at any part of the body and it can also present different severity grades depending on the injury level. Therefore, in order to classify SCI injuries depending on the remaining feeling or movement in different parts of the body after the injury several scales have been created [47][48].

The American Spinal Injury Association (ASIA) developed the International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI), known as ASIA scale, that is the most typical scale to classify motor and sensorial impairments after SCI. It is an universal classifier that performs a sensory and motor evaluation to finally determine the grade of remaining functionality at each side of the body, the single neurological level of injury, and if injury is complete or incomplete. It is recommended to perform this examination as soon as possible, if it is possible at acute hospital admission, in order to obtain a baseline against which to compare the results obtained during posterior follow-up. The evaluation tool is represented in Figure 24 and the results obtained are provided using three neurological summary scores that are ASIA motor score, ASIA sensory score and ASIA impairment scale grade. The results of this ASIA impairment scale are:

- ASIA A: it is a complete SCI where all the parts of the body, even those regulated by the lowest sacral nerves, have no sensation or movement. As it is a very severe condition, even after rehabilitation, the 80% of these patients will remain being class A while only 20% will convert into ASIA B and ASIA C classification [42].

- ASIA B: it is a sensory incomplete SCI where parts of the body below the level of injury, even sacral segments controlling bowel and bladder function, have sensory but no motor function. After a suitable rehabilitation, these patients recover 33% of their gait capacity [42].
- ASIA C: it is a motor incomplete SCI where parts of the body below the level of injury have feeling and movement. The majority of the major muscles below the neurological level are able to move, but only less than half of these muscles have a muscle grade higher than 3 allowing the movement against gravity. After a suitable rehabilitation, these patients recover 75% of their gait capacity [42].
- ASIA D: it is an motor incomplete SCI where parts of the body below the level of injury have feeling and movement. In this case, at least half (half or more) of the key muscles below the neurological level have a muscle grade equal or higher than 3, so they can move even against gravity. After a suitable rehabilitation, most of these patients are able to walk again one year after the injury [42].
- ASIA E: After having had prior deficits, the areas below the level of injury have normal feeling and movement.

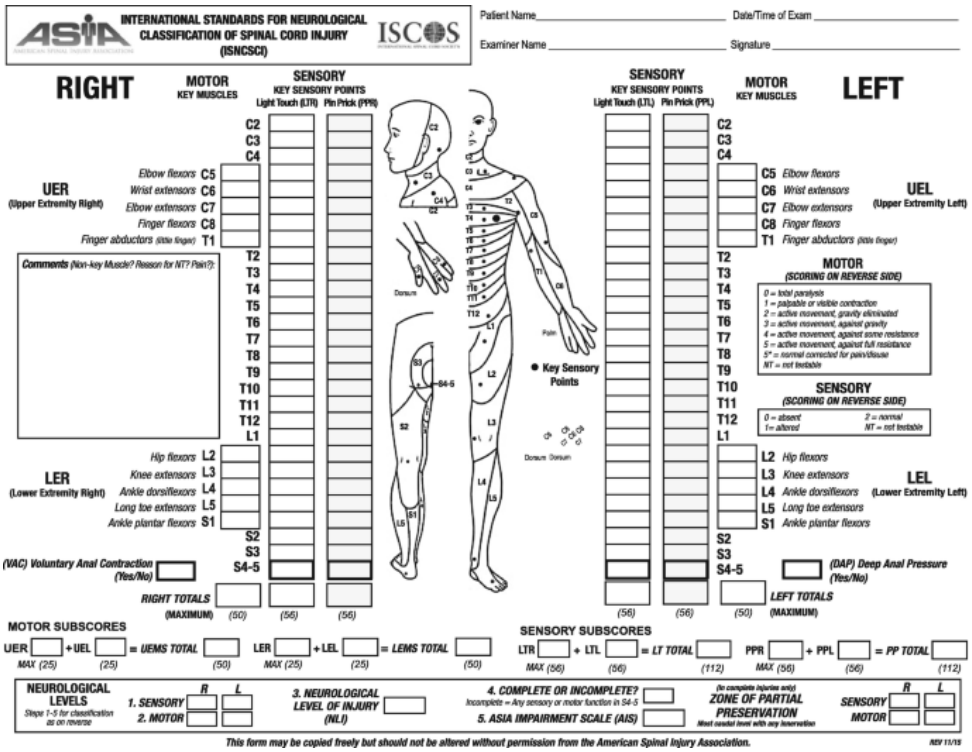

Figure 20: ASIA motor and sensory evaluation sheet. Retrieved from [48].

In spite of the fact that ASIA scale is the gold standard evaluation of functionality in SCI patients. There are other scales necessary to measure and evaluate other aspects of SCI patients. The Spinal Cord Independence Measure (SCIM) scale evaluates the capacity of SCI patients to perform daily life activities and therefore their independence. The Walking Index for Spinal Cord Injury I (WISCI II) scale evaluates the walking gait improvement of SCI patients from inability to walk to ability to walk at least 10 meters without needing walk assistance. The Short-Form Health Survey (SF-36) scale that evaluates the quality of life of SCI patients with mobility problems. Thanks to these physical and psychological evaluations that easy decision-making on treatment and rehabilitation, the most suitable treatment and rehabilitation program for each SCI patient is established [42].

3.2.2 Current approaches to the rehabilitation of SCI

Once the difficulties related to acute and subacute phases are overcome, that patients start to be psychologically and emotionally stable, it is time to minimize the remaining impairments and to learn how to live with the long-lasting or permanent ones. These processes is done by means of the SCI rehabilitation that is a very long-lasting process whose main goal is to maximize the functional recovery, prevent complications, improve the levels of independence and society integration to increase the patients' quality of life. The rehabilitation approaches are composed of an interdisciplinary team composed by different professionals such as physiatrist, nurses, psychologists, social workers, and occupational therapists among others.

The rehabilitation therapy is individualized and adapted to the necessities of each patient depending on the results obtained in the scales presented at Section 3.2.1. In addition, it is recommendable to vary the training session increasing the difficulties, in order for the patient to benefit from experiential learning [44].

These therapies vary depending on the phase of SCI at which they are applied. Therapies applied during the acute phase of injury are focused on increase body strength and stability, recover communication skills, and reduce complications. It is the most plastic phase, so the maximum number of exercises have to be performed [45], using passive exercises with the physiotherapist and assistive devices help. In the case of therapies applied to the chronic phase of injury the goal is to reintegrate patients into society by recovering motor and sensorial and motor skills such as walking [42]. Finally, the life long treatment and rehabilitation occurs in the home environment [45] thanks to house modifications.

As explained in 3.2.1 a powerful therapy has to solve the three main factors causing walking impairment in order to improve the functional locomotion. In addition, the introduction of activity-based therapies with the use of treadmill, weight-support, or cycling, help to restore and maintain muscle properties and reinforce the appropriate synaptic connections, producing neuroplasticity [44]. The most common therapies used during SCI rehabilitation are:

- Pharmacological treatments: The lack of endogenous supply of neurotrophic factors due to the disruption of brain and spinal cord produces loss of synaptic communication generating function loss, so exogenous administration of glycinergic and GABAergic agonists is an effective treatment to restore synaptic communication [44]. This restoration potentates other activity-based treatments such as locomotor training in combination with spinal cord stimulation allowing the recovery of motor function [44].

- Physical therapy: It is based on exercises that reinforce muscle strength, communication skills, and mobility. The repetition and correct performance of these exercises produces a potentiation of remaining connectivity between brain and spinal cord that generate neuroplasticity resulting in functionality recovery. Some of these activities are force training, stretching, cardiovascular and respiratory exercises and transfer or mobility training. These exercises are essential to improve patient mobility and prevent complications such as cardiorespiratory condition and pressure ulcers.

  In the case of more severe incomplete patients, that can not even stand by their own, there is a weight-supported locomotor training using assistive devices such as Hocoma's Locomat.

- Occupational therapy: It is composed of exercises that restore fine motor skills necessary for performing activities of daily living. The idea is to integrate adaptive devices into into the daily life or patients or to perform compensatory strategies that allow to perform daily activities using the remaining abilities.

- Vocational therapy: Its goal is to help patients to get back to work searching its optimal work position by determining their capabilities to be employed. To help this decision, it is essential to identify the work skills, physical, and cognitive capabilities of the patient as well as the assistive devices needed to create an optimal workspace.

- Educational therapy: Its main goal is to teach patients how to live with their new conditions by establishing new ways to perform activities. The idea is that, in spite of their disabilities, patients will be able to participate in activities, such as educational classes, hobbies, family and community events.

- Recreation therapy: Its main goal is to encourage patients to perform sports and activities adapted to their capabilities. The idea is to obtain a more balanced lifestyle, more socialization and self-expression occasions.

- Psychological therapy: Its main goal is to work on shock, denial, depression, anxiety, and anger emotions that appear after SCI producing a negotiation and a finally adaptation in both the patients and the relatives. The possible treatments are the use of psychotherapy groups, and in severe cases pharmacological treatment. The improvement of all the previous therapies produce a improvement of psychological state of SCI patients and relatives.

In spite of the benefits of these traditional therapies, there are some limitations on the extend of recovery that have to be overcome by new strategies and technologies. These treatments focus on spinal cord repair using spinal nerve regeneration and remaining nerves enhancement, instead of focusing on the adaptation of patients to their new life. These researches are mainly focused on strategies of:

- Neuroprotection that protect the surviving nerve cells by using drugs and lowering the body temperature, which improve the functional recovery.

- Repair and regeneration of nerves based on the human intrinsic ability to repair and regrowth nerve cell projections that were lost after injury. This ability is stimulated by cell transplants, natural substances to promote re-growth, and bio-engineered growth scaffolds.

- Cell-based therapies that substitute damaged nerves by other types of cells such as stem cells that have are able to enhance neuronal growth and create new connections between cells.

- Neuroplasticity is the most promising SCI rehabilitation strategy. It is based on the idea that thanks to the retraining of spinal circuits, it is possible to enhance remaining nerve connections and create new ones recovering some body functions. The most common rehabilitation techniques that after repetition generate neuroplasticity improving voluntary muscle movement and coordination are [38]:

Physical rehabilitation: It is the most traditional strategy that repeat different strength, range of movement and stretching exercises to improve joint mobility and flexibility, as well as a neuronal plasticity.

Electrical stimulation: It is a very promising area that prevent muscle deterioration and maintain skeletal musculature in optimal conditions to regain standing and walking ability. In addition, there are promising results regarding the use of Deep Brain Stimulation (DBS) to decrease depression [45]. There are different types of electrical stimulation are explained in 3.2.5.

Neuroprosthetic brain-computer interfaces: It is based on the implantation of a device on motor cortex that thanks to Functional Electrical Stimulation (FES) activates implants located in the desired muscle groups once brain thinks about it. It is a functional control of movement bypassing the spinal

cord. Thanks to this technology, it has been possible to develop neural prosthetics that are artificial body parts that, thanks to a electrical device located on the remaining nerves, are activated each time these nerves have the intention to move a determined body part [49].

Robot-assisted training: This area is having a rising role in the functional recovery after SCI. Exoskeletons robots are devices used to extend the functional capabilities of patients; however, most of them have bot been established as a real solution because they only compensate the motor impairment but they do not take into account the individual necessities of each patient [49]. Most of exoskeletons are used in research, another big quantity are on the market, while only few of them have been officially approved as rehabilitative equipment [49] such as ReWalk, Ekso, and Indego.

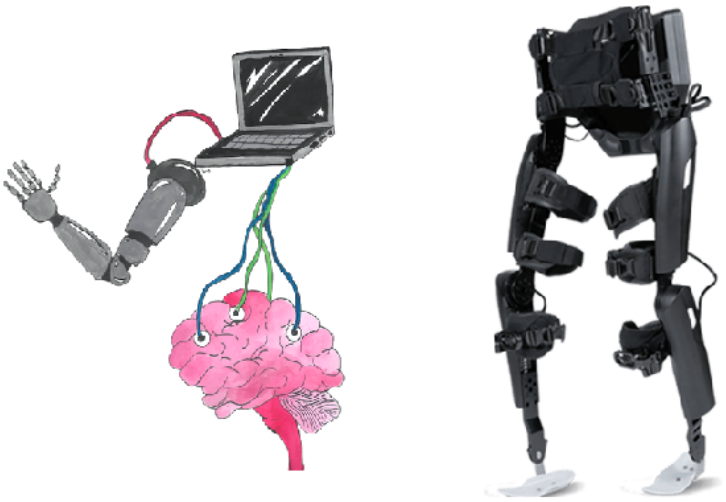

Figure 21: New technologies on SCI rehabilitation. Left image: Neuroprosthetic brain-computer interfaces. Right image: ReWalk exoskeleton robots officially approved as rehabilitative equipment. Retrieved from Google Images.

The duration of these approaches' effects depend on the functional state of locomotor circuitry during the application of therapy [44]. The longer-term effects, weeks or even moths, are reached when therapies are applied on step training therapy. In addition, there is a phase-dependent processing of inputs that allows the coordinated activation of motoneurons generating normal walking.

The future work related to these neuromodulatory approaches is to reduce the number of training session necessary to induce neuroplasticity, to make these technologies viable for their daily use, and to combine different approaches to optimize long-term effects of locomotor recovery in SCI patients [44]. Thanks to these combinations of therapies, the current expectations for locomotion recovery are extremely higher for SCI patients [44].

3.2.3 Neural plasticity in the spinal cord

One essential factor in the rehabilitation of SCI for the walking recovery is the already mentioned neuroplasticity [50]. Neuroplasticity is the CNS's ability to adapt and rewire its neural circuitry generating new and reorganizing existing synaptic connections as a response to learning, experience, or injuries such as SCI. Thanks to these adaptive changes in which spinal cord reassigns affected functions through remaining neural pathways, it is possible to relearn and recover body functions affected after injury, such as muscle contraction necessary to walk again [50].

As already explained, after a SCI, the interruption in the communication pathway between the brain and the rest of body can be complete or incomplete generating motor and sensor impairments [50]. The complete interruptions results in a complete loss of sensation an movement below the level of injury, while the incomplete interruptions where there are some remaining connections between the brain and areas below the level of injury resulting in a decreased but existing motor control and sensation below the injury level [50]. These remaining connections are essential for the SCI recovery because are the pathways that neuroplasticity uses to create adaptive changes. Therefore, only incomplete SCI patients benefit from neuroplasticity to recover the functions impaired, producing a **restorative rehabilitation**. However, the complete SCI patients only can focus on learning compensatory methods to be as functional as possible with their impairments and try to prevent further complications, this is **adaptive rehabilitation** [50].

There are three main components for promoting neuroplasticity after a SCI [50]:

- Specificity: it is important to apply a specific training depending on the desired motor recovery because each movement is associated to a specific neural pathway. Every time a specific activity-based therapy is performed, the demand for its specific motor function increases, performing a suitable rewire of spinal cord. This specificity is obtained by using activity-based therapies on treadmill or cycling machine.

- Repetition: it is based on the application of high repetitions of the training sessions in order to enforce the re-learning process of neuroplasticity. Everytime a movement is repeated, the neural pathways corresponding to that movement is reinforced becoming a really strong pathway. Once the pathway becomes strong, the movement associated with that pathway is performed easier and more natural.

- Intensity: the increase of training sessions intensity by means of modifications on resistance, speed, repetitions, duration accelerate the locomotor recovery. These modifications have to be performed progressively at each training session in order to constantly challenge the patient to stay engaged. However, intensity should not be extreme because if not patient is going to quit the therapy due to overwhelm and frustration.

The levels on neuroplasticity change during the recovery time period. During the acute phase of SCI, there is an increase in neuroplasticity levels because the spinal cord is trying to be stabilized. It is essential to take advantage of these increased levels by initializing the rehabilitation process as soon as possible. However, although the most recovery appears during the first months after the injury, neuroplasticity never disappear allowing the continuous functional recovery of patients as long as patients continue with the recovery programs.

Several promising therapies are under research in order to get treatments that activate this neuroplasticity in an efficient, specific and non-invasive manner. After SCI the interruption in supraspinal and segmental fibers result in a reduction or absence of reciprocal Ia inhibitory which produces a poor coordination in the contraction of agonist-antagonist muscles and therefore, walking impairments. Due to the importance of this inhibition for walking, the idea of neuroplasticity strategies is to create and reorganize synaptic connections in the reciprocal Ia inhibitory circuits in order to recover the voluntary and coordinated muscle movement necessary to improve walking ability in SCI patients [38].

This theory is the basis of the walking recovery strategy developed during this project. This strategy applies repeated and patterned electrical stimulation in the CPN to mimic the sensory inputs coming from Ia afferents during walking enhancing neuroplasticity of the involved neural pathways.

3.2.4 Gait training

During the whole life of healthy subjects there exist an activity-dependent plasticity in the spinal cord. The peripheral and brain inputs send orders to spinal cord regarding how to perform a determined movement, when these orders are sends continuously neuroplasticity starts to appear, learning how to perform during determined movements. This means that plasticity explained in previous section is essential in the acquisition and maintenance of motor function in healthy subjects [51]. For this reason, after the interruption on some spinal cord nerves in incomplete SCI that produce walking impairments, it is necessary to promote this neuroplasticity to re-learn and maintain motor skills.

One essential component to promote neuroplasticity is activity-dependent specificity, as explained in 3.2.3. The repetition of these specific training activate a operant-conditioning learning process that is based on a association between an activated spinal circuit with a determined effect, that can be an effective movement or pain. This means that spinal cord learn that each activation has a determined consequences and adapt the body to activate specific pathways related to the desired movement avoiding those that produce poor spinal connections generating neuropathic pain [51]. This neuroplasticity generates physical but also behavioral changes at supraspinal sites, demonstrating the multiple location of neuroplasticity that is essential to ensure the continuous application of previously acquired skills [16][52]. This theory is the basis to inspire new functionality restoration approaches after SCI.

Gait training is one of these activity-dependent training that induce plasticity and also maintain muscle properties allowing the walking recovery. There are different types of activity-depend gait training that when repeated induce neuroplasticity such as treadmill, weight-support exoskeletons such as Lokomat, and cycling training [42]. In addition to neuroplasticity induction, gait training is also needed to avoid the weakness and spasticity of muscles after SCI [52].

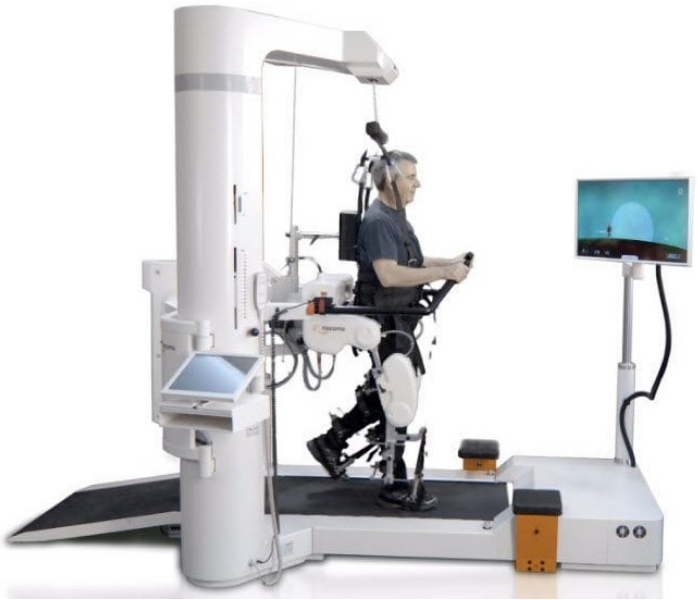

Figure 22: Gait training training using weight-support exoskeletons such as Lokomat. Retrieved from Google Images.

The idea of gait training is to recover different types of walking, one by one [52]. First, there is only non-ambulatory walking that is when patients only move thanks to a wheelchair. Then, exercise walking once or twice a day is necessary to eliminate wheelchair. The posterior walking recovery is household walking that is the ability to walk at home, while in the rest of situations use the wheelchair. Finally, the main goal is to recover community walking that is the ability of patient to walk at different situations of the life, in house and in the outside.

This training requires a lot of time and energy, for this reason the possibility to perform the therapy, its initialization, and its intensity depends on the capabilities of the patients. Depending on the phase of gait training, different assistive devices are used. During the early phase of injury and in the more severe cases, to improve balance and joint protection, different equipment are used such as parallel bars, exercises in a pool, robotic devices, and body-weight support devices. Posteriorly, once this early phase improve patient balance and strength, it is time for the patient to perform complete gait training by walking on the ground without any body-weight support device, just using assistive devices as braces in the required cases [52].

The achievement of the ultimate goal in gait training, recover walking ability in any community environment without the need of any assistive device, depends on factors such as: injury level, severity, time since injury, age, level of fitness, complications, level of sensation, spasticity, joint complications and pain [52].

It has been estimated that only 6.4% of patients who arrive at hospital with an SCI classified as ASIA A, at the time of hospital rehabilitation discharge are able to walk with some assistive device but no physical assistance from another person. This percentage increases until 23.5% for patients accepted as ASIA B, until 51.4% for patients accepted as ASIA C, and until 88.9% for patients accepted as ASIA D [52].

3.2.5 Electrical stimulation techniques for neurorehabilitation

There are different types of electrical stimulation therapies that when used independently or in combination with activity-dependent therapies, induce plasticity and improve the patient walking recovery. These therapies use an external electrical current to activate muscles or nerves generating action potentials that produce muscle contractions or spinal circuits modulations [4] and neuroplasticity. To estimate which parts of the body are more suitable for the application of electrical stimulation is essential to know that in humans there are different systems that control the movement, such as unconscious, brainstem, spinal, and sensory. Once these systems are electrically activated in SCI therapies, there can be reduction of pain, spasticity, and an improvement of locomotion in SCI patients [44][53].

Transcraneal Electrical Stimulation (tES): it is a non-invasive electrical stimulation of the brain cortex in order to alter neuronal excitability changing any brain function. The current delivered at this technique has not enough power to activate action potential and it only affects the cortical excitability and not deeper structures to ensure patient safety. This technique is sometimes replaced by TMS of the brain [54], a non-invasive brain stimulation that induce electrical current in a brain area by changing the magnetic field and produce more long-lasting effects.

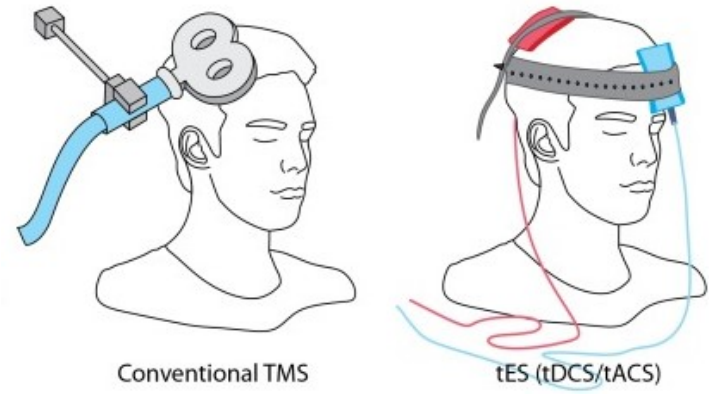

Figure 23: Brain stimulation therapies: tES and TMS. Modified from [55].

Transcutaneous Electrical Nerve Stimulation (TENS): it is a non-invasive peripheral nerve stimulation technique where electrical currents are applied to activate afferent neurons [56]. The activation of these afferent pathways generates spinal neuroplasticity, block the transmission of painful stimulus by other fibers, and stimulate the release of endogenous opiates and analgesic pathways. It is the most prevalent electrical stimulation technique to induce neural adaptations and modulate inhibitory spinal circuits such as reciprocal Ia inhibition resulting in a reduction of spasticity, pain [4], and improving balance and proprioception in SCI patients.

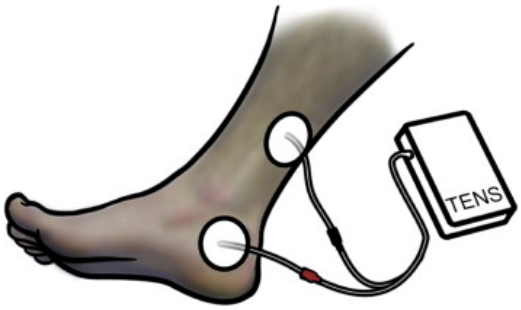

Figure 24: TENS therapy. Retrieved from Google Images.

Neuromuscular electrical stimulation (NMES): It is a non-invasive or invasive (depending on the location of electrodes) stimulation technique that activate the intact lower motoneurons or muscles in order to provoke a contraction of the affected muscle [4]. The idea of this stimulation is to relax muscle spasms, prevent muscle atrophy, increase blood circulation, maintain or even increase range of motion of joints, and re-educate the neuromuscular system after neural injury accelerating the muscle recovery of SCI patients.

Functional electrical stimulation (FES): it is an non-invasive or invasive if electrodes are implanted on desired muscle, electrical stimulation to activate necessary and weak muscles to perform a determined activity such as eating, gripping, writing, standing, stepping, or walking [4]. In addition to muscle, it can also stimulate organs in order to improve cardiorespiratory system or bowel and bladder systems. This increment of organs/muscle mobility and activity, reduce the organs/muscular atrophy and the continuous decrement of fatigue resistance after SCI. It is a very effective rehabilitation strategy because it turns contractions into the desired functional movements enhancing the restoration of neuromuscular, sensory, and autonomic systems.

Spinal cord stimulation: it is an invasive electrical stimulation of different parts of spinal cord with the idea to help in walking recovery and manage chronic pain of neural conditions such as SCI [44]. The first strategy is the most used one and it is composed of low-intensity stimulation of epidural surface that activates the CPGs circuits [44]. The second strategy is the direct generation of muscle movement applying a supra-threshold stimulation in the motoneurons. However, as it overpass the spinal inhibitory circuits, there is not a coordination between agonist and antagonist muscles necessary during walking.

Several studies, such as the ones explained in 2.3, have analysed the effects of these stimulation therapies, in isolation or in combination with activity-based strategies such as treadmill or cycling, on the walking recovery of neuropathological patients. The conclusion is that the application of electrical stimulation in SCI patients, is necessary to obtain effects such pain reduction, spasticity reduction, and changes in muscular power. All these changes induce the walking recovery as well as the performance of daily living activities, which improve the patients' quality of life and their re-integration on society.

4 Materials and methods

4.1 Experimental protocol: Gait neuromodulation platform design and development

The idea of this thesis, as explained in Section 2.3, is to create a gait neuromodulation platform based on the application of afferent electrical stimulation on CPN during the different phases of GC to induce neuroplasticity of reciprocal Ia inhibition.

The design of this platform is based on three different processes. First of all, an 'off-line' analysis that allow to distinguish the different phases of GC while analysing the EMG activation patterns of TA and SOL muscles at each of these phases. This analysis helps to affirm that the phase in which stimulation is applied to the CPN innervating the TA corresponds to the activation or deactivation of this muscle. The second process is the afferent stimulation intervention during a treadmill training session of 10 minutes. The CPN is stimulated during swing or stance phase of GC producing and 'In-phase' or 'Out-phase' stimulation strategy. There are also control sessions in which stimulation to CPN is not really delivered to check effectiveness of stimulation. The third and final process is the SOL H-reflex analysis to evaluate the effectiveness of afferent CPN stimulation on modulation of reciprocal Ia inhibition.

Once the design and development of this platform are properly performed, it can be applied to healthy subjects. If the results regarding modulation of reciprocal Ia inhibition are promising, this platform could be established in a future as a therapy to recover walking ability after SCI.

4.1.1 Equipment

In order to develop this designed platform is necessary the application of different devices, as well as its proper manipulation. The required devices to create the gait neuromodulation platform are, in order of appearance during the experiment:

Treadmill: The treadmill used during the whole project is the Domyos TC 450 treadmill. It is used during the previous gait analysis and also during the application of phase-dependent afferent stimulation at treadmill training. This device has different modes to be used; however, in this experiment only natural velocity walk depending on the comfort of the patient is required, so any treadmill could have been used.

Figure 25: Domyos TC 450 treadmill.

Force Sensitive Resistor (FSR) sensors: These force or pressure sensors (FSR UX 400 Series, 402, Interlink Electronics, California, USA) are placed on the sole of the foot, one on the heel and other on the tip. These sensors are connected to the microcontroller and this one with the EMG amplifier "Quattrocento", that will be explained later. These connections allow the visualization of the signal acquired by the FSR and therefore, the correct detection of the different phases of the GC.

Figure 26: FSR UX 400 Series, 402.

Recording and stimulation electrodes: On the one hand, to record muscle activity, surface electrodes of 22x35mm, that allow to record in a non-invasive way are needed. In the case of this project, the activity of two muscles is recorded, the TA and the SOL muscles. The placement of these electrodes follows the indications of the Surface ElectroMyoGraphy for the Non-Invasive Assessment of Muscles (SENIAM) project. On the other hand, surface stimulation electrodes of 3.2cm of diameter are also needed, both for afferent stimulation of CPN and PTN, to deliver the stimulation needed to analyse the SOL H-reflex. The location of these stimulation electrodes and the intensity of stimulation provided by them is chosen using a bar electrode. In the case of this project, recording electrodes are from Nissha (USA) while stimulation electrodes used are from Axion (Spain) and Prim Physio (Spain). However, any supplier of surface electrodes is valid.

Figure 27: Recording and stimulation electrodes. From left to right: Recording surface electrodes, stimulation electrodes, and bar electrode.

Microcontroller: The Arduino UNO microcontroller (Arduino LLC, Italy) has different functions during this experiment. The first function is to perform the 'off-line' gait analysis prior to the stimulation intervention itself. Once Arduino receives information from the FSR sensors regarding the phases of the GC, it sends this information to the EMG amplifier 'Quattrocento' for the proper visualization of gait phases in combination with each muscle activation. Its second function is controlling the stimulation intervention based on the phase information it receives from the FSR sensors. Once it receives information related to the phase of GC, it sends it to the computer that is in charge of controlling the stimulation device. Finally, it is also used during the SOL H-reflex analysis since it will be responsible for sending orders to the computer that controls the stimulation platform, indicating when to perform test or conditioning stimulation.

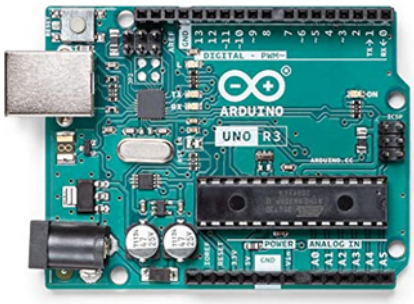

Figure 28: Arduino UNO.

EMG amplifier: The EMG amplifier used in this experiment is the Quattrocento (OTBioelettronica, Milano, Italy) that uses a sampling rate of 1000 Hz. This device is capable of measuring 400 channels of EMG, plus auxiliary input channels that will be used to align muscle activation signals with the respective phases of the GC. This amplifier has two fundamental functions during the project, receive the muscle activation information during the 'off-line' gait analysis and during the SOL H-reflex analysis prior to and post afferent stimulation intervention.

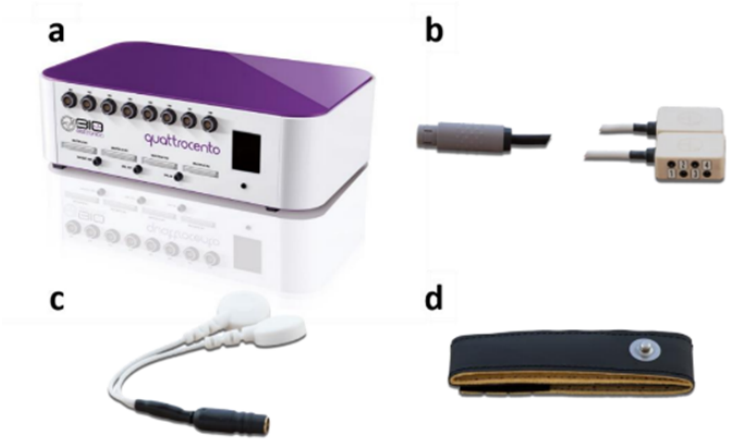

Figure 29: EMG Quattrocento amplifier and its accessories. (a) Quattrocento, (b) 16 channel bipolar adapter jack connector, (c) concentric pressure adapter and (d) wristband to which reference electrode is connected.

Afferent electrical stimulation device: For the stimulation intervention, RehaStim 1 (HASOMED GmbH, Mageburg, Germany) is used. It is an 8-channel commercial biphasic stimulator. It has an execution mode that allows the user to control the device through a computer via USB communication, and you can specify both the amplitude of current stimulation, as well as the width of the pulse and frequency required. This will be very useful as the intensity of stimulation changes according to each participant motor threshold.

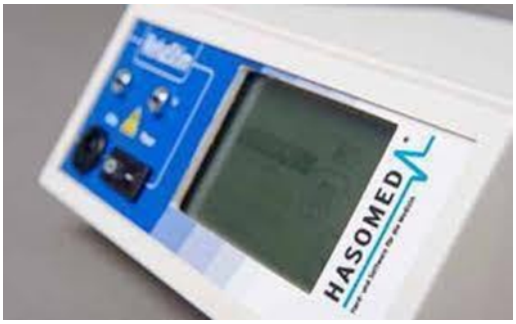

Figure 30: RehaStim.

Direct current stimulation device: Two different direct current stimulators are used for the study of SOL H-reflex. On the one hand, the DS8R Digitimer that is used to generate the test stimulus on SOL muscle, stimulating the PTN; and on the other hand the DS7A Digitimer that generates the conditioning stimulus between the TA and SOL muscles, stimulating the CPN and the PTN at different time intervals. These are discrete, multimode, direct current and isolated pulse stimulators for use in human subjects in a research environment. Both safely provide short-lived current pulses (50-2000 μs) for TENS and activation of nerves and muscles through surface electrodes. In addition, they have an execution mode that allows the user to control the device through a computer via USB communication, and you can specify both the amplitude of current stimulation, as well as the width of the pulse and frequency. This will be very useful as the intensity of stimulation changes according to each participant motor threshold.

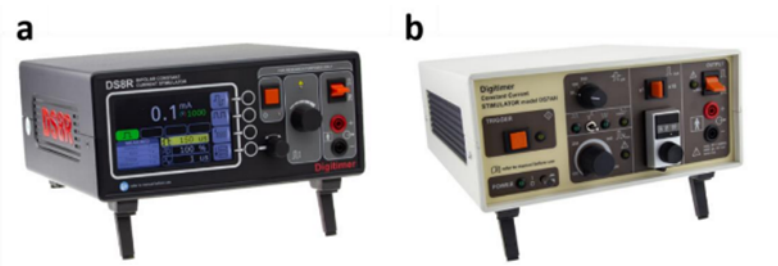

Figure 31: Direct-current stimulators. a) Digitimer DS8R y b) DS7A

Personal computer: The computer used to extract, control and analyse the data coming from all the processes of this experiment is a personal Lenovo IdeaPad 3 15ITL6. One of the software used during the experiment in this computer are the OT Biolab+ software that allow the visualization of signals such as EMG and FSR signals. Then, the Arduino IDE is used to connect FSR sensor signals with OT Biolab+, to control Rehastim stimulator thanks to these FSR sensor signals, and to also control the sequence of test and conditioning stimulation necessary for SOL H-reflex analysis. Finally, programming platforms such as Matlab and Python have been used to analyse, visualize and represent the results obtained during this experiment.

Graphical User Interface (GUI) of MatLab: The MatLab platform is of especial interest because the GUI used during this experiment is a data processing tool developed with MATLAB by the NRG of the Instituto Cajal. This tool allows us to import, process and display recorded or pre-recorded EMG data, as well as extract and evaluate H-reflex components. This graphical interface is designed for the use of inexperienced users, but also includes specific variants that allow us a deeper and more advanced analysis.

Figure 32: GUI example for the visualization of reflexes.

4.1.2 Platform characteristics

Once all the equipment is prepared, it is necessary to connect properly the devices in order to obtain the suitable systems. Depending on the phase of the project, three different systems are going to be created.

During the 'off-line' gait analysis, the idea is to measure the activity of two antagonist muscles during the GC, the plantarflexor TA and the dorsiflexor SOL muscles. In order to know at which phase muscle is activated is necessary to automatically differentiate the phases by using two footswitch or FSR sensors, one in the toe and another in the heel.

This first system created is the easiest one as it is the one with less number of devices. First of all the subject is walking normally in the treadmill, at the most comfortable speed for him/her. While walking, on the one hand, the sEMG electrodes positioned in the TA and SOL sends the information though a cable united to 'Quattrocento' EMG amplifier using all the Figure 29 devices. On the other hand, both footswitch placed on the sole of the foot, send pressure information to Arduino microcontroller. This microcontroller is connected to the computer by an USB-C cable that provides power to the Arduino circuit. Then, Arduino connects to the AUX inputs of the EMG amplifier through BNC cables. Once all the information is on the EMG amplifier, it connects to the computer through a USB cable. Using the OT BioLab+ software, it is possible to visualize in a synchronous way both EMG signals and the pressure signals provided by the FSR sensors. This visualization helps us to understand how muscle activate during each GC and compare if experimentation coincide with literature where it says that TA activates during the swing phase, while SOL activates during the stance phase.

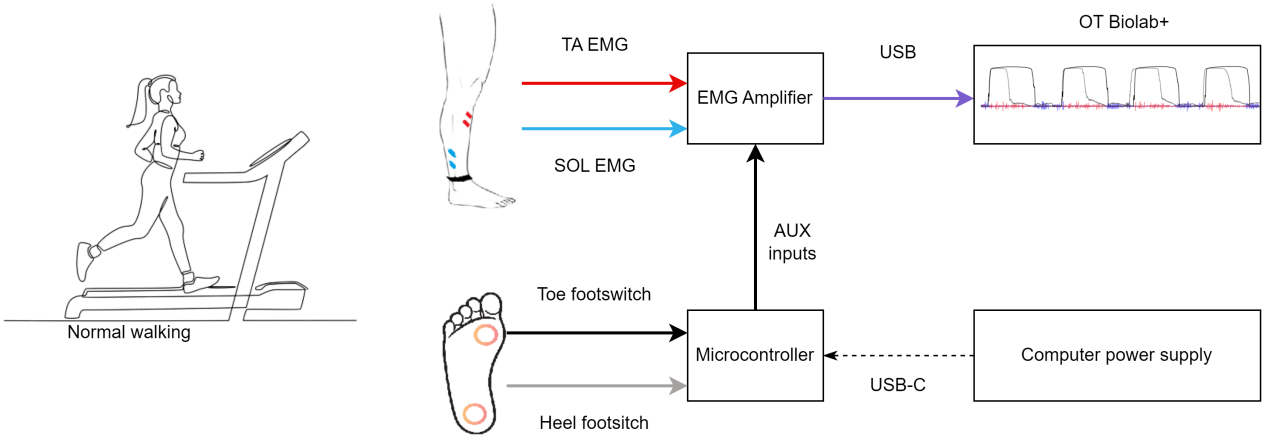

Figure 33: Diagram representing the 'off-line' gait analysis system.

During this process there is a complete synchronization between EMG signals and footswitch signals arriving to the amplifier. This perfect synchronization between all the signals received in the amplifier is possible thanks to the IN and AUX inputs that receive the amplifier without any time delay. In addition, the OT Biolab+ software includes a huge quantity of configuration and visualization options, for this reason it is essential to get familiar with this platform. First thing to do in this platform is to adjust the configuration determining which of all the channels are going to receive data and which type of data is entering, EMG signals, force signals, pressure signals... Then, it is necessary to adapt the visualization screen depending on how the signals have to be displayed, the order, the amplitude, the frequency...this will create the proper representation of the data. Once the data is acquired, this software gives to opportunity to process data and also record it in order to posteriorly save in the local computer. It is a very useful software as once you record and save any signal, the posterior processing can be performed at any time of the experiment.

The following process during this experiment is the application of afferent stimulation on the CPN. This nerve, as explained before, is the one innervating the plantarflexion muscles such as TA. The stimulation is going to be applied at three different strategies; first of all an In-phase stimulation that is performed when CPN is stimulated during the TA contraction which is during the swing phase, second strategy is Out-of-phase that is the stimulation of CPN while the TA muscle is deactivated which is during stance phase, and finally the Control stimulation in which there is not stimulation at all just to see if there are differences between stimulation and not stimulation.

The idea is that when stimulating the CPN with a low-intensity stimulus, the afferent fibers of TA are activated making the action potential to travel though these fibers to the spinal cord. Once in the spinal cord, the action potential activates the inhibitory Ia interneurons. If these interneurons are activated, they inhibit the activation of antagonist muscle motoneurons. This inhibition is the key point of reciprocal inhibition in humans, when an agonist muscle such as TA is activated, its antagonist muscle in this case SOL is deactivated thanks to inhibitory Ia interneurons. In SCI patients there might be problems regarding this inhibition because in damaged spinal cord, there is not a proper activation of interneurons, inhibiting the deactivation of antagonist muscle. The idea of this intervention is to analyse this treatment in healthy subjects, as a first step to develop a therapy that helps to reactivate this inhibitory path. Ultimately, the goal is to avoid the co-contraction of antagonist muscles in pathological subjects.

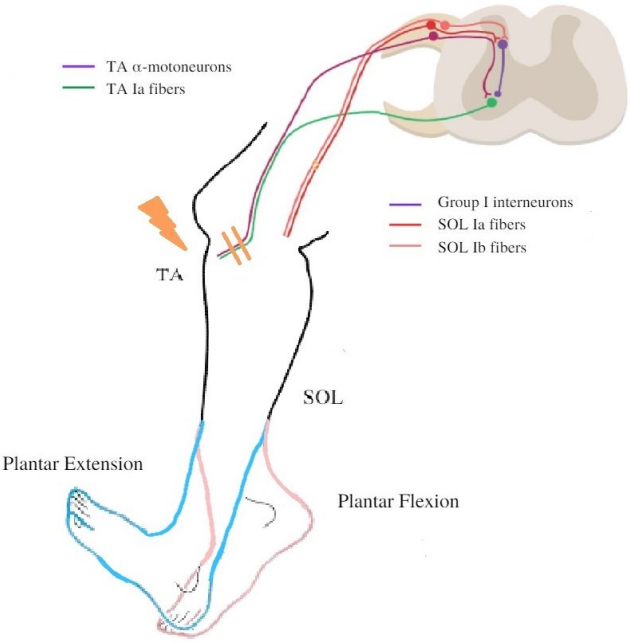

Figure 34: Reciprocal inhibition pathway during walking. Modified from [57].

To perform this process, the system created is more complicated than the previous one. The stimulation is applied during the normal walking of the subject. In this case, there are recording electrodes that measure the activity of antagonist muscles previously mentioned, the same two footswitch on the sole of the foot as explained previously, and now there are stimulation electrodes positioned over the CPN.

Both footswitch are connected to the Arduino microcontroller which is in charge of capture the data regarding the pressure exerted on these footswitch. This data is send from Arduino to Matlab codification where a programmed script controls the pressure data and automatically differentiate between swing and stance phases of GC. Depending on if stimulation is wanted to be delivered on swing or stance phase, the Matlab Software is connected to RehaStim stimulator to send to order of activation. If Matlab send the order to stimulate on swing phase, the RehaStim will send a low-intensity stimulus to the CPN during the swing phase, when foot is on air; however, if the order is to stimulation on stance, RehaStim will send the same stimulus to CPN during the stance phase, when foot is in contact with ground. At the same time, EMG signals are being recorded to evaluate if the stimulation is delivered properly. The EMG amplifier receives the signals from surface recording electrodes and from footswitch and represent it on the OT Biolab+ software. Thanks to this representation, it can be checked that In-phase stimulation is properly delivered to the CPN when TA is activated it means, during swing phase. In the Out-of-phase stimulation, the CPN has to be stimulated when TA is deactivated, it means during the stance phase.

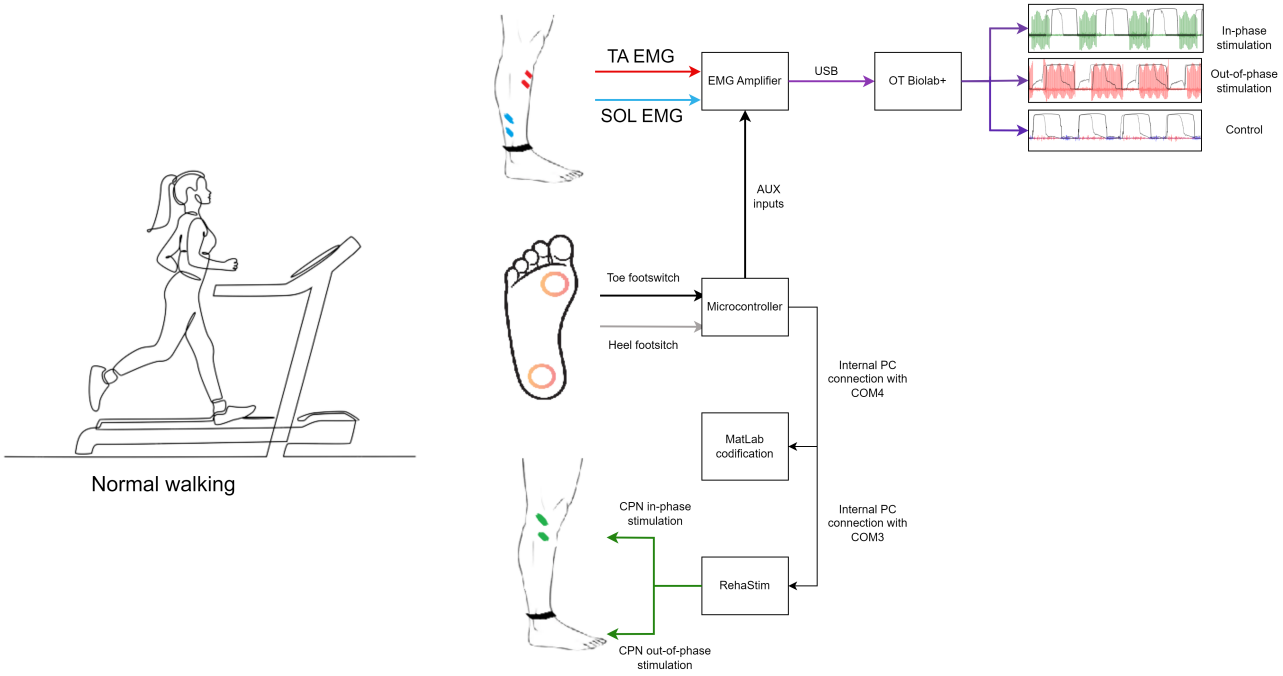

Figure 35: Diagram representing the afferent stimulation intervention system.

Finally the last and most complicated process in terms of both, design of the platform and evaluation of the process, is the evaluation of SOL H-reflexes. The idea of this process is to measure the H-reflexes previous to the afferent stimulation intervention and posterior to the intervention to conclude if it has been effective. The H-reflexes are analysed by performing test and conditioning stimulus. The test stimulus occurs when stimulus is only delivered to the nerve innervating the SOL muscle, the PTN, while the conditioning occurs when stimulus is first delivered to CPN and some milliseconds later to the PTN. The hypothesis is that conditioning stimulus should elicit a smaller SOL H-reflex than test stimulus because as explained before, when TA is contracted the SOL relaxes. In addition, this difference between conditioning and test stimulus have to be even more increased after the afferent stimulation intervention where a strengthening of reciprocal Ia inhibition has occurred. If this hypothesis is proved, it could be concluded that afferent stimulation is a good intervention to modulate reciprocal inhibition in the spinal cord strengthening the coordination between antagonist muscles.

This system is the most difficult one as it the one that entails more devices and more difficult connections and synchronization between them. In this case the patients are seated in a chair with the feet in a footrest, it means with the knee angle around 135º. In the leg of patient there are surface recording electrodes that record the activity of TA and SOL muscles. Then, there are also stimulation surface electrodes in both nerves, CPN innervating plantarflexor and PTN innervating dorsiflexor. The EMG amplifier GUI establishes the required parameters for the test-conditioning protocol, determines the sequence of stimulus applied, and the time interval between stimulus. These parameters are send to the Arduino microcontroller that carry these orders to the Digitimer stimulators, DS8R and DS7A. These devices perform the stimulation of the nerves in the way has been established, when there is a test stimulus the DS8R only stimulates the PTN as represented by the orange arrow in the Figure 36, and when there is a conditioning stimulus both stimulators activate both nerves at different coordination and times as represented by the green arrows. Both stimulators are connected to EMG amplifier by BNC cables, in order to synchronize the stimulus trigger with the data recorded by the surface electrodes. These information acquired by the amplifier is then visualize and save on the computer thanks to the USB connection between EMG amplifier and GUI of MatLab. These GUI allow to visu-

alize the SOL H-reflex each time a stimulus is delivered to any of the nerves, performing in this case a comparison between test and conditioning stimulus, pre and post afferent stimulation intervention.

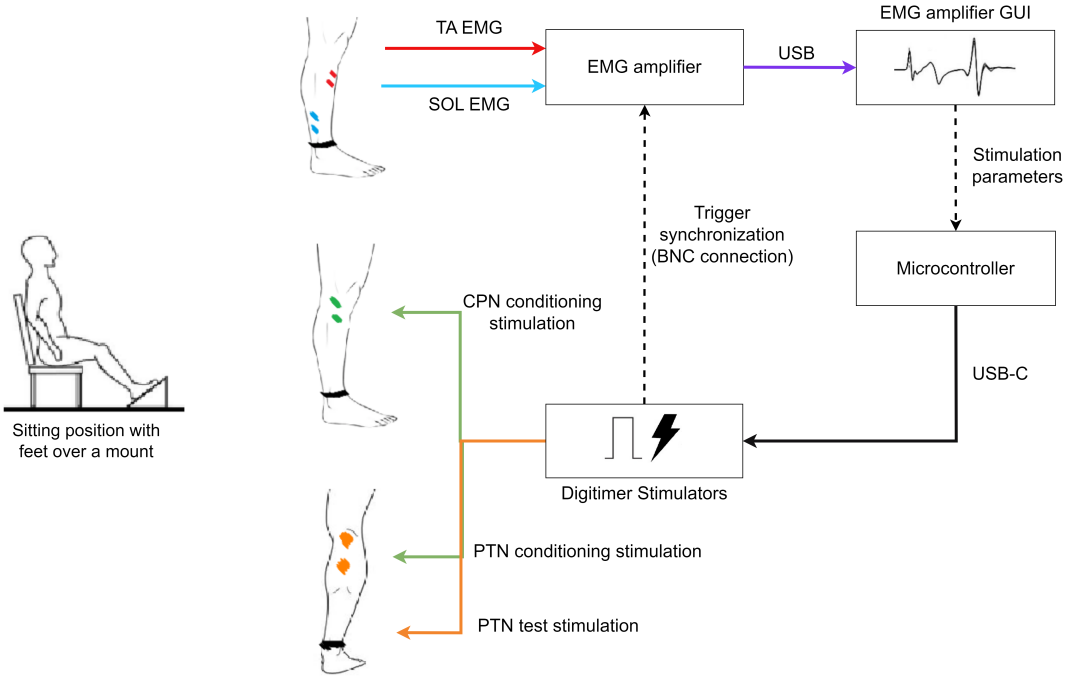

Figure 36: Diagram representing the spinal excitability testing system.

4.1.3 Bio-inspired stimulation strategy

The idea of this project is to apply the afferent electrical stimulation at different GC phases. The reason is that, according to the literature presented in 2.2, when afferent stimulation of CPN nerve innervating TA is produced while this muscle is activated (swing phase), the effect of stimulation on the strengthening of reciprocal Ia inhibition is stronger. However, if the afferent stimulation of CPN is applied when this muscle is deactivated (stance phase), the strengthening of reciprocal Ia inhibition will be lower or absent. This is the reason why, it is said that this stimulation follows a bio-inspired strategy, because it depends on the agonist and antagonist muscle activation.

The first step when performing this strategy is to check if the theory regarding muscle activation during GC, explained in 3.1, is real. For this reason, the 'off-line' gait analysis is performed at the beginning of the experiment. The signal visualization presented in Figure 37 is the result obtained at the OT BioLab+ software once TA and SOL muscle activity, as well as toe and heel footswitch data are recorded.

In this figure the first row correspond to the SOL muscle activity and the second row to the TA activity, both measured in millivolts. The last two rows of the figure correspond to the toe and heel footswitch signals, correspondingly. These footswitch signals are measured in volts because in the EMG amplifier, when using the AUX channels, the A/D converter input range is $0 \div 5$ V, and the gain factor is 0.5 V/V since the inputs accept $-5 \div 5$V dynamics. This is better explained in OT Biolab+ User Manual or in configuration instructions [58]. When any of the footswitch is activated, it means there is stance phase because the foot is in contact with the ground; however, if none of the footswitch is activated, there is swing phase because the foot is completely on air.

The global visualization of these four signals makes it easy to understand the pattern of activation of each muscle at the different phases of GC.

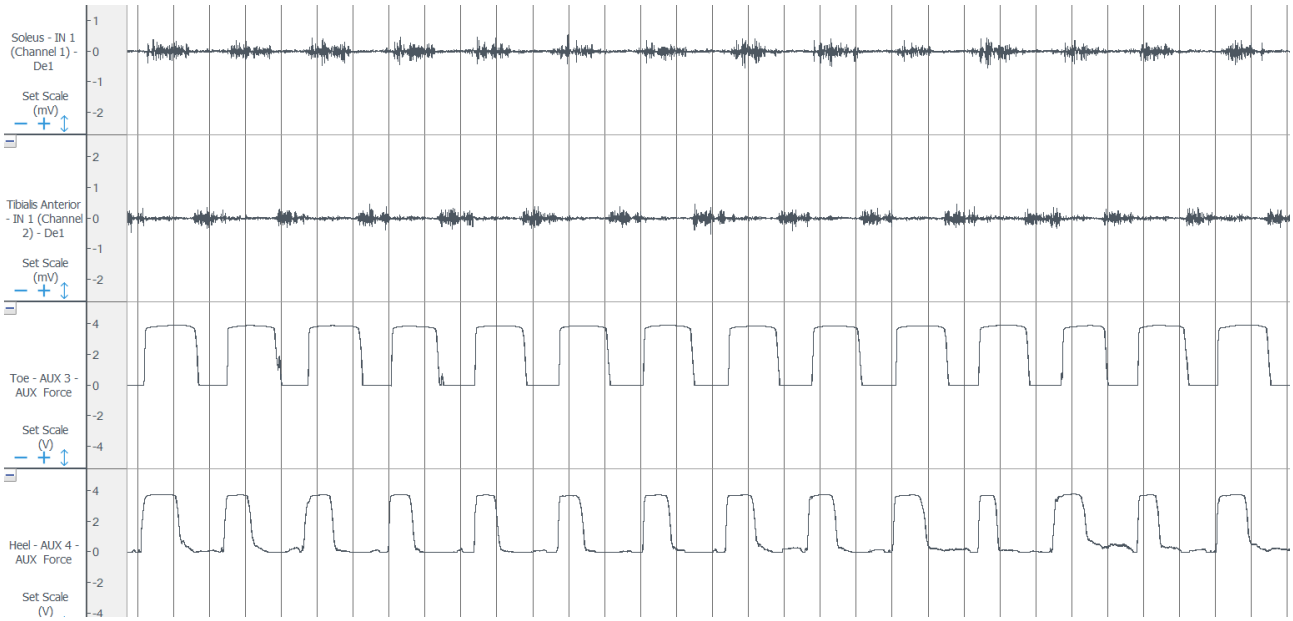

Figure 37: OT Biolab+ screen representing SOL activation (row1), TA activation (row2), toe footswitch signal (row3), and heel footswitch signal (row4) over time during several GC.

However, when typically analysing the muscle activation during the GC, it is better to create a representation of the mean envelope of each muscle during each GC instead of over the whole time. For this reason, a processing is performed over these acquired signals, dividing all the time period into GC and generating the envelope of the EMG signal at each GC. The processes applied to a EMG signal to obtain its envelope are: perform the periodogram of the signal, apply a band pass filter with a high frequency of 450Hz and a low frequency of 20Hz, apply a notch filter, demean the resulting EMG signal, rectify it, and finally apply a low pass filter of 5Hz to this resulting signal. Once the envelope of each cycle is created, it is necessary to interpolate and generate a mean signal. The resulting signal is the represented in Figure 38 where the EMG envelopes of each GC are represented as black lines and the mean envelope of all of them is represented in red line. This process has been done for both muscles, SOL and TA, as it can be seen in the representation.

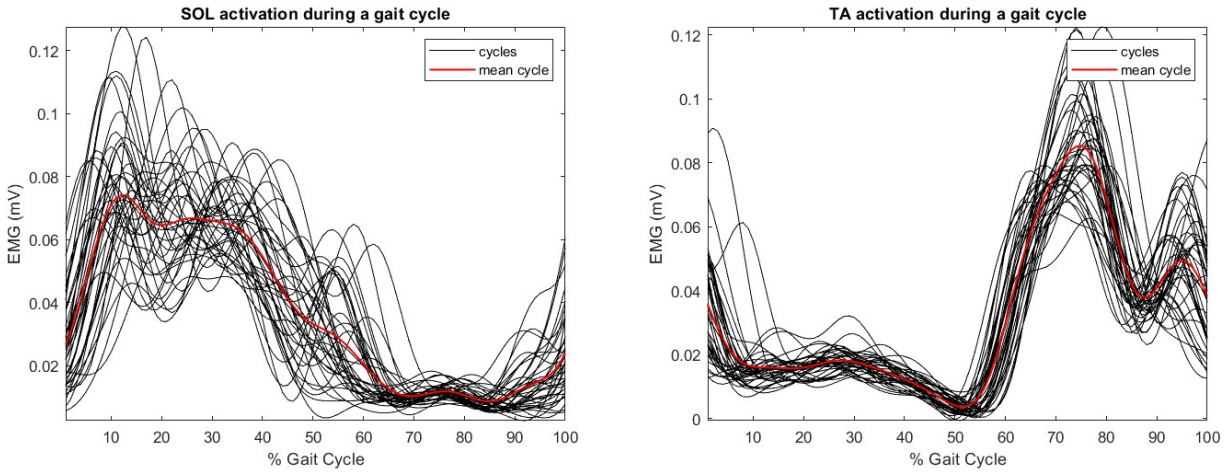

Figure 38: Muscle activation during GC, where black line represents each of the envelopes and red line represents the mean envelope for a GC. The left image corresponds to SOL activation during GC while right image corresponds to TA activation during GC.

Thanks to this analysis it can be determined at which phase each muscle activates. In this way, three different strategies of afferent stimulation can be applied as it is observed in Figure 39. First of all, an In-phase strategy where low-intensity stimulus is applied to CPN when its innervated muscle, TA, is activated. This means that stimulation is delivered during the swing phase of GC. Then, a Out-of-phase stimulation where stimulus is delivered when this same muscle is deactivated, stance phase of GC. Finally, a Control stimulation in which electrical stimulus is not delivered, and it is only used to see if there are real changes between outcomes after stimulation and outcomes without stimulation.

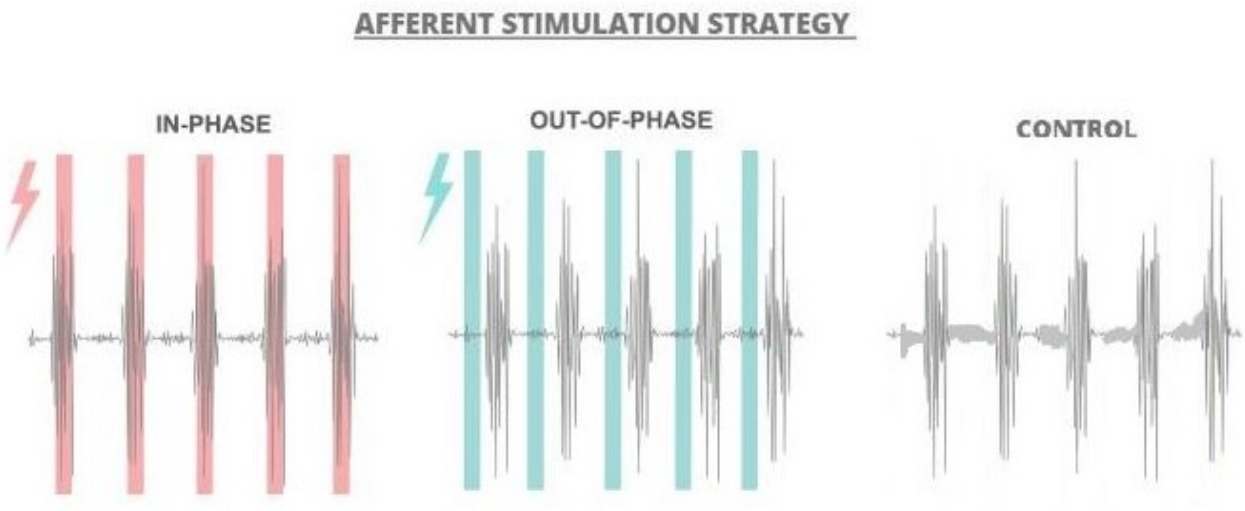

Figure 39: Types of afferent stimulation strategies performed during experiment. Retrieved from [57].

This is the reason why this stimulation is a bio-inspired strategy, because it is based on the activation or deactivation of muscles such as the TA in order to deliver the low-intensity stimulus. In these types of strategies is essential to automatically identify and determine the gait phases to apply the stimulus at the suitable time. In case that stimulation was not applied exactly at swing or stance phase, the experiment would be useless.

Theory and studies presented during the first part of the thesis claim that when stimulation is applied at the swing phase, when muscle is contracted, there is a major improvement of reciprocal inhibition because there is a extra powerful activation of Ia afferent fibers reaching the inhibitory Ia interneurons; therefore providing a stronger inhibition of SOL motoneurons. In this way, when the stimulation is delivered at swing phase, the antagonist SOL muscle relaxes even more generating an improvement of reciprocal inhibition between antagonist muscles during walking.

The idea of using these three strategies is first check if afferent stimulation intervention has any effect on reciprocal inhibition compared to control strategy; then, check if literature conclusions are correct and there is a higher improvement in reciprocal inhibition when stimulation is applied during the swing phase of GC.

4.2 Afferent stimulation intervention in healthy subjects

Once the design of the experimental protocol has been created and the set-up is completed, it is time to apply the protocol into healthy subjects. It is necessary to first use healthy subjects in order to see if the afferent stimulation intervention gives the hypothetical results regarding modulation of reciprocal Ia inhibition. In case the results coincide with literature and the hypothesis of the project, the next step will be to adapt the protocol to the application of afferent stimulation intervention in SCI patients to improve their reciprocal inhibition and in this way their walking recovery.

4.2.1 Subjects

The participation in this experiment is completely voluntary; however, it is important to reach a minimum sample size of 10 participants in order to obtain representative, accurate results, and perform relevant descriptive statistics. However, to participate in the experiment there are some inclusion and exclusion criteria that have to be met. Regarding the inclusion criteria is essential to present:

- No motor or neurological injury.
- Do not have skin problems such as sores that prevent the use of equipment.
- Age between 18 and 60 years old.
- Height between 150 and 195 centimeters.
- Weight maximum 100 kilograms.
- Ability to follow instructions well and demonstrate learning skills.

However, the most important criteria is the exclusion criteria because if any of the participants present any of these characteristics previous or during the experimentation, they are completely excluded from the experiment. This criteria for exclusion is:

- Pregnant woman, breastfeeding or postmenopausal.
- People suffering from epilepsy.
- Have been hospitalized due to a heart attack, surgery or acute heart failure within 3 months prior to study adherence or the presence of major cardiovascular disease or deep vein thrombosis in the lower extremities.
- People who have a pacemaker in their heart.
- People who have a peripheral nerve injury.
- Persons who belong to or have some connection with the research group.

Considering these criteria, for performing this experiment, 20 healthy subjects were recruited; 11 women participants and 9 male participants. None of them have signs or symptoms of neurological disease. The age range was between 19 years for the youngest participant to 56 years for the oldest participant, with a mean age of 27.9 years. These twenty participants were divided into three groups depending on the stimulation intervention applied to them, eight participants were introduced into In-phase stimulation, other eight participants into Out-of-phase stimulation, and the other four participants received the Control strategy. All these participants voluntarily decided to participate into the experiment.

All volunteers were given all the necessary information, both verbally and in written form. Once all doubts were clear, they signed an informed consent form giving their consent to the experimental procedures before participation to the study. The experiments were performed in accordance with the Declaration of Helsinki (1964), and both the experimental protocol and its application on healthy subjects were approved by the ethics committee of the CSIC and NRG.

The experimental sessions assessed the effects of a 10 min afferent electrical stimulation paradigm, having the total experimental session an approximate duration of 3 hours.

4.2.2 Protocol

The experimental protocol is divided into five different processes that are: Preliminary gait analysis, pre-assessment of H-reflex, afferent stimulation intervention, post-assessment of H-reflex, and post 30 minutes assessment of H-reflex. These processes are conducted in the order illustrated in Figure 40.

In this section of the thesis, only experimental protocol is going to be explained including preliminary gait analysis and afferent stimulation intervention itself. The evaluation of the effectiveness improving reciprocal Ia inhibition thanks to this technique, it means the assessment of SOL H-reflex, is going to be explained posteriorly in Section 4.2.3.

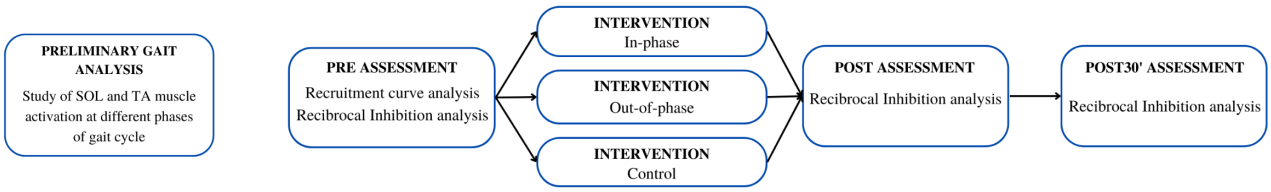

Figure 40: Protocol followed during the experiment.

The first part of the study is the recruitment of subjects following the inclusion and exclusion criteria listed in 4.2.1. Before the start of the experiment, participant should receive information on the structure, duration, and other significant and relevant aspects of the experiment. Once the participant decides to participate in the experiment, he/she must be asked to sign informed consent form. Once participant has signed the consent, the experiment can begin.

The first step of the experiment is the preparation of the set-up explained before as well as of the software necessary for the complete process. All the devices required have to be turned on and properly connected as in the diagrams explained in 4.1. The next step is to prepare the four Surface Electromyography (sEMG) electrodes, the four stimulation electrodes, the reference ankleband, and the bar electrode.

Muscle activity is recorded during all the processes of this experiment, for that reason it is necessary to connect the cables of the sEMG electrodes to the Quattrocento amplifier. Those cables recording SOL activity are connected to channel 1 of amplifier, whereas the ones recording TA activity connect to the channel 2 of amplifier. The toe and heel footswitch cables are connected to Arduino that thanks to a defined electrical circuit provides the necessary power and resistance to make the footswitch work. From this Arduino, toe and heel footswitch are connected by BNC cables to the AUX 3 and AUX 4 channels of the amplifier, correspondingly.

The next step and one of the most important ones is the placement of sEMG and stimulation electrodes on the patient right leg. The first step is the preparation of the skin where participant has to be shaved, if necessary, and the skin cleaned with abrasive alcohol.

When placing the bipolar sEMG electrodes is necessary to find the TA and SOL muscles by asking the patient to perform plantarflexion and dorsiflexion correspondingly. These electrodes are placed following the SENIAM recommendations [59] as illustrated in Figure 41. For positioning electrodes in the SOL muscle it is necessary to have patient sit with the knee approximately 90º flexed and the right foot on the floor. In this position, when patient is asked to lift the heel from the floor, it is the best moment to find SOL muscle. The electrodes are then placed at 2/3 of the line between the medial condylis of the femur to the medial malleolus with a electrode distance of 20mm. In the case of TA electrode positioning, it is necessary to have the participant in supine or sitting position. In this position, when participant is asked to perform dorsiflexion of foot, it is the best moment to find TA muscle. The electrodes are then placed at 1/3 on the line between the tip of the fibula and the tip of the medial malleolus with also an electrode distance of 20 mm. Once the electrodes are positioned, it is necessary also to place the wet reference ankleband and some adhesive tape in order to fix better the electrodes and reduce the environmental artifacts. The electrode positioning varies a lot depending on the patient anatomy and physiology, being easier to locate them in young and athletic participants.

Figure 41: SENIAM recommendation for sEMG electrode position when measuring SOL (left image), and TA (right image) muscle activity. Retrieved from [59].

In the case of stimulation electrodes, there is not a guide to know is exact location as with the sEMG electrodes. However, most of previous experiments have a similar electrode position in their protocol. First of all, it is necessary to use the bar electrode in order to properly search for the point of the leg that have the highest effects to stimulation. The feeling of the patient when stimulation is applied must be the feeling of the current travelling through the body but it must not be painful or harmful. It is important to remember that the current always goes from the anode to the cathode, and that in stimulators the anode corresponds to the red socket or red cable, while the cathode corresponds to the black socket or white cable, following the Digitimer manuals.

When bipolar stimulation is applied to the CPN innervating the TA muscle, the stimulation or cathode electrode (-, white electrode) is placed over the nerve between the fibular head in the lateral side of the leg and the TA muscle belly. The anode electrode (+, red electrode) on a neutral part of the same limb, 3 cm above the cathode one. This point or location of stimulation electrode has to be the one that when high intensity is applied eliciting the TA Mmax, there is no a co-activation in the M-wave of peroneal muscles.

In the case of stimulating the PTN innervating the SOL muscle, the stimulation/cathode electrode (-, white electrode) is placed as medial and as distal in the popliteal fossa as possible, in order to reduce the risk of activating afferent fibres in the CPN. Whereas, the anode electrode (+, red electrode) is placed 3 cm below this cathode electrode, in the neutral part of the posterior leg. Once the electrodes

are positioned, it is necessary also to place some adhesive tape in order to fix better the electrodes. In addition, as the PTN is a very deep muscle in the fossa poplitea, and it is necessary to stimulate only this nerve and not others around it such as gastrocnemius nerves, an extra strap is usually require to be more precise in the stimulation of this nerve. The Figure 42 shows the final location of all the electrodes, sEMG and stimulation electrodes in the right leg of a participant.

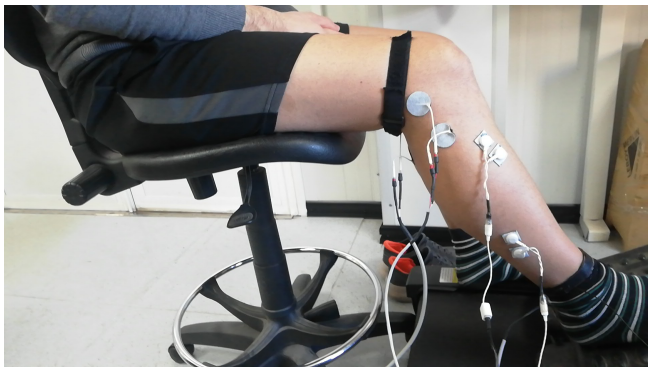

Figure 42: Final electrode position during the experiment protocol.

One surface electrodes are positioned, is also time to locate the toe and heel footswitch in the sole of the right foot. Due to the sensitivity of these sensors, it is essential to fix them in order to prevent their damage or even rupture. Once, electrodes and sensors are positioned is necessary to check the quality of both EMG and footswitch signals before starting with the preliminary gait analysis.

During the preliminary 'off-line' gait analysis, participant walks in a treadmill at more comfortable speed and more natural possible way, without putting the arms in the treadmill. During these process, only EMG recording and footswitch are working, whose signals are recorded by OT BioLab+ during a period of 5 minutes. These signals are saved to perform a posterior analysis in which activation of each muscle at the different phases of GC is determined. The idea of this process is, thanks to both FSR sensors, automatically differentiate the GC phases while recording sEMG of agonist and antagonist muscles during walking. This allows to see which muscle is activated or deactivated during both phases of the GC and thus be able to affirm that the phase in which we are going to afferent stimulate corresponds to the activation of a certain muscle.

The next process is the most long and tedious one, that is the searching of suitable intensities for both, afferent electrical intervention itself and H-reflex assessment using conditioning-test paradigm. During this process the participant has to sit with the feet in a footrest producing a 135º flexion of the knee, as illustrated in Figure 42. The idea of this process is to obtain the recruitment curves of CPN and PTN by applying different intensity stimulus and capturing the TA and SOL responses, correspondingly. These responses are the EMG signals composed by M-wave that appears between the 10 and 27 milliseconds of EMG signal, and H-reflex appearing between 30 and 60 milliseconds. As it was explained in 3.1.2, at a certain stimulus the H-reflex starts to appear but the M-wave does not appear until the stimulation is increased. However, when stimulation is increased over a determined threshold, the H-reflex disappears and the M-wave reaches a plateau state. The recruitment curve represent the appearance of both waves depending on the stimulation applied in order to determined with is the optimal intensity to apply to obtain sensorial but no motor stimulation, it means that there was H-reflex but not M-wave.

The recruitment curve of each nerve is produced independently, by using in both cases the DS8R Digitimer. The first curve to obtain is the one corresponding to the SOL muscle; for this reason, cables corresponding to stimulation electrodes of the PTN are connected to the DS8R Digitimer. The following step is to decide which are the intensities that are going to be applied in order to generate

the recruitment curve, in most of the experiments, only 5 intensities have been applied from lowest to highest and each of them applied 6 times. The Matlab GUI is run and at each intensity delivery, a file is recorded and saved with the 6 trials performed for each intensity, as well as the mean trial of these ones. The delivery of these 6 trials at each intensity is done manually by pressing the trigger button of the Digitimer, with a rest between each trial of 5 seconds. This process is repeated for each of the intensities. Once this data has been saved, using a MatLab programming script created by previous students of the NRG and adapted for this experiment, the SOL recruitment curve can be analysed. In the same way, and using exactly the same process, the recruitment curve for the TA is obtained. In this case, the cables corresponding to the stimulation electrodes of CPN are the ones connected to the DS8R Digitimer and other five intensities are choosing depending on the participant motor threshold. Once both recruitment curves are obtained, the intensities for conditioning-test paradigm and for afferent stimulation intervention are selected.

Figure 43: Afferent electrical stimulation during experiment.

The protocol for the afferent electrical stimulation itself is really simple because RehaStim stimulator is really simple to control and understand. During this process, participant walk normally in the treadmill with sEMG electrodes, CPN stimulation electrodes, and footswitch. The function of footswitches in this process is essential because they are going to determined the period of the GC where afferent stimulation has to be applied, swing or stance phase. In this case EMG recording is only applied to ensure that stimulation is being properly delivered during these phases. During this process, Digitimer stimulators are turned-off and CPN electrodes are connected to RehaStim cables. Depending on the phase at which stimulation is going to be applied, one of the two different MatLab scripts created during this thesis is run; in-phase stimulation or out-of-phase stimulation. Once participant starts to walk in the treadmill, sEMG electrodes start to record muscle activity plotting them on the OT BioLab+ software. In order to dissociate sensory and motor effects of stimulation, nerve electrical stimulation is delivered at a intensity lower the motor threshold that is selected using the recruitment curves, and it is applied when footswitch detect the corresponding gait phase. The stimulation frequency is 40 Hz and with a pulse width of 350 μs. This intervention during one of the phases of GC is performed during 10 minutes where participant has to walk completely normal. Once this time is finished, the stimulation is stopped.

In addition, it is essential to highlight that although the use of afferent stimulation intervention has low incidence of adverse reactions because its intensity is even below the motor threshold [60], to ensure the safety and comfort of the patient, an emergency button that immediately stops the electrical stimulation completely is also provided.

Once the experiment is finished, including the analysis of the effectiveness of this intervention following explained in 4.2.3, all the electrodes and footswitch sensors are removed, the skin of participant is cleaned and the devices are turned-off. Finally, at any other moment, it is necessary to analyse all the data acquired during these processes, studying the muscle activation at each phase and checking if stimulation has been properly delivered at the determined gait phase.

It also important to highlight that several rests are allowed to the participant in order to reduce his/her stress and allow participant to be more comfortable during the long-lasting process.

4.2.3 Testing spinal reflex excitability

To analyse the effectiveness of afferent electrical stimulation intervention into the strengthening of reciprocal Ia inhibition, it is necessary to test the spinal reflex excitability pre and post intervention. The idea is to perform a previous assessment of SOL H-reflex (PRE) to establish a baseline to posteriorly to the intervention, perform another assessment of same reflex and compare both. Post stimulation intervention, there are two assessments, one immediately after the stimulation (POST) and another one 30 minutes after the stimulation intervention (POST30).

For this process, patient has to be sit in a chair with the feet in the footrest producing a knee angle of 135º, as illustrated in Figure 42. This H-reflex is very sensitive to changes in the external environment and body position; this is the reason why it is essential to maintain the same body position during the entire experiment and at different experiments, as well as perform the assessment in a quiet environment to reduce noise affecting. The eight electrodes, four sEMG and four stimulation electrodes are necessary during this process. The DS8R Digitimer is connected to the SOL nerve, PTN, while the DS7A Digitimer is connected to TA nerve, CPN. The configuration of EMG amplifier is the same as the previously explained.

Once electrodes are positioned and recruitment curves are obtained, there is a PRE1 assessment of SOL H-reflex using the conditioning-test paradigm. The Matlab GUI generates a random vector containing the values of the time intervals between conditioning (TA) and test (SOL) stimuli. These values are -5,-3,-1,0,1, and 99 milliseconds with a ISI of 3.5-5 seconds between each of the stimulus and with each type of stimuli repeated 10 times. It means a random vector containing 60 values. It is essential to understand each value and how Digitimer actuates at each value. The negative values indicate that conditioning stimulus on CPN is delivered 5,3 or 1 millisecond before the test stimulus on PTN. The 0 value means that both Digitimers are activated at the same time providing a simultaneous stimulus on CPN and PTN. On the other hand, 1 value means that test stimulus on PTN is delivered 1 millisecond before than conditioning stimulus applied to CPN. Finally, the 99 value means that only test stimulus to PTN is delivered, creating the baseline test. In this way, the SOL H-reflexes elicited by the rest of values are compared to the SOL H-reflex obtained after 99 value stimulation. These are the typical intervals values that produce disynaptic reciprocal Ia inhibition. In addition, the time between stimulus is important because delivering stimuli too close together decreases the amplitude of the H-reflex due to the previous activation in Ia afferents and depletion of neurotransmitters, producing post-activation depression.

Once Matlab GUI creates this random vector, the vector is introduced into the Arduino software, as Arduino is the device which send the control information to both Digitimers. The following step is decide the stimulus intensity delivered by each Digitimer, process that has to be done regarding the recruitment curve. The intensity selected for DS8R Digitimer stimulating the PTN has to be one corresponding to the ascendant curve of the SOL H-reflex, being a 50% of Hmax or 25-35% of the Mmax. As sometimes during experimentation is really difficult to find the Mmax, it is better to select the 50% of Hmax. However, the intensity that must be selected for DS7A Digitimer stimulating the CPN has to be exactly before the TA motor threshold. In some cases, it is necessary to adjust this stimulation 1.1 or 1.2 times the TA motor threshold because the amount of reciprocal inhibition depends on the conditioning stimulation intensity and if this is not enough, there is not reciprocal inhibition effect.

Once all the configuration is prepared, MatLab GUI is started and a file starts to be recorded and saved. This is the moment where Arduino script containing the random stimulation vector as well as other orders of stimulation is run and Digitimers start stimulating at a coordinated manner, following the order established by Arduino code. When all the 60 stimulus have been delivered, Matlab GUI is stopped and the recorded file is saved. Using different Matlab scripts created by previous NRG students and modified for this project, it is possible to visualize the EMG representing the SOL H-reflex for each condition and also the resulting reciprocal inhibition at each condition. The idea of this

Matlab scripts is to measure the SOL H-reflex amplitude of each of the cases and make a final diagram showing the conditioning results compared to the baseline or test SOL H-reflex, as represented in the first column of Figure 63. Theoretically,the amplitude of the test (99) H-reflex has to be a little bit higher than the one obtained after conditioning stimulus because when antagonist muscle, TA, is stimulation there is a generation of reciprocal Ia inhibition, reducing the SOL H-reflex.

In order to confirm the reliability of these results, a PRE2 SOL H-reflex assessment is performed. The idea is to ask the participant to walk in the treadmill during 5 minutes in a natural and conformable way. After these walking minutes, the participant sit again in the same position explained before and the procedure explained during the PRE1 SOL H-reflex assessment is repeated. However, in this case, instead of using the six different values for creating the random vector, only the three ones with the best results during the PRE1 assessment would be used repeating each of them 20 times. In this case, the 99 value is always introduced as it is the baseline, in addition to other two selected values with the best results. In the same way, the random vector of 60 values, is created in the MatLab GUI and posteriorly introduced into the Arduino script following the same procedure as previously. The results obtained in the assessment should be equal or quite similar to those obtained on the three best results of PRE1 assessment. In the case of having significant differences, the procedure and electrodes should be re-adjusted until both PRE1 and PRE2 assessment coincide.

Once after the afferent stimulation intervention, is essential to evaluate the modulation of SOL H-reflex, it means the modulation of reciprocal Ia inhibition as it is represented by the second column of Figure 63. For this reason a POST SOL H-reflex assessment is performed immediately after the stimulation intervention. This assessment follows exactly the same procedure as PRE2 SOL H-reflex assessment. The best three values obtained previously are repeated randomly 20 times to create the random stimulation vector. This vector is introduced into the Arduino software and the stimulation starts. In some cases, after 10 minutes of treadmill walking, the electrodes or the strap are moved or poorly adjusted. For this reason, it important to try to mimic the SOL H-reflex obtained during the PRE assessments using the Matlab GUI 'Plot tracing' function that allows to load and visualize the previous H-reflexes. Once the same amplitude of test H-reflex is obtained, the POST SOL H-reflex assessment can be performed in the same way as previous ones.

If stimulation is applied during swing phase (In-phase strategy) as represented by the first row of Figure 63, these conditioning POST SOL H-reflexes should be smaller in amplitude than the once obtained by conditioning PRE SOL H-reflexes, meaning that there is a increase of reciprocal Ia inhibition. However, if stimulation is applied during stance phase (Out-of-phase strategy) as represented by the second row of Figure 63, these conditioning POST reflexes should be higher in amplitude than conditioning PRE reflexes, promoting a worsening of reciprocal Ia inhibition. In the case of applying a Control stimulation strategy, as in third row of Figure 63, the conditioning PRE and POST SOL H-reflexes are stable or almost stable.

Finally, to check the duration of the effect of afferent stimulation intervention, a POST 30 minutes SOL H-reflex assessment is performed as represented by third column of Figure 63. The protocol of this process is exactly the same as the previous POST SOL H-reflex assessment. The idea is that after 30 minutes the effects of the intervention have disappeared; however, in most cases there is not a complete absent of effects but just a decrease.

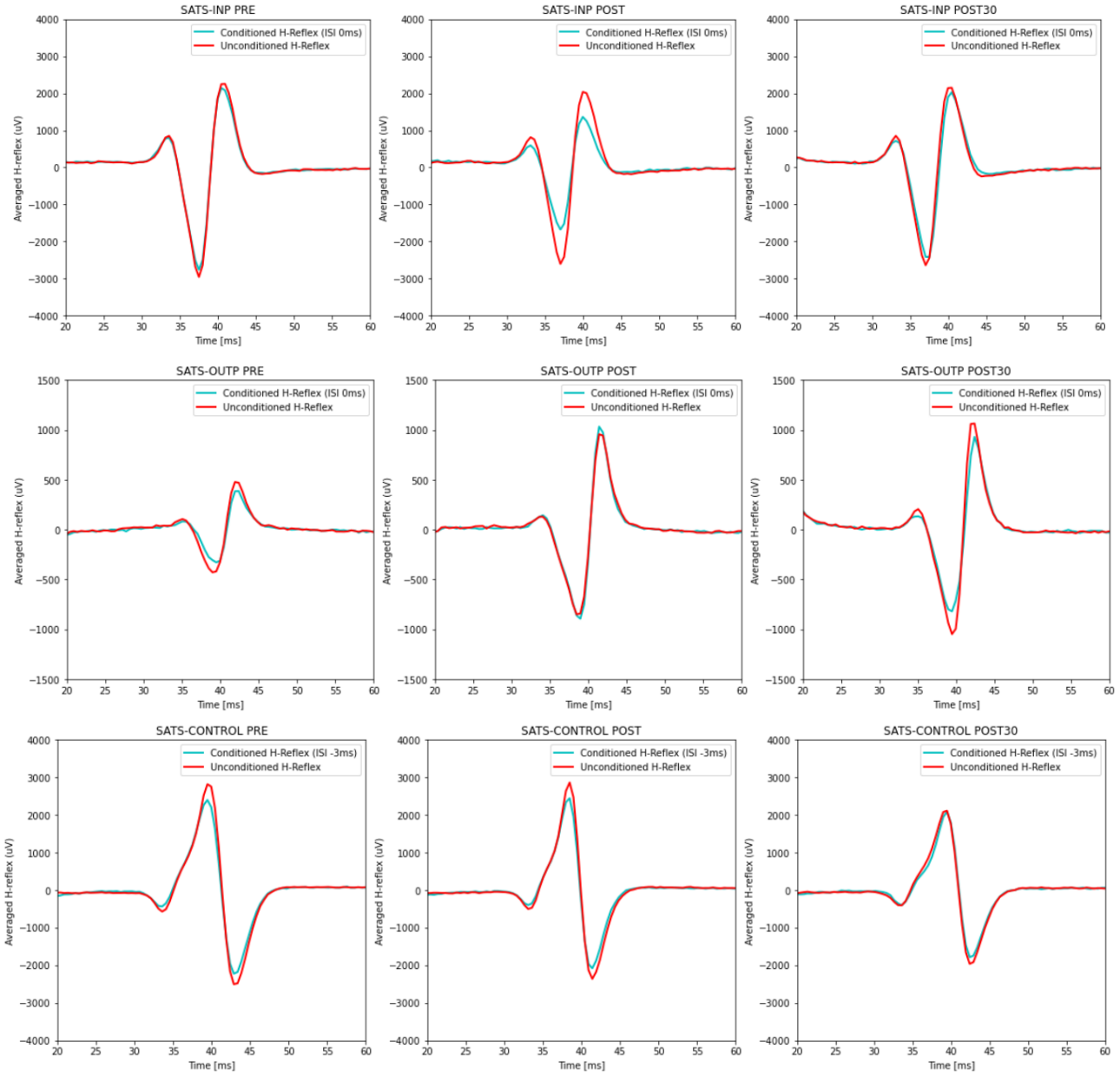

Figure 44: SOL H-reflex differences between test (red line) and conditioning (blue line) stimulus at different assessment tests (columns representing PRE, POST ,and POST30), and at different stimulation strategies (row representing In-phase, Out-of-phase, and Control).

4.2.4 Data analysis

Once all the experimental protocol is finished, it is necessary to perform a posterior data analysis of the obtained EMG data. This data analysis is performed by using two different softwares, MatLab and Python codification.

The main activities during this data analysis are the the extraction and the evaluation of the recruitment curves in order to check if the selection of the stimulation intensities has been properly performed and also to determine which is the motor threshold of each participant. Then, it is also necessary to confirm that afferent electrical stimulation intervention has been provided in the correct phase of GC. Finally, and the most important data analysis is the evaluation of the reciprocal inhibition mod-

ulation after the electrical intervention. It is also important to check that reciprocal Ia inhibition modulation is performed according to the theory that explains that it depends on the stimulation strategy used: In-phase, Out-of-phase, or Control.

To perform the data analysis of the reciprocal Ia inhibition is necessary to create some variables including the value of reciprocal inhibition obtained at each strategy and at each part of protocol; PRE, POST, and POST30. This value of reciprocal inhibition is obtained by dividing the mean value of SOL H-reflex amplitude obtained using the conditioning value with better results (-5000, -3000, -1000 or 0) by the mean value of SOL H-reflex amplitude obtained using test stimulus. Performing this operation for all of the necessary variables, the database used for reciprocal Ia inhibition and statistical evaluation is created with the variables being: preInphase, postInphase, post30Inphase, preOutphase, postOutphase, post30Outphase, preControl, postControl, and post30Control. The first six variables contain eight values that represent the reciprocal inhibition value obtained by each of the participants performing each strategy; however, the last three variables only contain four values that also represent the reciprocal inhibition value obtained by each of the participants performing this strategy. In other cases, instead of using these variables, it is necessary to have only three variables regarding the strategies: In-phase, Out-of-phase, and Control. In this case, each variable is composed by 24, 24, and 12 values representing the three different reciprocal inhibition values (PRE, POST, and POST30) associated to each patient.

These three variables are used to represent the changes in PRE, POST, and POST30 reciprocal Ia inhibition in each of the three strategies. The changes at POST and POST30 reciprocal Ia inhibition are also represented with respect to the amount of reciprocal Ia inhibition during the PRE assessment of the experiment. In addition to the representation of reciprocal Ia inhibition, the differences between PRE, POST, and POST30 EMG signals at each of the three stimulation strategies used are also analysed by plotting the EMG signals in each of the cases.

Once this analysis of the results has been performed, it is essential to apply a statistical analysis in order to evaluate if the differences between PRE, POST, and POST30; as well as the differences between In-phase, Out-of-phase, and Control strategies are statistically significant to determine that afferent electrical stimulation of CPN is a promising strategy to modulate reciprocal Ia inhibition.

First of all, in order to determined which statistical test has to be used to compare independent variables described above, it is necessary to apply a normality test. During this process, different normality check methods have been applied to each variable to check its normality, such as visual methods and statistical methods. The visual normality test represents the normalized histogram, the boxplot, and the QQ Plot of each variable to see if they follow a normal distribution. These three methods analyse the probabilistic distribution of variables, comparing them to a normal distribution represented in red. If the obtained results coincide with the red lines representing the normal variable, it could be concluded that variable follows a normal distribution. However, if it differs from normal variable, the analysed variable would be concluded to be a non-normal variable.

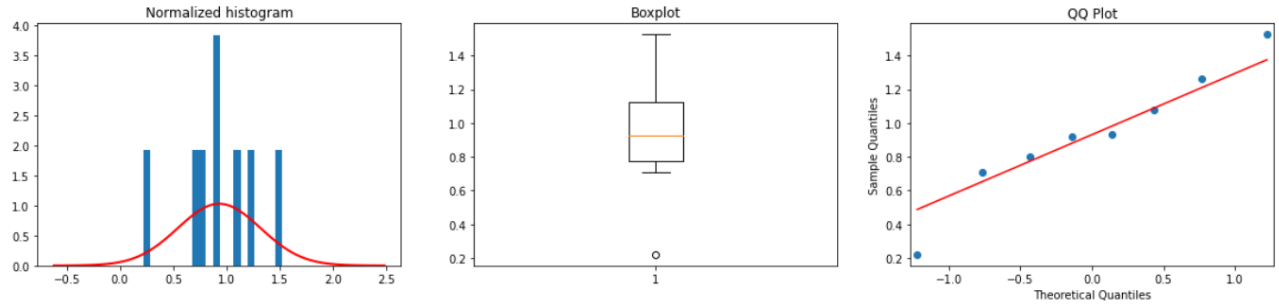

Figure 45: Example of normality test using visual methods.

Although, this visual inspection of the distribution may be used for assessing normality, this approach is usually unreliable and does not guarantee that the distribution is normal. For this reason statistical methods are performed using a statistical test to compare the scores in the sample to a normally distributed set of scores with the same mean and standard deviation. These test are based on two different hypothesis, H0 that claim that sample data are not significantly different than a normal population, and H1 that claim that sample data are significantly different than a normal population. These methods check if the null hypothesis (H0) is confirmed or rejected. The statistical methods used are:

- Kolmogorov–Smirnov (KS) test: This method performs a test of the distribution of an observed random variable S(x) against a given standard normal distribution F(x). Under the null hypothesis, the two distributions are identical, S(x) = F(x). To know if both samples are identical, it calculates the maximum difference between both variables, following the left formula of Figure 46. If this maximum difference is equal or less than a determined value Dn, then it could be assumed that variable follows a normal distribution because it fits properly with F(x).

  This test, although well-known, has not much power and is really sensitive to outliers. This means that a large number of observations (n>50) is necessary to reject the null hypothesis. The results given by the test are in terms of statistics and p values. If the p value is higher than 0.05, the variable looks like a normal distribution; otherwise, it is a non-normal variable.

- Shapiro–Wilk (SW) test: This method is based on the correlation between the data and the corresponding normal scores. It tests the null hypothesis that a sample came from a normally distributed population.

  Its advantage is that it provides better power than the KS test (it works best for data set with n<50), being the most powerful test when testing for a normal distribution. It has been developed specifically for the normal distribution and it cannot be used for testing against other distributions like for example the KS test. This test gives the results in terms of statistics and p values. If the p value is higher than 0.05, the variable looks like a normal distribution; otherwise, it is a non-normal variable.

$$D_n = \max_x |F(x) - S_n(x)| \qquad \max_x |F(x) - S_n(x)| \leq D_{n,\alpha} \qquad W = \frac{\left(\sum_{i=1}^{n} a_i x_{(i)}\right)^2}{\sum_{i=1}^{n}(x_i - \overline{x})^2},$$

Figure 46: Formula corresponding to Kolmogorov Smirnov (left and middle formula) and to Shapiro Wilk (right formula) normality tests. Retrieved from Google Images.

Once normality test are performed and concluding, as it will be explained in 5, that some of the variables do not follow a normal distribution it is time to apply non-parametric statistical methods. These methods require no or very limited assumptions to be made about the data. The idea of these methods is to evaluate if there is a statistical significant difference between variables containing the reciprocal inhibition values obtained at each assessment and at each strategy, without showing which variables are different and which not. There are several non-parametric methods being the most important ones [61]:

- The sign test: it is the simplest non-parametric methods and it is used to compare a single sample with some hypothesized value. This method assigns a sign positive or negative to each observation depending if observation is higher or lower than the hypothesized value. It is the analogous of one-sample or paired t-test of parametric methods. Its main drawback is that it simply allocates a sign to each observation but it does not consider the observation magnitude. For this reason, this method results inefficient and has a reduced statistical power.

- The Wilcoxon signed rank test: This test is an alternative of sing test that consider the magnitude of the observations. However, the procedure is the same, once the observations are ranked, it assign a positive or negative sign to each observation depending if they are higher or lower than hypothesized value.

  These two previously explained non-parametric methods are useful to replace one-sample and paired t-tests, it means to analyse two dependent groups. However, if the idea is to find an alternative to unpaired t-test the following method is required.

- Mann-Whitney test: This statistical method is performed when comparison is made between two independent groups. This method gives as result a calculated statistic that must fall into a determined range to reach a level of significance. If p-value obtained is lower than 0.05, there is evidence that both groups analysed have a significant differences. However, if p-value is greater than 0.05, the test provides no evidence that groups analysed are different.

- Friedman test: This statistical method is really similar to sign or Wilcoxon signed tests because it is used to compare dependent samples; however, in this case it can compare three or more dependent groups, not only two.

- Kruskal-Wallis test: This statistical methods is similar to Mann-Whitney test because it is used to compare independent samples that can have different sizes; however, in this case it can compare three or more groups.

Regarding all these methods it can be concluded that the one that best suit the type of variables of this thesis is the Kruskal-Wallis non-parametric test because the variables are independent samples belonging to nine different groups.

Therefore, the Kruskal-Wallis test is computed for all the nine independent samples. Its null hypothesis is that the population median of all of the groups are equal, meaning that there are not significant changes between the variables. Once the test is performed, the result are a statistic and a p-value or probability value. This value describes how likely the results would have occurred if the null hypothesis where true. If p-value obtained is higher than the significance level (0.1/2=0.05), the null hypothesis is affirmed meaning that samples are equal. However; if p-value obtained is lower than 0.05 the null hypothesis is rejected indicating that there are significant differences between groups. The smaller the p-value, the more significant the results are.

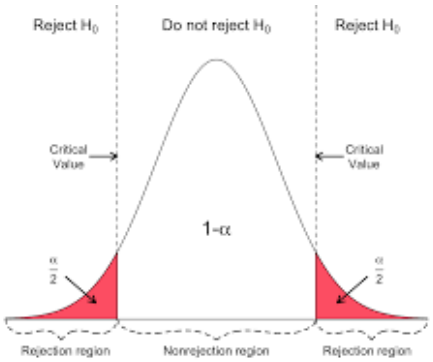

Figure 47: Theory of hypothesis testing. Retrieved from Google Images.

The significance level or alpha level explains the probability of wrongly rejecting a true null hypothesis. This level is selected by subtracting the confidence level form 100%. This means that in this case, the confidence level is 95% and the alpha level is 5% or 0.05. For this reason, if p-value is lower than this alpha level, there is a low probability that results would have occurred if the null hypothesis where true, meaning that is necessary to reject the null hypothesis, being including in the rejection part of the Figure 47. In the other case, if p-value is higher than alpha level, there is a high probability that results would have occurred if the null hypothesis where true, meaning that is necessary to accept the null hypothesis, being including in the confidence interval of the Figure 47.

The problem of this method, is that rejecting the null hypothesis does not indicate which of the groups differs between them. For this reason, post hoc comparisons between all the groups are required, in

order to determine which are those groups that differ the most. The test used to perform this pairwise multiple comparison is a test used in non-parametric studies called Conover's test. Performing this test, it is necessary to choose a method to adjusts the p-value for multiple comparisons. In this case a bonferroni adjustment was made in which the alpha or significance level is divided by the number of comparisons being made. The result of this pairwise multiple comparison is a table in which the resulting p-value of each pairwise comparison is represented. In the same way, when p-value is lower than 0.05, the null hypothesis is rejected meaning that there are significance differences between both groups.

In addition to compare and find the differences between each of the nine variables; preInphase, postInphase, post30Inphase, preOutphase, postOutphase, post30Outphase, preControl, postControl, and post30Control; differences between PRE, POST, ans POST30 assessments at each stimulation strategy have also been statistical analysed. Analysing in this way the differences between preInphase, postInphase, post30Inphase; between preOutphase, postOutphase, post30Outphase; and between preControl, postControl, post30Control. In this case, as the differences are being studying into three dependent variables, because they belong to same patient, the non-parametric test performed has been Friedman Chi-square non-parametric method. In the same way as previously, if the p-value is lower than 0.05, the null hypothesis is rejected meaning that there are significant differences between the variables. In the same way as Kruskal-Wallis test, the rejection of null hypothesis does not indicate at which point differences are. For this reason, post hoc comparisons are required using Conover's test.

Once statistical analysis is performed, if the results confirm that there are statistically significant differences between variables such as previous reciprocal Ia inhibition and posterior inhibition after the stimulation at any of the gait phases, it will mean that afferent electrical stimulation is a good way to modulate reciprocal Ia inhibition in healthy subjects.

5 Results

5.1 Effect of afferent stimulation intervention in healthy subjects

Once the experimental protocol was performed, all the acquired data were analysed in order to determine several points such as the performance of the developed gait neuromodulation platform, the usability and comfort of subjects while using this developed platform, and the effects of sensory electrical stimulation on spinal plasticity by the modulation of reciprocal Ia inhibition.

These three aspects were analysed during the three main processes of the gait neuromodulation platform. During the 'off-line' analysis both EMG and FSR signals were processed and analysed in order to determine the activation and deactivation of TA and SOL muscles. The ideal results of this process would be the activation of the TA during the swing phase of the GC and the activation of the SOL during the stance phase.

Then, regarding the afferent electrical intervention itself, the idea was to obtain EMG signals that, in combination to footswitch signals, demonstrated that during the In-phase strategy, stimulation was being applied at the TA during the swing phase of the GC and at Out-of-phase strategy, it was being applied during the stance phase of GC. In addition, these signals were also important to demonstrate that in Control experiments there was not any type of stimulation on the TA muscle.

Finally, most of the results were extracted from the data obtained at the reciprocal Ia inhibition analysis using the SOL H-reflex assessment. During this process, SOL EMG data acquired at the conditioning test assessments was analysed in terms of the H-reflex amplitude to measure the quantity of reciprocal Ia inhibition at each assessment time. It is essential to remember that SOL H-reflex appears at between the 30-60 milliseconds of the SOL EMG signal. The results of this SOL H-reflex amplitude analysis add more evidence to the literature, occurring an increase in reciprocal Ia inhibition (decrease of SOL H-reflex amplitude) if the afferent stimulation was performed during the swing phase of GC, and occurring a decrease in reciprocal Ia inhibition (increment of SOL H-reflex amplitude) if the afferent stimulation was performed during the stance phase of GC.

Thanks to all these results, it will be concluded if the developed gait neuromodulation platform is useful to modulate reciprocal Ia inhibition in healthy participants by applying afferent or sensory electrical stimulation. If the results determine that there is an improvement in reciprocal Ia inhibition after stimulation at the swing phase of GC, this platform could be tested on SCI subjects in order to see if similar results are obtained. The future objective of this project will be the creation of a useful rehabilitation platform to increase reciprocal Ia inhibition in SCI patients, improving their walking recovery.

5.1.1 Performance of the gait neuromodulation platform

At this section, the results regarding the performance of the gait neuromodulation platform are exposed. As written before, the platform performance was analysed differentiating the three different processes of the platform. However, in general, it should be mentioned that all the processes had a fairly correct functionality, in some cases exceeding the expectations placed before carrying out the healthy participants experimentation. In addition to the functionality success, it is also important to highlight the ease to use this platform was, that with a brief explanation could be completely understood and applied.

The results obtained at the off-line analysis, presented in 4.1.3, on the one hand demonstrated the activation of TA muscle during the swing phase of the GC and its deactivation at the stance phase. On the other hand, demonstrated the activation of SOL muscle during the stance phase and its deactivation at swing phase. These results, presented in Figure 38, coincide with the literature demonstrating that TA

activates during swing phase and that to perform different stimulation strategies, CPN innervating TA should be stimulated at different phases of GC. In order to perform an In-phase stimulation strategy, CPN should be stimulated while its innervated muscle is contracted, it means during the swing phase. However, in the case of performing a Out-of-phase stimulation strategy, this same nerve should be stimulated while TA is relaxed, during the stance phase. These results regarding the activation of TA and SOL muscles during the different phases of GC coincided in all the 20 healthy participants of the study. In some of them, the muscle activation was strongly differentiated between muscles at the different phases. However, in other participants, although the activation and deactivation of muscles occurred in the same way depending on the phase of GC, the EMG signal was not so properly and strongly activated and represented. The reason of these differences on EMG amplitude were based on two different factors. First of all the anatomy of participant, when there were young, muscular, and athletic participants, the EMG signals were stronger than if participants were older, fat and had a sedentary lifestyle. The second factor was the acquisition of EMG signal, in some cases electrodes could be located in a less optimal position, be less attached to the participant skin, skin could be also worse prepared increasing the impedance of the signals, or noisy in the laboratory could be stronger due to external factors. However, these second factors were always minimized as much as possible in order to allow a proper EMG detection in all the participants.

The result obtained in the following process of the experiment was the extraction of recruitment curves of each participant thanks to the evaluation of M-wave and H-reflexes at different stimulus intensities. As previously explained, this part was essential in order to determine which stimulation intensity had to be applied to CPN and PTN during the reciprocal Ia inhibition assessments. The intensity of the CPN had to be this intensity just before the onset of M-wave, while the PTN intensity had to be 50% of the maximum H-reflex.

During the evaluation of M-wave and H-reflex amplitude at the SOL muscle, using the DS8R Digitimer stimulation, both the SOL and TA activity during PTN stimulation at different intensities was recorded. The EMG activity of both muscles during this nerve stimulation is presented in Figure 48.

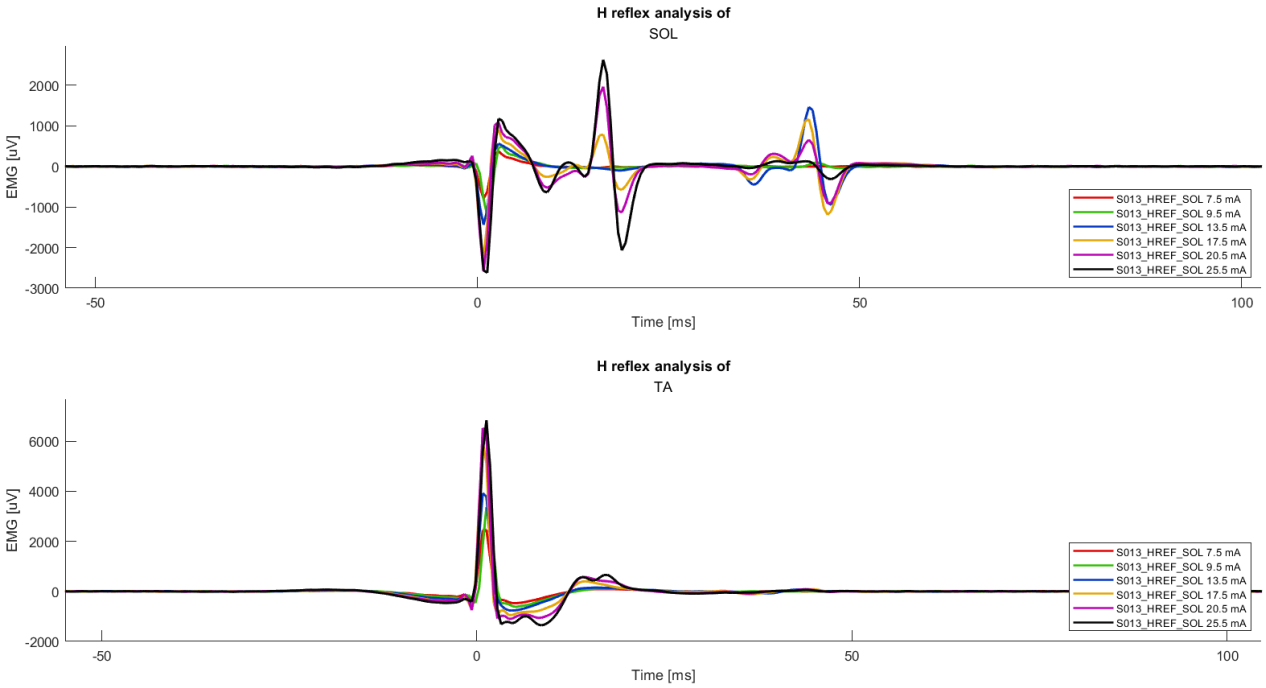

Figure 48: EMG activation of both SOL muscle (upper graph) and TA muscle (lower graph) when PTN is stimulated. The color of each line represents the stimulation intensity delivered to elicit M-wave and H-reflex.

Regarding this mentioned figure, it can be observed how during PTN stimulation, the SOL muscle was activated resulting in M-wave and H-reflex depending on the stimulus intensity. However, the TA muscle was deactivated only being represented the stimulation artifact at the 0 millisecond. These results were expected as the PTN is the nerve in charge of innervating the SOL muscle. In this way, its stimulation only result in an activation of SOL muscle producing M-wave and H-reflex wave in accordance to the theory represented in 3.1.2.

However, these EMG signals did not properly illustrate which was the suitable intensity to select in order to stimulate PTN during reciprocal Ia inhibition assessment. For this reason, the graphical representation of SOL recruitment curve was essential as represented in Figure 49. This curve represents the amplitude of M-wave and H-reflex in terms of the stimulus intensity applied.

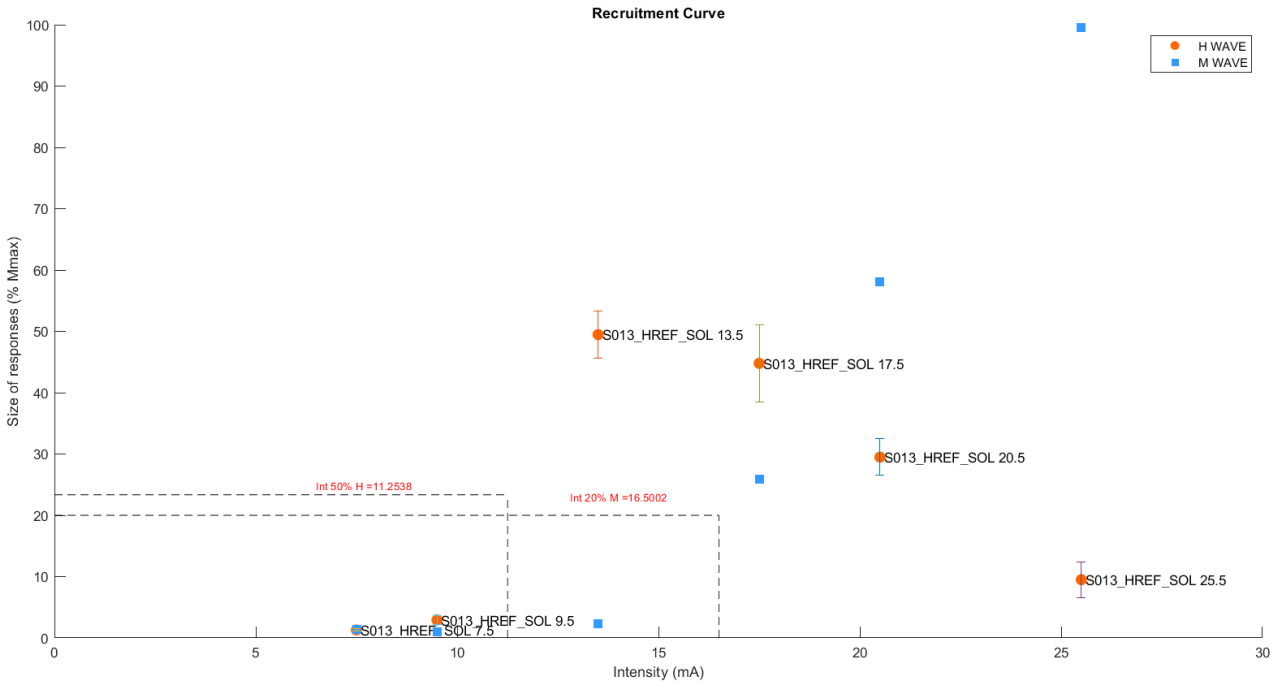

Figure 49: Recruitment curve of SOL muscle. Orange points represent the amplitude of the H-reflex while blue points represent the amplitude of the M-wave. Black dashed lines represent the desired PTN stimulus intensities with the value of these intensities in red color.

This previous graph represents the amplitude of M-wave and H-reflex (based on 100% of Mmax) in terms of stimulus intensity. It can be seen how these curve are similar to the one explained in the theory, such as Figure 13, highlighting the proper functioning of this experiment process. In this graph it can be seen how when stimulus intensity was really low, there was no neither H-reflex neither M-wave because low stimulation was not able to stimulate neither afferent nor motor fibers. However, once intensity started to increase, there was also an increase of H-reflex amplitude because afferent fibers of SOL muscle were being activated. Once the maximum amplitude of H-reflex was reached, posterior increases in intensity generated the disappearance of this reflex and the appearance of M-wave due to the activation of both afferent and motor fibers producing a muscular contraction that resulted in an increase of M-wave, and an antidromic collision that resulted in the reduction of H-reflex amplitude. This process continued to occur until a point of high stimulus intensity, where H-reflex completely disappeared due to the stronger effect of antidromic volley than orthodromic volley during the collision. When stimulation was extremely high, there was no H-reflex and the M-wave started to create a plateau, it means to have a continuous amplitude. All this process can be properly represented by this figure.

In addition, as it has been previously explained, the desired intensity of the PTN during the reciprocal Ia inhibition assessment was 20% of Mmax or 50% of Hmax, as represented by the dashed black lines and the red sentences indicating the required intensity in both cases. However, due to the difficulty in some cases to reach the Mmax value, it has been stipulated that PTN stimulation intensity had to be the 50% of the Hmax.

This evaluation of M-wave and H-reflex was also performed at the TA muscle by stimulating the CPN. This evaluation was also performed by using the DS8R Digitimer stimulator that delivered stimulus at different intensities while measuring both the SOL and the TA activity. In the same way as the previous evaluation, the EMG activity of both muscles during this nerve stimulation is presented in Figure 50.

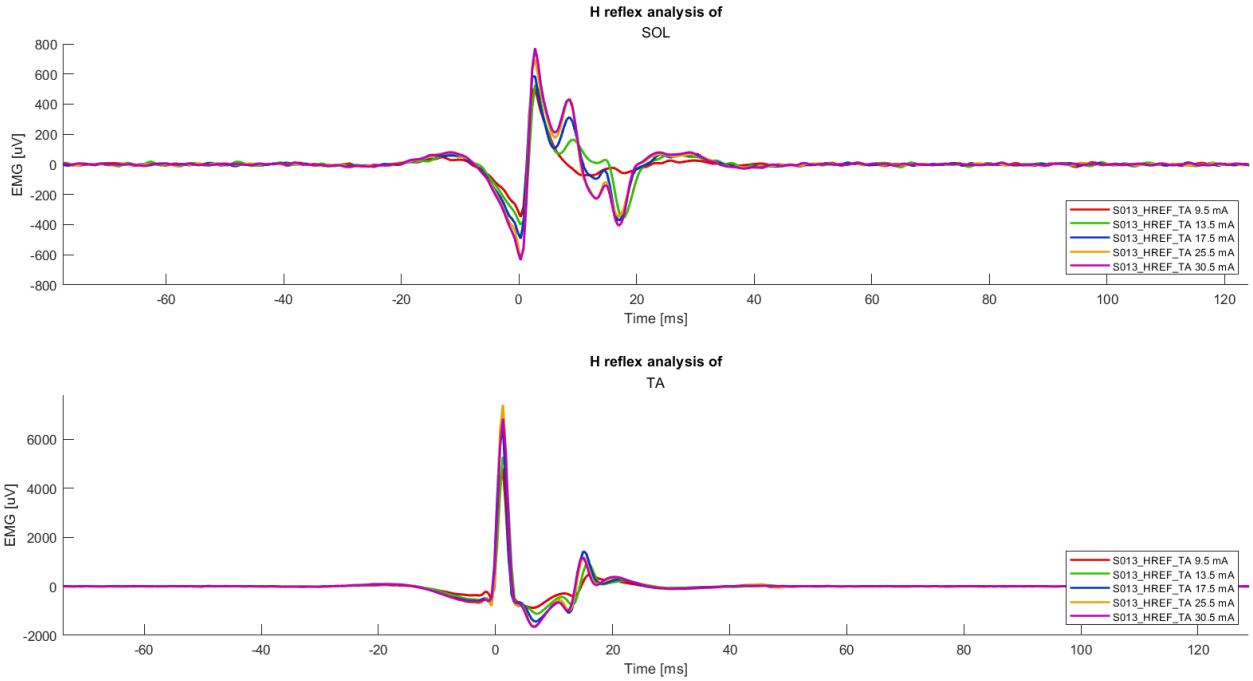

Figure 50: EMG activation of both SOL muscle (upper graph) and TA muscle (lower graph) when CPN is stimulated. The color of each line represents the stimulation intensity delivered to elicit M-wave and H-reflex.

In this previous figure, it can be observed how during CPN stimulation, the SOL muscle was deactivated with the only representation of the stimulation artifact in the first milliseconds of the EMG signal. However, the TA muscle seemed to be activated but only resulting in a M-wave whose amplitude varied depending on the stimulus intensity. These results coincide with the literature that claims that stimulation of CPN result in the appearance of M-wave providing TA contraction, but the H-reflex is extremely difficult to be found on the EMG signal. This affirmation also coincide with the knowledge provided from the HNP professionals, who advised about the difficulty of analysing the H-reflex of TA muscle. However, during this project only M-wave was necessary in order to know which was the motor threshold of each participant. For this reason, the absent of TA H-reflex did not suppose a problem for the development and continuity of the project.

In the same way as with the evaluation of SOL activation, the EMG signals did not properly illustrate which was the suitable intensity to select. For this reason, TA recruitment curve was also represented, as illustrated in Figure 51.

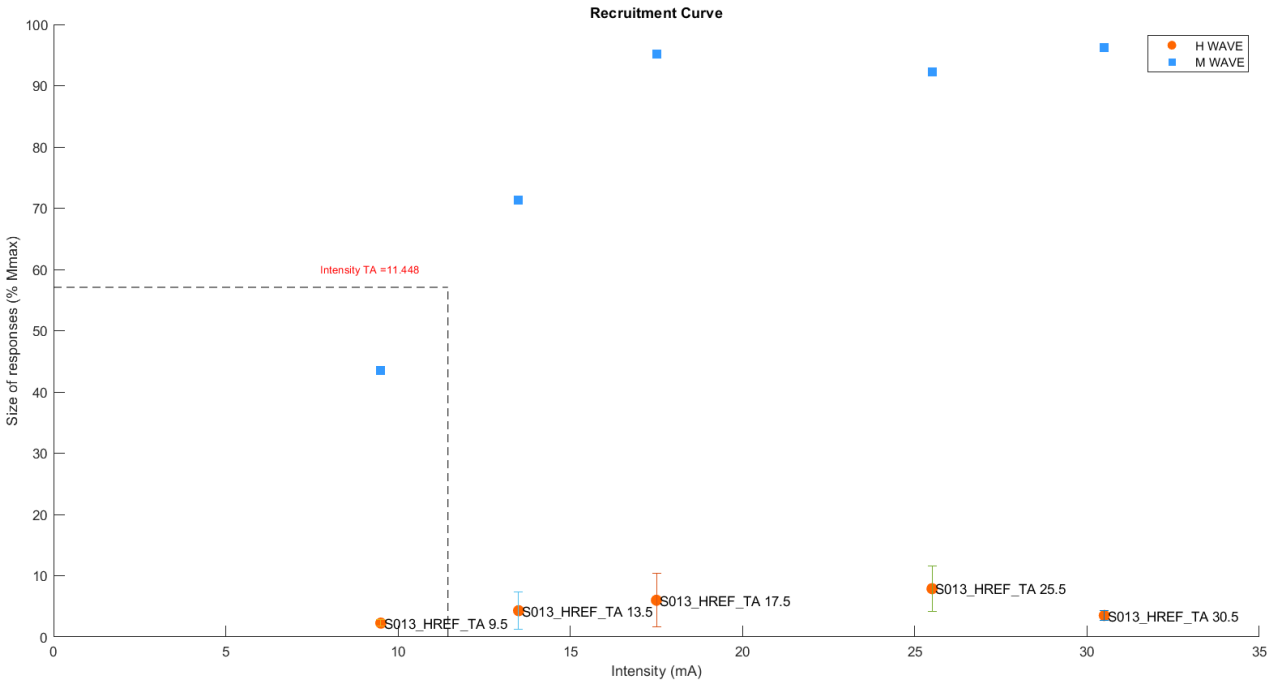

Figure 51: Recruitment curve of TA muscle. Orange points represent the amplitude of the H-reflex while blue points represent the amplitude of the M-wave. Black dashed line represents the desired CPN stimulus intensity with the value of this intensity in red color.

This previous graph representing TA recruitment curve, illustrates how H-reflex was insignificant or almost absent due to the difficulty to elicit H-reflex on the TA muscle. In addition, it can be seen how only M-wave was elicited representing the motor threshold of the participant. This configuration, absent of H-reflex and only representation of M-wave, was present in the recruitment curve analysis of all the participants concluding that actually it is extremely difficult to elicit the H-reflex on the TA muscle. As it has been previously explained, the desired intensity of the CPN during the reciprocal Ia inhibition assessment was just below the motor threshold. However, as explained, in some cases this intensity was not enough to provide reciprocal inhibition of the antagonist muscle. This fact made necessary to change the CPN intensity to 1.1-1.2 times the intensity of the motor threshold, as represented by the dashed black line and the red sentence indicating the required intensity to stimulate CPN inducing reciprocal Ia inhibition.

Once stimulation intensities were obtained for both CPN and PTN, the afferent electrical stimulation intervention was performed. Electrical stimulus were delivered to the CPN in a determined strategy: In-phase stimulation during swing phase, Out-of-phase stimulation during stance phase, or Control strategy where no stimulation was applied. During this afferent stimulation, EMG signals for both the TA and SOL muscles were recorded as well as footswitch sensor signals. These signals would help us to determine if the afferent stimulation was been performed during the established phase of GC.

The Figure 52 shows how stimulation was properly delivered at the desired phase of GC at different experiment. Remember that each experiment only comprised one type of stimulation strategy, so this graph is a representation of three different experiments with three different participants.

In this figure, it can be observed how during the Control stimulation strategy where electrical stimulation was not delivered, the blue and red signals corresponding to the TA and SOL muscles respectively were normally activated. It can be seen thanks to the footswitch sensors, grey line is the heel sensor while dark black is the toe sensor, how TA activated when neither of both sensors were activated which means during the swing phase of GC. However, SOL muscle activated in those periods where

any of the sensors was activated which means during the stance phase. These results conclude that during Control strategy, as there was not stimulation, the muscles followed their normal activation pattern.

The second graph representing the In-phase stimulation intervention demonstrate how when afferent electrical stimulation was delivered at the CPN during the phase where TA was contracted, it means during the swing phase where both footswitch signals were deactivated, there was an extra activation of the TA activity represented by green lines. This graph ensures that electrical stimulation was only delivered during the swing phase of GC.

In the same way as In-phase stimulation strategy, the Out-phase stimulation was perfectly delivered at the CPN during the stance phase, as can be perfectly seen at the third graph of Figure 52. When any of the footswitch sensors was activated, it means during the stance phase of GC, the TA activity represented in red was increased. This illustration ensure that electrical stimulation was only delivered during the stance phase of GC.

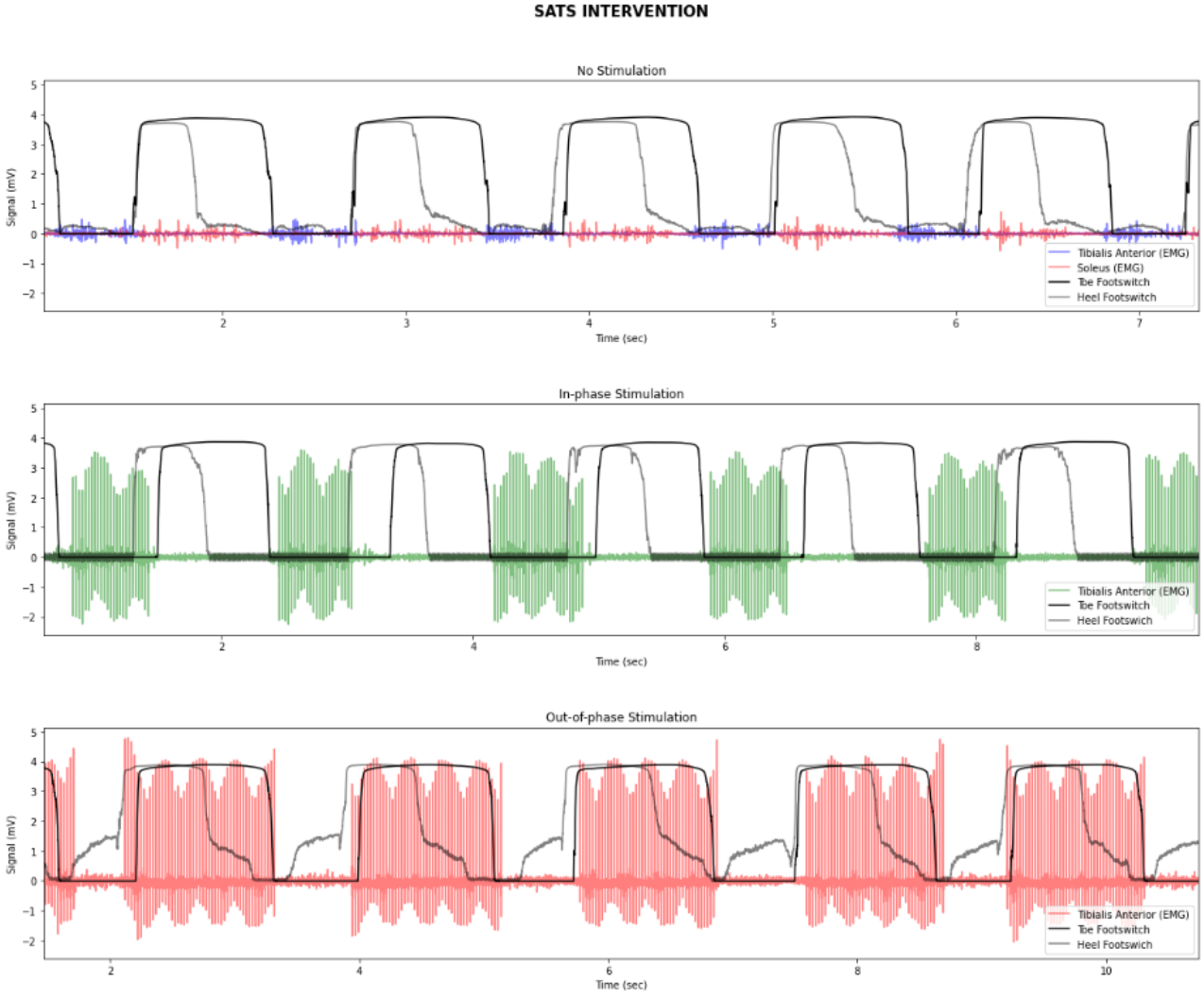

Figure 52: Selective and adaptive timely afferent electrical stimulation. First graph represents the Control strategy, second graph represents the In-phase stimulation strategy, and third graph represents the Out-of-phase stimulation strategy.

In addition to this graph, other method to ensure that stimulation was being applied at desired phase of GC, was the visualization of the led light presented in the RehaStim stimulation. This led illumi-

nated always than afferent electrical stimulation was being delivered to the CPN. However, one of the main ideas of this project was the automation of the gait phases detection in order to trigger the stimulation at the desired millisecond, reducing as much as possible the human or visual errors and delays. In both cases, EMG signal representation and Rehastim light visualization, the result is that stimulation was delivered at the desired phase of GC.

The final process where platform demonstrated its correct performance was the analysis of reciprocal Ia inhibition due to the SOL H-reflex assessment. During this process measurements of the SOL H-reflex amplitude were performed two times previous to the afferent stimulation intervention, immediately after the intervention, and 30 minutes after the intervention.

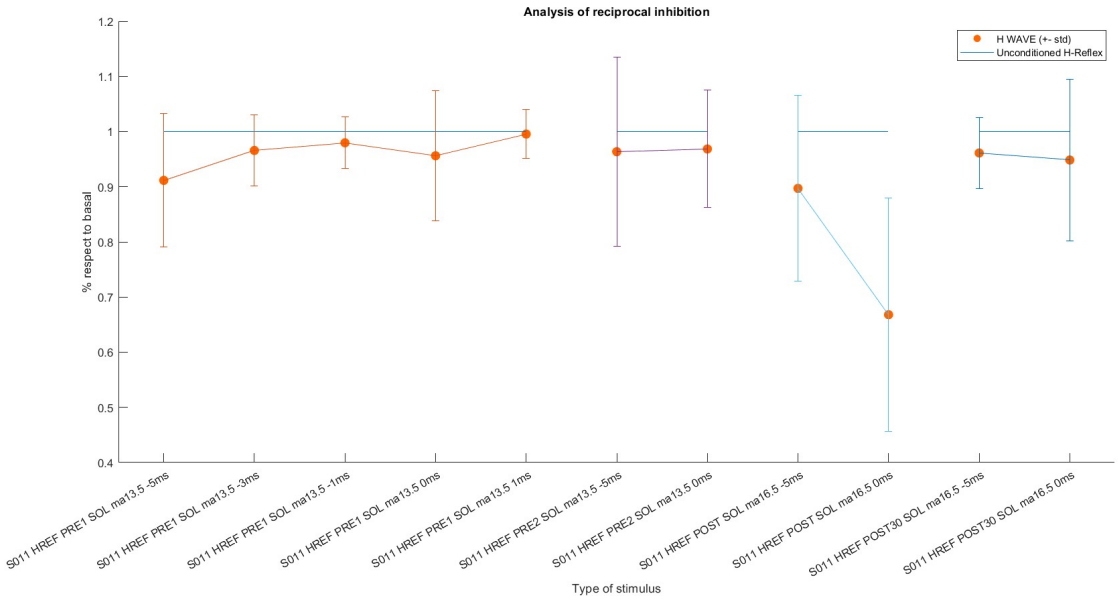

Figure 53: Results of the reciprocal Ia inhibition assessment in a In-phase stimulation strategy performed to one participant.

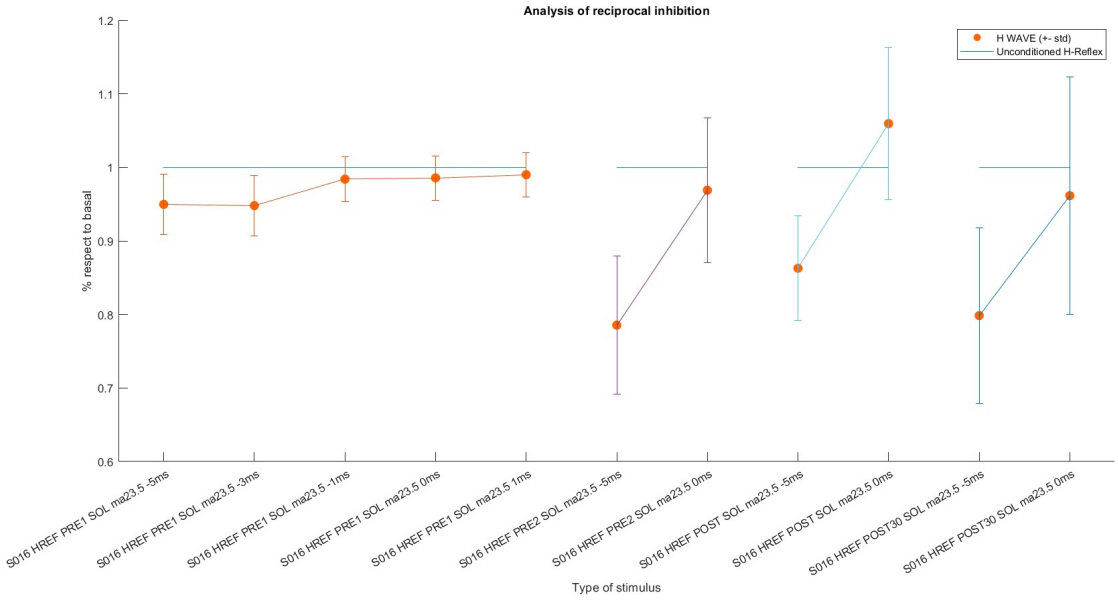

Figure 54: Results of the reciprocal Ia inhibition assessment in a Out-of-phase stimulation strategy performed to one participant.

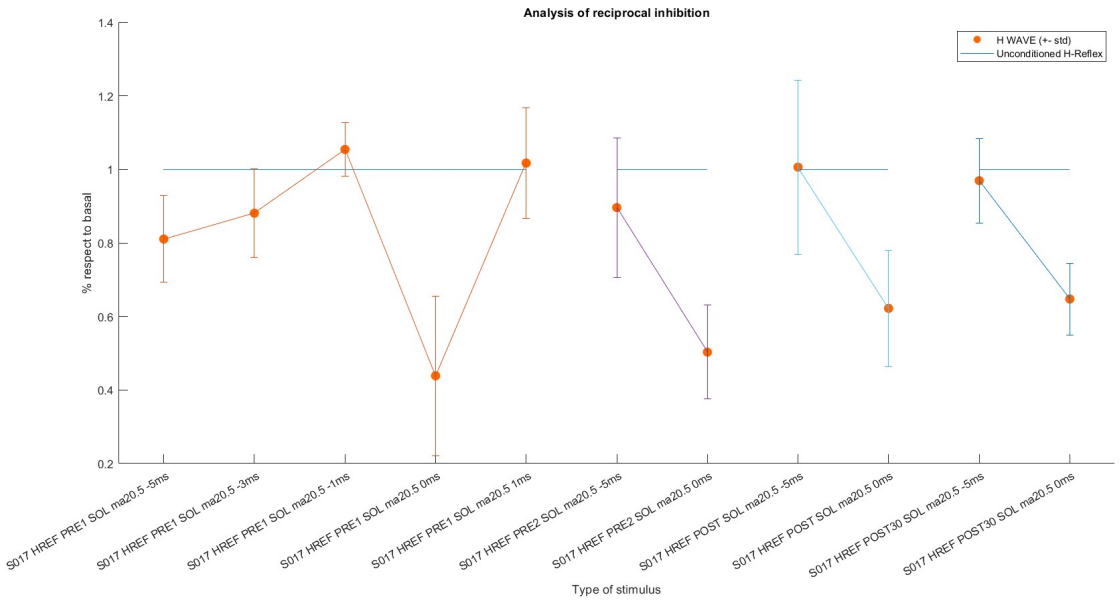

Figure 55: Results of the reciprocal Ia inhibition assessment in a Control strategy performed to one participant.

In the previous figures it can be observed how, during the PRE1 assessment, -5,-3,-1,0,1,99 milliseconds between conditioning-test stimulus were used in order to determine which ISI provided the lowest H-reflex amplitudes, it means the better reciprocal inhibition. It is important to highlight that, the 99 ISI provided only PTN stimulation generating the test H-reflex and that the rest of the ISI were compared to this test or basal H-reflex. In the case of PRE2, POST, and POST30 assessments only the 99 and the two best ISI of the PRE1 were applied, in order to increase the number of trials of each one reducing its variability. In all the graphs, the blue lines represent the test reciprocal Ia inhibition while the orange points represent the conditioning reciprocal Ia inhibition, then vertical lines represent the standard deviation or variability of trials.

In the Figure 53 it can be seen how after performing PRE1 assessment, the best reciprocal Ia inhibition results were obtained applying -5 and 0 millisecond ISI. In these cases there were a 10% and a 5% of reciprocal Ia inhibition increment with respect to the basal when conditioning stimulation was applied 5 milliseconds before test stimulation, and when both stimulations were delivered simultaneously, respectively. These results were more or less maintaining after performing the PRE2 assessment, reinforcing the repeatability of the assessment. After the afferent electrical stimulation, in the POST assessment, there was a 15% and 25% of increment in reciprocal inhibition with respect to the basal for -5 and 0 milliseconds respectively, concluding that there was a improvement of reciprocal Ia inhibition when afferent stimulation was applied during swing phase of GC, following In-phase strategy. Finally, the POST30 assessment that tried to study the durability of the afferent stimulation effects, demonstrated how 30 minutes after the stimulation intervention there was a recovery of the previous reciprocal Ia inhibition levels. At this point, the reciprocal Ia inhibition returned to be more or less 10% with respect to the basal.

Then, in the Figure 54, the PRE1 assessment shows how quite good reciprocal Ia inhibition results were obtained also by applying -5 and 0 millisecond ISI. In these cases there was a 5% and 3% of reciprocal Ia inhibition increment with respect to the basal respectively. In this case, PRE1 assessment results were not maintained during the PRE2 assessment where there was a increment of 27% in the -5 millisecond results obtained, indicating a variability in the H-reflexes obtained previous to the intervention. Posteriorly, it will be discussed the reason of this variability that occurred in some of the performed experiments. In the POST assessment, there was a 10% of reciprocal Ia inhibition decrement with respect to the PRE2 results in both of the ISI. These results conclude that there was

a worsening of reciprocal Ia inhibition when afferent stimulation was applied during stance phase of GC, following Out-of-phase strategy. Finally, the POST30 demonstrated how 30 minutes after the stimulation intervention there was a recovery of the previous reciprocal Ia inhibition levels. At this point, the reciprocal Ia inhibition returned to be more or less 27% and 3% and with respect to the basal.

Finally, in the Figure 55, the PRE1 assessment shows good reciprocal Ia inhibition results by also applying -5 and 0 millisecond ISI. In these cases there was a 20% and 55% of reciprocal Ia inhibition increment with respect to the basal respectively. At this experiment, there was a repeatability between PRE1 and PRE2 results maintaining the levels of reciprocal Ia inhibition. Regarding the POST assessment, there was also a maintenance of reciprocal Ia inhibition levels, around 55% in the 0 ms ISI. However, although the levels at -5 ms ISI were not so well-maintained, they were not so drastic being just around a 5% of modulation with respect to the PRE2 results. These results conclude that, more or less, there was a maintenance of reciprocal Ia inhibition when afferent stimulation was applied following Control strategy. In this case, as there were not stimulation effects, the POST30 results were also maintained around 10% and 55% with respect to the basal.

However, it is important to remember that these three graphics represent three different experiments performed into three different subjects and they are only a representation of results obtained by applying each strategy. In the following Section 5.1.3, the results obtained in the 20 different experiments will be presented analysing the percentage of reciprocal Ia inhibition improvement or worsening with respect to the basal. In addition, in the Section 6, the variability of the H-reflexes generating differences between PRE1 and PRE2 assessments will be also explained, as well as some ideas to solve it.

In general, removing this H-reflex variability aspect, the designed and developed gait neuromodulation platform had a quite good performance. This platform allowed to perfectly perform the three different process of the experiment providing quite good and promising results.

5.1.2 Usability and comfort of the developed gait neuromodulation platform

The perception of usability and comfort during the experiment had by each of the participants was also noted. During each experiment, there was a data sheet where all the information relevant to the participant and the experiment was collected, it can be seen at the 6.1. The main function of this data sheet was to collect data such as stimulus intensity, velocity of treadmill, and other variables regarding the experimental protocol. However, in the comments of each process, information regarding the usability and comfort of the patients while using the platform was also introduced.

The following Table 2 and Table 3 represent the data acquired where the participants responded to some statements regarding usability and comfort of the platform. The range of response of this questionnaire went from 0 being completely disagree, to 5 being completely agree. In order to better visualize the information, there are two different tables where the first one represents the responses of the first 10 participants and the second one represents those responses of the last 10 participants.

Table 2: Results regarding the usability and comfort of the platform from participant 1 to 10.

Statement	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
The duration of the experiment is adequate	4	3	4	4	3	4	4	4	4	4
Adequate information is given prior to the experiment	5	5	5	5	5	5	5	5	5	5
The space for conducting the experiment is adequate	4	3	3	4	2	3	2	3	3	4
The position of participant during the experiment is comfortable	5	4	4	4	3	5	5	5	4	4
Skin preparation prior to electrode placement is adequate	5	5	5	5	5	5	5	5	4	4
The location of the muscles prior to electrode placement is painless	5	5	5	5	5	5	5	5	5	5
Nerve location prior to electrode placement is painless	3	3	4	4	2	3	4	3	4	2
Footswitch position on sock is suitable and comfortable	5	4	4	4	5	3	3	4	4	3
The intensity of stimulation is adequate and painless	5	4	4	4	3	5	5	5	5	4
Use of treadmill to perform stimulation during the gait cycle is the ideal equipment	2	3	5	5	4	4	3	5	5	5
Days after experiment, there is irritation or dryness in the location of the electrodes	0	0	0	0	0	0	1	0	0	0
Days after experimentation, there are discomfort in the muscles and nerves activated	0	0	0	0	0	0	0	0	0	0

Table 3: Results regarding the usability and comfort of the platform from participant 11 to 20.

Statement	P11	P12	P13	P14	P15	P16	P17	P18	P19	P20
The duration of the experiment is adequate	5	4	2	4	4	4	4	3	3	3
Adequate information is given prior to the experiment	5	4	5	5	5	5	5	5	5	5
The space for conducting the experiment is adequate	4	2	2	3	2	3	3	2	3	3
The position of participant during the experiment is comfortable	5	4	5	4	4	4	4	5	5	5
Skin preparation prior to electrode placement is adequate	5	5	5	5	5	5	5	4	5	4
The location of the muscles prior to electrode placement is painless	5	5	5	5	5	5	5	5	5	5
Nerve location prior to electrode placement is painless	4	4	3	3	4	2	3	1	3	2
Footswitch position on sock is suitable and comfortable	5	4	4	3	4	4	4	4	3	4
The intensity of stimulation is adequate and painless	5	4	4	4	4	3	4	3	4	4
Use of treadmill to perform stimulation during the gait cycle is the ideal equipment	5	3	2	5	4	5	4	3	5	5
Days after experiment, there is irritation or dryness in the location of the electrodes	0	1	0	0	0	2	0	2	0	0
Days after experimentation, there are discomfort in the muscles and nerves activated	0	0	0	0	0	2	0	4	0	0

Regarding these tables, it cannot be properly concluded which is the mean response of all the participants because there are loads of numbers. For this reason, it was essential to perform a better visualization of these responses using visual graphics such as circular graphics. The Figure 56 represents the percentage of each value 0, 1, 2, 3, 4 and 5 for each of the statements presented in the table considering the 20 participants of the project. Each of the mentioned values is represented by a colour, being light blue the 0 value meaning completely disagree, and dark purple the 5 value meaning completely agree with the statement.

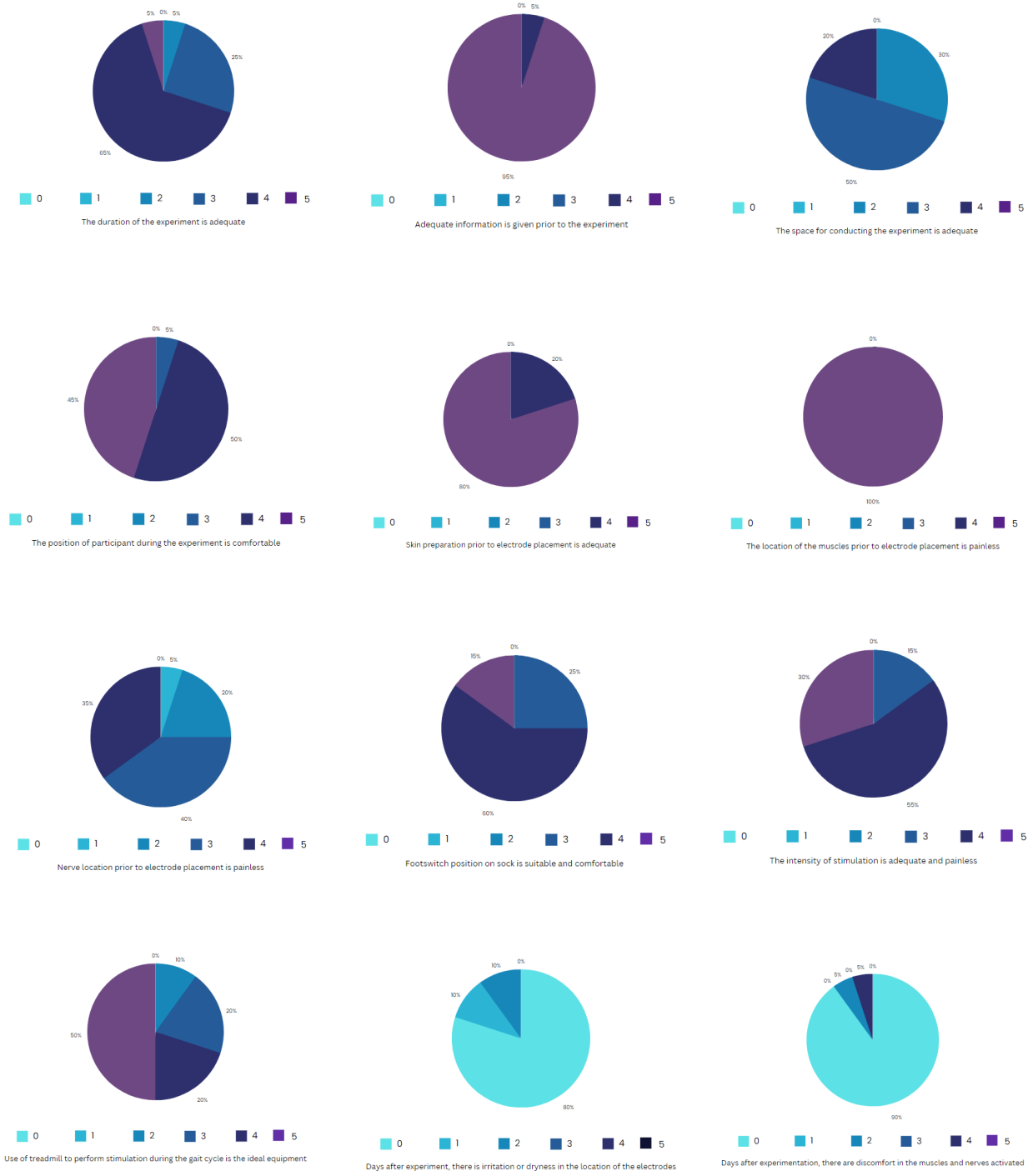

Figure 56: Results analysing the usability and comfort of the gait neuromodulation platform. From top to bottom and from left to right, the circular graphics related to each statement of the previous tables following a continuous order.

Analysing these circular graphics it can be seen how most of the participants considered that experiment had an adequate duration, that all the necessary information was provided, that the position required to perform the experiment was comfortable, that skin preparation before experiment was properly performed, that localization of the muscles to position electrodes was excellent, as well as the positioning of footswitch on the participants' foot, and that stimulus intensity was adequate and painless avoiding irritation, dryness, and discomfort in the activated zones days after the experiment.

However, some of the factors considered as improvable were the space of the lab where the experiment was performed as many participants considered it small, the nerve location prior to electrode placement as many participants considered it a painful process, and the use of treadmill to perform stimulation during GC as many of participants found it difficult to use. Nevertheless, it is important to mention that these last two factors varied depending on the participant. On the one hand, in the case of the treadmill use, most of the participants that considered treadmill was difficult to use were the oldest ones as they were not familiarized with its use. On the other hand, in the case of pain sensation while locating the nerve previous to electrode positioning, most of the participants that feel more pain were those with highest corporal mass compared to athletic ones because their nerves were deeper and therefore required more time to be found.

Thanks to these results it can be generally conclude, in spite of some tiny points that should be address when applying the platform to pathological subjects, that the gait neuromodulation platform is very easy to use and that it is comfortable for the participants.

5.1.3 Effects of sensory electrical intervention on spinal plasticity: modulation of reciprocal inhibition

The most important result regarding the gait neuromodulation platform is its effectiveness modulating reciprocal Ia inhibition of participants. In order to obtain the results regarding this modulation, the data belonging to the ISI with the best PRE1 or PRE2 reciprocal Ia inhibition was selected. It is important to highlight that the best PRE reciprocal Ia inhibition corresponded to that H-reflex whose amplitude was between the 'normal' or 'standard' ranges, that in healthy subjects usually is between 80% and 90% of the test H-reflex amplitude. This is clearer when observing Figure 53, Figure 54, and Figure 55, where the selected as 'best' reciprocal Ia inhibition were that where conditioning H-reflex amplitude was between the 80% and 90% with respect to the test or basal H-reflex amplitude represented by a blue line in the graphics. In most of the cases, the ISI selected to acquire the results was -5 or 0 milliseconds, being this last one the most typical one.

Once the most adequate ISI was selected, data corresponding to EMG at each condition was extracted. This corresponding data was based on EMG signal at this conditioning stimulation and the reciprocal Ia inhibition obtained when performing this stimulation. Remember that reciprocal Ia inhibition was obtained by comparing the difference between the amplitude of the SOL H-reflex when applying the conditioning stimulus to the amplitude of the SOL H-reflex after test stimulation.

On the one hand, if the percentage of this difference was below the 100%, there was a reciprocal Ia inhibition because SOL H-reflex at conditioned stimulation was smaller than SOL H-reflex at test stimulation. It means, when agonist muscle such as TA was activated at the conditioned stimulus, its antagonist muscle SOL deactivated reducing its H-reflex. This concluded that there was a reciprocal Ia inhibition occurring. On the other hand, if the percentage of this difference was above the 100%, there was a reciprocal Ia facilitation where activation of agonist muscle was activating the antagonist muscle, concluding that there was a malfunctioning of reciprocal inhibition mechanisms.

In order to extract and generate appropriate results, these measurements of the difference between conditioned H-reflex and test H-reflex were collected into three different dataframes represented in Figure 4. The upper dataframe corresponds to the In-phase experiments, the middle corresponds to Out-of-phase experiments, and the lower corresponds to Control experiments. In all these data, the value 1 corresponds to the amplitude of the test H-reflex, values < 1 corresponds to decrements in the amplitude of conditioned H-reflex and therefore inhibition, and values > 1 to increments in the amplitude of conditioned H-reflex and therefore facilitation.

Table 4: Dataframes with the values of difference between conditioned SOL H-reflex and test SOL H-reflex.

	Strategy	Time	Subject1	Subject2	Subject3	Subject4	Subject5	Subject6	Subject7	Subject8
0	InPhase	PRE	0.906	0.928	1.004	0.878	0.722	0.546	0.899	0.968
1	InPhase	POST	0.679	0.745	0.947	0.631	0.655	0.221	0.796	0.668
2	InPhase	POST30	0.958	0.884	0.980	0.858	0.773	0.473	0.875	0.948

	Strategy	Time	Subject9	Subject10	Subject11	Subject12	Subject13	Subject14	Subject14.1	Subject16
3	OutOfPhase	PRE	0.967	0.823	0.681	0.914	0.735	0.932	0.786	0.094
4	OutOfPhase	POST	1.159	1.016	0.932	1.642	0.970	1.601	0.863	0.473
5	OutOfPhase	POST30	1.079	0.920	0.710	1.264	0.934	1.525	0.798	0.221

	Strategy	Time	Subject17	Subject18	Subject19	Subject20
6	Control	PRE	0.896	1.007	0.605	0.924
7	Control	POST	1.006	1.038	0.606	0.906
8	Control	POST30	0.969	1.011	0.628	0.894

In this table it can be seen how first dataframe contains the data of the eight different subjects performing In-phase strategy, the second dataframe contains the data of the eight participants performing Out-of-phase strategy, and the third dataframe contains the data of the four participants of Control strategy. Each of these dataframes includes the data corresponding to the PRE2, POST, and POST30 assessments of the SOL H-reflex.

As it has been said above, in addition to this numeric data, the EMG signals of each participant obtained at the PRE2, POST, and POST30 assessments were collected. These signals belonging to three different participants are represented at Figure 57 illustrating the performance of conditioned SOL H-reflex at each stimulation strategy.

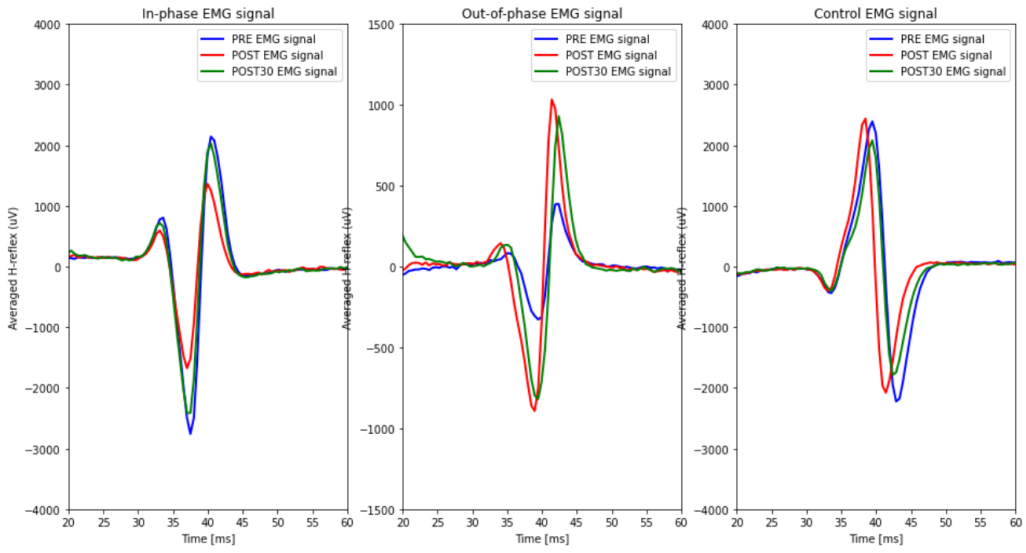

Figure 57: Conditioned SOL H-reflex at each stimulation strategy. From left to right: In-phase, Out-of-phase, and Control strategy. In each plot, blue line represents PRE assessment, red line represents POST assessment, and green line represents POST30 assessment.

The observation that can be made when regarding this figure is that only H-reflexes, between 20 and 60 milliseconds of the complete SOL EMG, were represented. As explained in the caption, in these three graphics the blue signal corresponds to the H-reflex obtained previous to the stimulation intervention, the red signal corresponds to the H-reflex obtained immediately after the stimulation intervention, and the green line corresponds to the H-reflex obtained 30 minutes after the stimulation intervention.

Regarding the SOL H-reflexes during the three assessments of the In-phase strategy (left plot in Figure 57), it can be seen how red signal has lower amplitude than blue and green signals. That means that immediately after the swing-phase stimulation, the SOL H-reflex decreased its amplitude compared to its amplitude before the In-phase stimulation. For this reason, regarding EMG signals, it could be concluded that In-phase stimulation produced an improvement of reciprocal Ia inhibition by reducing the SOL H-reflex when activating its antagonist. In addition, the figure demonstrates that 30 minutes after the stimulation, the H-reflex returned to its origin, being exactly the same as the blue signals.

During the Out-of-phase strategy, the SOL H-reflexes obtained at different assessments (middle plot in Figure 57) illustrate how the red signal increases notably its amplitude with respect to the original H-reflex or blue signal. This indicates that after applying CPN stimulation during the stance phase of GC, there was a worsening of reciprocal Ia inhibition or even an induction of facilitation because SOL H-reflex increased its amplitude when there was an activation of its antagonist. Besides, this figure also indicates that 30 minutes after the stimulation, the H-reflex tended to return to its origin, as it can be seen observing how the green signal starts to decrease again until reaching the level of the blue signal.

When analysing the SOL H-reflexes obtained at different assessments during Control strategy (right plot in Figure 57), however, it can be seen how all the three H-reflex stay more or less constant. The reason is that during this Control strategy, stimulation was not delivered at any point. Therefore, the amplitude of the PRE, POST, and POST30 H-reflex was unchanging as it can be perfectly observed in the figure.

These first observations indicate there is a modulation of reciprocal Ia inhibition depending on the stimulation strategy applied, being In-phase strategy an activator and Out-phase strategy an inhibitor of reciprocal Ia inhibition. Further inspection of reciprocal Ia inhibition was performed as follows:

Representation of reciprocal Ia inhibition for each participant at each assessment is shown in Table 4. This representation is illustrated in Figure 58 where reciprocal Ia inhibition is analysed by representing the percentage of conditioned H-reflex relative to the basal.

This figure is divided into three different sub-figures that represent the results of each strategy, left figure represents In-phase stimulation strategy, middle figure represents Out-of-phase stimulation strategy, and right figure represents Control strategy. The data of each participant include three different point corresponding to the PRE, POST, and POST30 assessments. All the exact data is represented by dark blue points, while the boxplot of each variable (preInphase, postInphase, post30Inphase, preOutphase, postOutphase, post30Outphase, preControl, postControl, and post30Control) containing the median, quartile, and outlier values is represented by light blue boxes. Finally, the median values from each variables are united by a red line that facilitate the visualization of differences between variables.

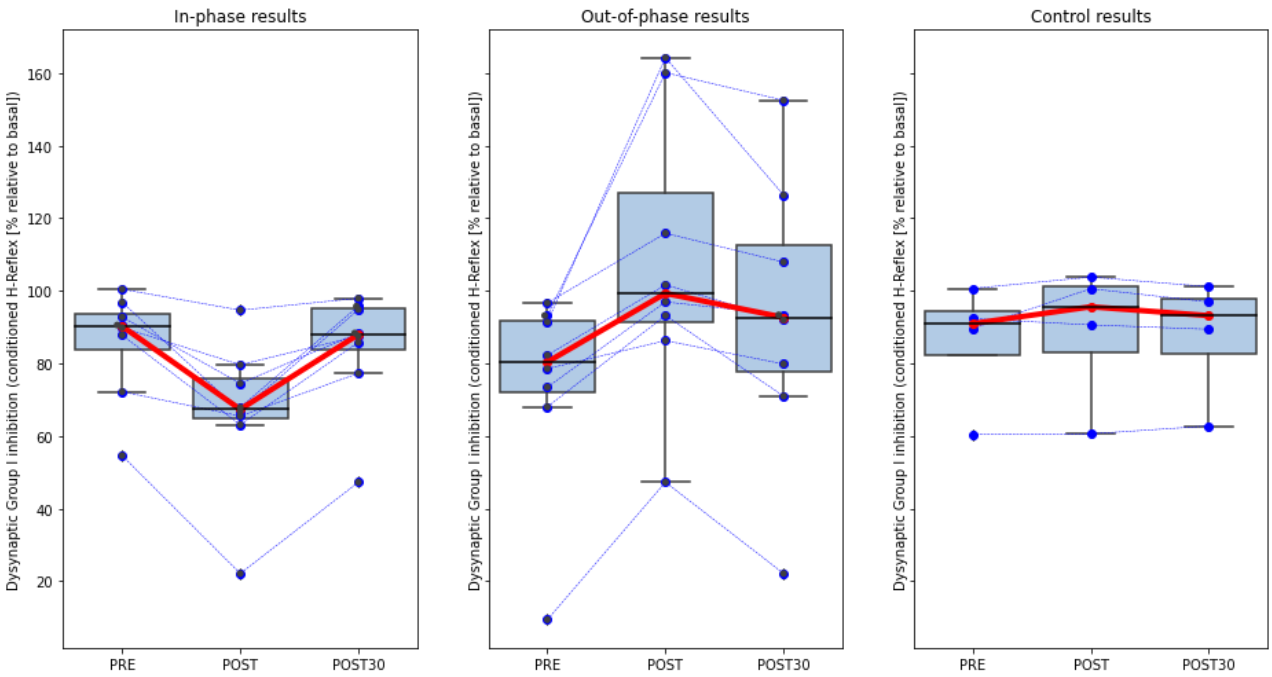

Figure 58: Results of reciprocal Ia inhibition with respect to basal. From left to right: In-phase, Out-of-phase, and Control strategies. In each figure PRE, POST, and POST30 assessments are represented.

The results in Figure 58 depend on the stimulation strategy performed. Regarding the In-phase results, it can be seen how except for one subject there was not excessive variability between the participants. In addition, all the participant followed the same trajectory: there was a previous value of reciprocal Ia inhibition, that immediately after the stimulation of CPN during swing phase improved more or less in a 20%. This is the same as saying that the percentage of difference between the conditioned H-reflex relative to basal previous to the intervention was of 10% (from 100% in basal to 90% in conditioned test) while immediately after the intervention this percentage increased to 30% (from 100% in basal to 70% in conditioned test). Then, 30 minutes after the application of stimulation, the percentage returned to the level previous to the stimulation, it means a percentage of difference between the conditioned H-reflex relative to basal of 10%. This percentage demonstrate that In-phase electrical stimulation during swing phase of GC, increased the reciprocal Ia inhibition of participants. In addition, it demonstrated the short duration of this effect by showing how 30 minutes after intervention the reciprocal inhibition levels returned to its origin.

In the case of Out-of-phase results, there was more variability between participants. However, in spite of the outliers that are out of the scope of this project, all the participant followed the same trajectory: the initial value of reciprocal Ia inhibition changed immediately after the stimulation of CPN during stance phase, worsening more or less a 20%. This means that percentage of difference between the conditioned H-reflex relative to basal previous to the intervention was of 20% (from 100% in basal to 80% in conditioned test) while immediately after the intervention this percentage decreased to 0% (from 100% in basal to 100% in conditioned test). These percentages demonstrate that Out-of-phase electrical stimulation during stance phase of GC, reduced the reciprocal Ia inhibition of participants producing a reciprocal facilitation. The short-term duration of intervention effects are also demonstrated by showing that 30 minutes after the application of stimulation, the percentage tended to return to the level previous to the stimulation, it means a percentage of difference between the conditioned H-reflex relative to basal of 20%.

Analysing the Control results, it can be seen how only one of the subjects had results that vary with respect to other participants. However, as in the previous strategies, all the participants followed the same trajectory: the initial value of reciprocal Ia inhibition remained constant at the POST and POST30 assessments, with a percentage of difference between the conditioned H-reflex relative to basal of 10% (from 100% in basal to 90% in conditioned test). These percentages demonstrate that stimulation had effect as when there was not stimulation the percentages stayed constant.

The same analysis could be done by representing values obtained at POST and POST30 assessments with respect to the ones obtained at PRE2 assessment. The Figure 59 illustrates the differences in reciprocal Ia inhibition between the assessments performed after the stimulation intervention and the assessment performed previous to the intervention. This illustration and the red line of Figure 58 are performed considering the median values of variables instead of the mean values. Following the theory, median values are more robust than mean values because the mean is sensitive to all elements in the sample, whereas the median uses one or two sample values. For this reason, due to the high variability in our experiment between participants in some variables such as postOutphase, median values are used.

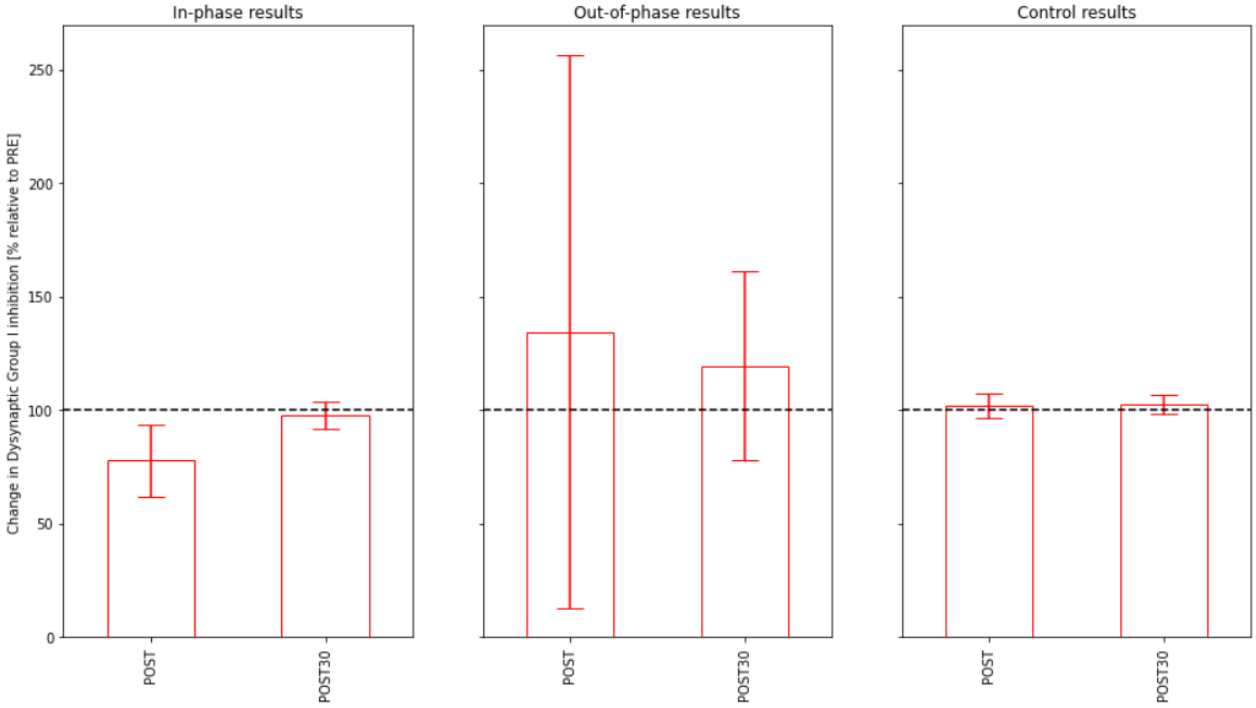

Figure 59: Results of reciprocal Ia inhibition with respect to PRE assessment. From left to right: In-phase, Out-of-phase, and Control strategies. In each figure POST and POST30 assessments are represented with basal or PRE represented by dashed line at 100%.

In the In-phase strategy, when electrical stimulation was delivered to CPN during swing phase of GC, there was a 20% of improvement of reciprocal Ia inhibition (from 100% in PRE to 80% in POST assessment). This also demonstrates the short-term effect of In-phase stimulation as POST30 results returned to the exact values of PRE assessment. At the Out-of-phase strategy, when electrical stimulation was delivered to CPN during stance phase of GC, there was more than a 50% of worsening of reciprocal Ia inhibition (from 100% in PRE to 150% in POST assessment). In Out-of-phase intervention there was also a tendency of POST30 to return to the origin, but it seemed to require more time to recover the levels previous to the intervention. Finally, it is clearly seen how the Control intervention did not modulate the levels of reciprocal Ia inhibition, demonstrating that afferent electrical stimulation has a real effect.

Although these results demonstrate that electrical stimulation has real effects and that these effects generate differences between PRE and POST values of both therapies, In-phase and Out-of-phase, it was necessary to perform a statistical analysis to conclude that these differences were statistically significant.

As explained in 4.2.4, the first step of statistical analysis was to check if the variables followed a normal distribution in order to know which type of statistical test apply. The results of the visual methods to check normality are illustrated in Figure 60. This method analysed the histogram, the boxplots, and the QQ plot of each of the variables with respect to the normality represented by a red line in all the figures.

Regarding Figure 60, it can be concluded that some variables such as post30Outphase followed a normal distribution. However, the scarce number of samples in the Control strategy (only 4 participants), made it difficult to check normality in some variables such as preControl, postControl, and post30Control. For this reason, it was necessary to use not only visual methods but also statistical methods such as Kolmogorov–Smirnov test and Shapiro–Wilk test. The results offered by these both methods are represented in Table 5.

Table 5: Results Kolmogorov–Smirnov and Shapiro-Wilk normality test.

Variable	KS Statistic	KS p-value	KS Conclusion	SW Statistic	SW p-value	SW Conclusion
preInphase	0.311	0.348	Normal distribution	0.837	0.071	Normal distribution
postInphase	0.300	0.389	Normal distribution	0.855	0.107	Normal distribution
post30Inphase	0.288	0.441	Normal distribution	0.775	0.015	NOT Normal distribution
preOutphase	0.283	0.461	Normal distribution	0.749	0.008	NOT Normal distribution
postOutphase	0.197	0.861	Normal distribution	0.923	0.455	Normal distribution
post30Outphase	0.146	0.985	Normal distribution	0.974	0.926	Normal distribution
preControl	0.349	0.606	Normal distribution	0.857	0.250	Normal distribution
postControl	0.290	0.801	Normal distribution	0.845	0.211	Normal distribution
post30Control	0.299	0.774	Normal distribution	0.857	0.251	Normal distribution

Both methods used in the normality test tried to analyse if the null hypothesis (H0), that states that a variable follows a normal distribution, was confirmed or rejected. The results provided by both test were the Statistic and the p-value. The important value during this normality test was the p-value that determined if the variable followed a normal distribution or not. When this value was > than 0.05, there were not significant differences between normal distribution and the distribution of the variable, concluding that variable followed a normal distribution. However; when p-value was < than 0.05, there were significant differences between normal distribution and the distribution of the variable, concluding that variable did not follow a normal distribution.

The results presented in Table 5 are in agreement with the theory, that establishes that Kolmogorov-Smirnov test has not much power and is really sensitive to outliers, needing a large number of observations (n>50) to reject the null hypothesis. This is the reason why, as in this experiment there were only 8 number of observations for In-phase strategy or Out-of-phase strategy and only 4 observations for Control strategy, the Kolmogorov-Smirnov test was not useful. This can be confirmed by the table where this test determined that all the analysed variables followed a normal distribution.

However, Shapiro-Wilk is more powerful working best for data set with less than 50 number of observations. This is the reason why, this was the test that must be used during this experiment. It can be seen how, using this test, two different variables (post30Inphase and preOutphase) did not follow a normal distribution.

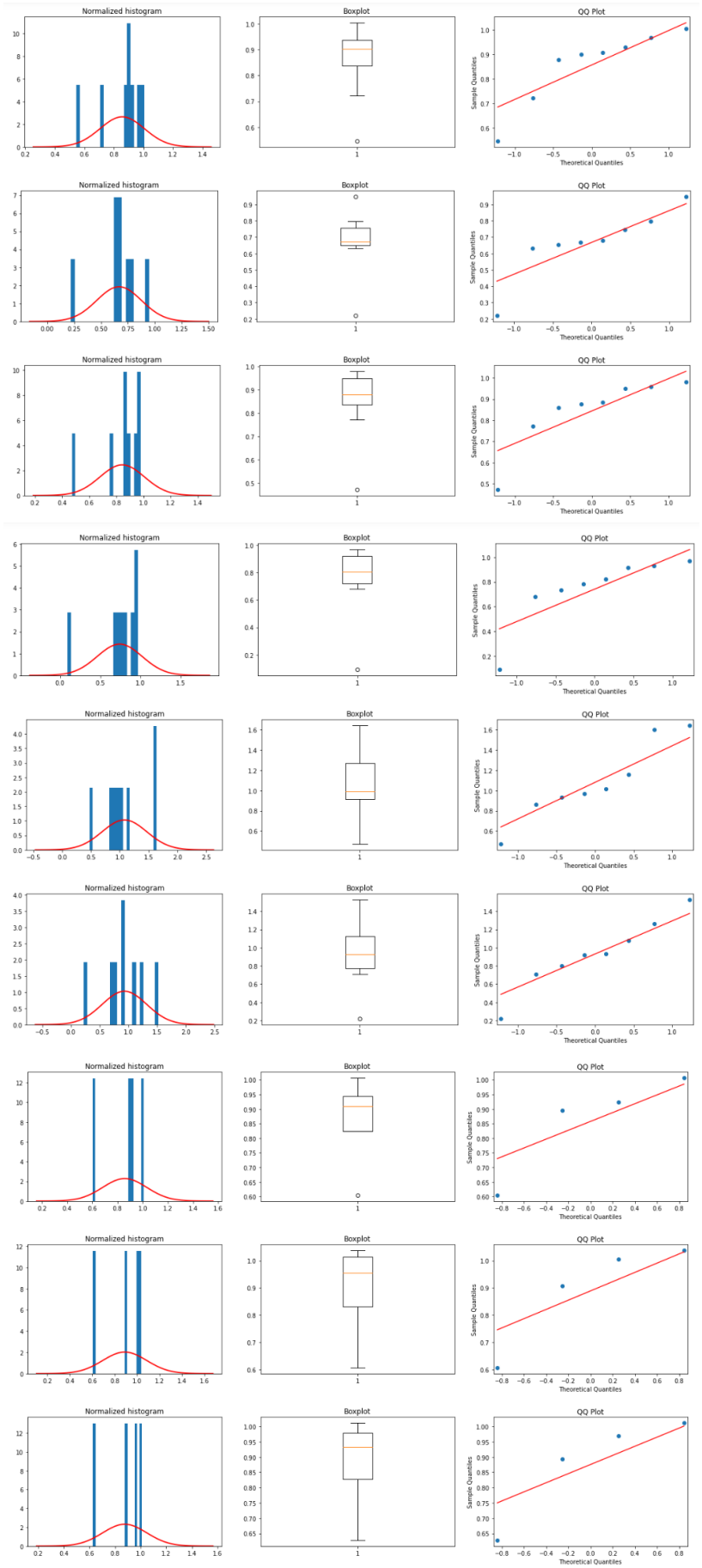

Figure 60: Results of normal distribution analysis of variables, from top to bottom: preInphase, postInphase, post30Inphase, preOutphase, postOutphase, post30Outphase, preControl, postControl, and post30Control.

Once it was confirmed, thanks to the Shapiro-Wilk test, that some variables did not follow a normal distribution, it was necessary to apply a non-parametric statistical test. This test would evaluate all the variables and determine if there were some differences between variables. However, it did not specify which were the variables that were statistically different. First of all a Friedman Chi-Square test was performed to compare dependent samples of three or more dependent groups. This means that it was applied in order to check if there were significant differences between PRE, POST, and POST30 variables within the same stimulation strategy. The results provided by this test are represented in Table 6.

Table 6: Results of Friedman Chi-Square test.

Strategy	Statistic	p-value	Conclusion
In-phase	13.0	0.0015	There are significant differences
Out-of-phase	16.0	0.0003	There are significant differences
Control	2.0	0.3679	There are NOT significant differences

The results confirm that there were significant changes between PRE, POST, and POST30 variables of In-phase and Out-of-phase stimulation strategy. In addition, they confirm that, as p-value was > 0.05, there were not significant differences between Control variables. These results agree with the previously explained, the stimulation intervention has a real effect on modulation reciprocal Ia inhibition and this modulation depends on the phase of GC where stimulation was applied. However, as previously explained, this test did not provide information about which were the variables with statistical differences. For this reason, a post hoc comparison using Conover's test was performed. This test was applied to the combination of PRE and POST variables of both strategies, In-phase and Out-of-phase. The results of this test are provided by Table 7 and conclude that the combination of preInphase and postInphase had a p-value of 0.0551 which was higher than 0.05, concluding that there were not significant differences between these variables, and that the combination of preOutphase and postOutphase had a p-value of 0.0213 which was lower than 0.05, concluding that there were significant differences between these variables.

Table 7: Results of Conover's multiple comparison test I.

Strategy	Combination	p-value	Conclusion
In-phase	PRE and POST	0.0551	There are NOT significant differences
Out-of-phase	PRE and POST	0.0213	There are significant differences

In order to analyse differences between all the variables of all the differences strategies, it was necessary to perform a test that compared independent samples that could have different sizes and was able to compare three or more groups, such as the Kruskal-Wallis test. This test was applied only to preInphase, postInphase, post30Inphase, preOutphase, postOutphase, and post30Outphase variables because previous test determined that Control variables did not present differences. The result of this test was a Statistic value of 11.4521, and a p-value of 0.0431. As this p-value was lower than 0.05, this means that there were significant differences between these variables studied. However, as previously explained, this test did not provide information of which variables presented higher differences. For this reason, another post hoc comparison using Conover's test was performed. The most significant results of this multiple comparison (different than 1) are provided by Table 8.

Table 8: Results of Conover's multiple comparison test II.

Combination	p-value	Conclusion
Post30In and PostIn	0.9998	There are NOT significant differences
Post30Out and PostIn	0.2809	There are NOT significant differences
PostIn and PostOut	0.0225	There are significant differences
PostIn and PreIn	0.8314	There are NOT significant differences
PostOut and PreOut	0.3794	There are NOT significant differences

The results of this table conclude that the combination of postInphase and postOutphase variables had a p-value of 0.0225 which was lower than 0.05, concluding that there were significant differences between these variables.

The general conclusion of all these results analysing modulation of reciprocal Ia inhibition after afferent stimulation intervention is that there are significant changes between preOutphase and postOutphase variables. This means that stimulation during stance phase produces significant changes in the reciprocal Ia inhibition. However, the combination of preInphase and postInphase gave a p-value that was in the limit of significant and not significant differences. However, as it was higher than 0.05 (by thousandths) it is established that swing phase does not produce significant changes in reciprocal Ia inhibition. This conclusion could be changed to significant changes if more observations were performed, the lack of participants make the results not as optimal as desired. Finally, it is also concluded that there are significant differences between postInphase and postOutphase, demonstrating that the effects produced by stimulation at swing and stance phase are completely opposite. This coincide with the idea that swing phase stimulation generates reciprocal Ia inhibition while stance phase stimulation generates reciprocal Ia facilitation. These final conclusions can be visualized in Figure 61 where significant differences are represented by a black line with an asterisk uniting the variables that present significant differences.

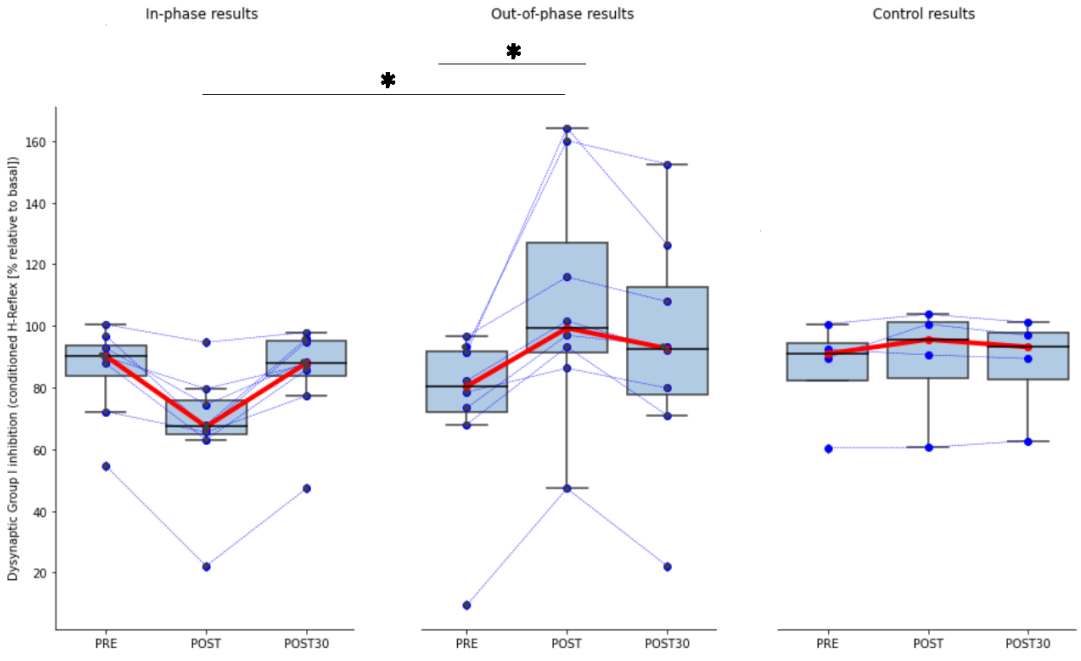

Figure 61: Final results regarding modulation of reciprocal Ia inhibition after intervention.

6 Discussion

The main objective of this project was to evaluate the effects of afferent electrical stimulation on modulating reciprocal Ia inhibition. However, this general objective was decomposed in particular objectives of the project.

The first process of the project was the analysis of muscle activation during GC. The designed and developed gait neuromodulation platform has demonstrated to provide accurate results in the identification of both phases of GC as well as in the recording of muscle activation at these phases. The results obtained from this gait analysis coincide with the theory explained in section 3.1. On the one hand, the recordings of the TA muscle concluded that this muscle was activated only during the first 10% of stance phase in order to provide support to the limb, and during the whole swing phase in order to allow the limb to advance. On the other hand, the recordings of the SOL muscle concluded that this muscle was activated only during the stance phase of the GC where it supports the body weight in one limb. This means that TA muscle works as a plantar-extensor and SOL muscle works as a dorsi-extensor during the GC in 'healthy' subjects.

The results in Section 5.1.1 demonstrate that gait neuromodulation platform based on two footswitch located at toe and heel of foot and sEMG electrodes located over the studied muscles is extremely efficient to perform an analysis of muscle activation depending on the phases of GC. In addition to its efficiency, this process is very easy to perform and very comfortable for the participants making it a possible gold standard to the analysis of muscle activation during the GC. In this project, only TA and SOL muscles have been analysed because the idea was to evaluate an agonist muscle in coordination with its antagonist muscle. However, this analysis could be performed at any muscle of the human body, determining the activation pattern of each muscle.

The next process was the application of afferent electrical stimulation on the CPN innervating the TA muscle during walking. The developed gait neuromodulation platform has demonstrated to properly identify swing and stance phases and based on this, deliver afferent stimulation at the required phase of GC depending on the strategy performed at each experiment. The results provided by the EMG signals of the TA muscle acquired during the stimulation intervention and illustrated in section 5.1.1 confirmed that stimulation at In-phase strategy has been delivered during the swing phase of GC, and that stimulation at Out-of-phase strategy has been delivered during the stance phase of GC. In addition, it has demonstrated that Control strategy did not deliver any kind of afferent stimulation.

The adequate combination of footswitch, sEMG recording electrodes, stimulation electrodes, RehaStim stimulator, and controlling softwares such as Arduino and MatLab, confirms that gait neuromodulation platform provides a robust performance delivering afferent electrical stimulation. In addition, the application of the stimulation using this platform is really easy for professionals and very comfortable for the participants, making it an excellent options to the stimulation of any kind of nerve in the human body at different stimulation patterns.

The final process related to the main objective of this Master Thesis was the assessment of reciprocal Ia inhibition after the application of afferent electrical stimulation. The results obtained during this evaluation demonstrate that application of sensory electrical stimulation had a real effect on the modulation of reciprocal Ia inhibition. This coincide with literature such as the study conducted by Thierry Paillard (2021) [8] or the study performed by Monica A.Perez et al (2004) [9] that conclude that application of afferent electrical stimulation in a repetitive and patterned manner produce changes in the plasticity of spinal circuits producing a modulation of reciprocal Ia inhibition. In terms of type of modulation depending on the phase of GC where afferent stimulation is delivered, there were two different results as represented in Figure 58. On the one hand, when electrical stimulation was delivered during the swing phase of GC, there was an improvement or strengthening of reciprocal Ia inhibition.

This means that when stimulation was applied to the TA muscle while it was activated, the antagonist muscle deactivated even more thanks to a potentiation of reciprocal Ia inhibition. On the other hand, when electrical stimulation was delivered during the stance phase of GC, there was a worsening of reciprocal Ia inhibition. This means that when stimulation was applied to the TA muscle while it was deactivated, the antagonist muscle was more activated due to a decrease of reciprocal Ia inhibition. These results completely agree with results obtained by several studies such as the study performed by Obata et al (2018) [10], the study performed by Monica A.Perez et al (2003) [11], or the study conducted by Clarissa Crone et al (1986) [12]. These studies demonstrated higher improvement of reciprocal Ia inhibition after the application of afferent electrical stimulation during the swing phase of GC as well as the importance of providing this stimulation in combination to cyclic movements such as walking that provide a sensory feedback that potentiate the patterned-effects of stimulation.

Another point of the obtained results that coincide with the literature is the short-term duration of effects provided by electrical stimulation. In the results provided by Figure 59 it can be seen how 30 minutes after the application of stimulation intervention, the levels of reciprocal Ia inhibition returned to the PRE assessment levels. This coincide with the study performed by Tomofumi Yamaguchi et al (2016) [15] and the study conducted by Berthe Hanna-Boutros et al (2015) [16] that concluded that to induce long-term effects, it was necessary a combination of brain stimulation such as tDCS or TMS with patterned afferent electrical stimulation.

The results obtained in this project demonstrate that the developed gait neuromodulation platform has an appropriate performance and it is an efficient method evaluating the modulation of reciprocal Ia inhibition after afferent stimulation intervention.

6.1 Limitations of the current work

In spite of its performance and efficiency, this gait neuromodulation platform still presents limitations that should be solved to test it with pathological subjects.

During the first process related to analysis of muscle activation during the GC, the main limitation presented was the use of fragile footswitch sensors. Due to this characteristic, in order to solve the problem, it was required to generate numerous sensors in order to have replacement in case of rupture during the experiment, and to position very carefully these sensors in the sole of the participants' foot to prevent its early damage. Another solution to the problem, but more difficult to implement, would be the use of Inertial Measurement Units (IMUs) that capture velocities and accelerations related to the different phases of GC.

The use of sEMG electrodes could present another limitation during the process due to the low-quality signals that are captured in some cases. The solution followed during this project and that could be even improved, was the acquisition of more precise signals and the reduction of background noise. These processes were achieved by improving the electrode position localizing the muscles in a more meticulous manner, performing a more exhaustive skin preparation, and applying extra processing methods to the acquired signals.

Some limitations presented during the application of the afferent stimulation intervention were also related to the fragility of footswitches. Once they are damaged, the detection of phases of GC is poorer and more erroneous. For this reason, it was essential to check the correct functioning of each sensor previous to the intervention and to have several sensors in order to replace them when needed. However, the main limitation presented during this stimulation intervention, was the co-activation or simultaneous stimulation of nerves other than CPN.

This simultaneous activation occurs due to the anatomical characteristics of participant or due to the improper location of stimulation electrodes. During this experiment the location of these electrodes

were properly performed; however, depending on the leg anatomy of participant, the stimulation was more focused on the CPN or it could softly activate other nerves presented around the knee. Better stimulation results were obtained when stimulation was applied to skinny, young, and athletic participants. In the rest of participants, it was necessary to add a strap in order to properly fix the electrodes to the correct location.

During the assessment of the modulation of reciprocal Ia inhibition, thanks to the application of afferent electrical stimulation, the main limitation presented was the high variability between the H-reflexes of some participants. The main cause of this high variability was the position of the participants' leg during the assessment of SOL H-reflex. The leg under study should be fixed in order to prevent its movement and therefore, the variation of these reflexes. In addition to the reduction of variability in reflexes, this fixation would also help to reduce variability between participants by fixing the position required during the assessment. However, although the creation of a rigid structure to fix the participant's leg is necessary, during this experiment, a specific position was tried to be maintained for all the participants trying to reduce the variability of experiment.

Another important limitation regarding this process was the location of the PTN in order to elicit the test and conditioning SOL H-reflex. This nerve is very deep and surrounded by other nerves that innervate SOL antagonist muscles. For this reason, as it was essential to locate very careful and specifically this nerve, in some cases it was necessary the use of strap in order to deliver the stimulation to the proper nerve. Therefore, a very exact location of PTN has to be tried in order to obtain proper results. During this project, a really long time of experiment has been dedicated to the location and proper assessment of this nerve, giving as result proper SOL H-reflex and consequently proper results.

The results obtained using statistical analysis did not indicate all the expected consequences of afferent electrical stimulation on the modulation of reciprocal Ia inhibition, as explained and illustrated in section 5.1. This lack of expected results was also due to some limitations such as the reduced number of participants enrolled in the experiments, as well as the variability of SOL H-reflexes between participants explained before.

In spite of the statistical results, analysing all the graphs presented in 5.1.3, it can be seen how the application of afferent electrical stimulation provides important modulations on reciprocal Ia inhibition and that this developed gait neuromodulation platform is an effective method to apply and evaluate it.

Conclusions

This project related to my Master Thesis had different objectives that were described in Section 2.3. The main objective was the design and development of a gait neuromodulation platform that, in a future, could by applied to SCI patients in order to potentiate their reciprocal Ia inhibition allowing their walking recovery. However, the scope of this project only entailed the design, development, and validation of platform on healthy participants. The validation of this platform on pathological participants will depend on the results obtained at this project. The Figure 62 represents the conclusions obtained after the realization of the project based on each of the objectives proposed previous to the initialization of the project.

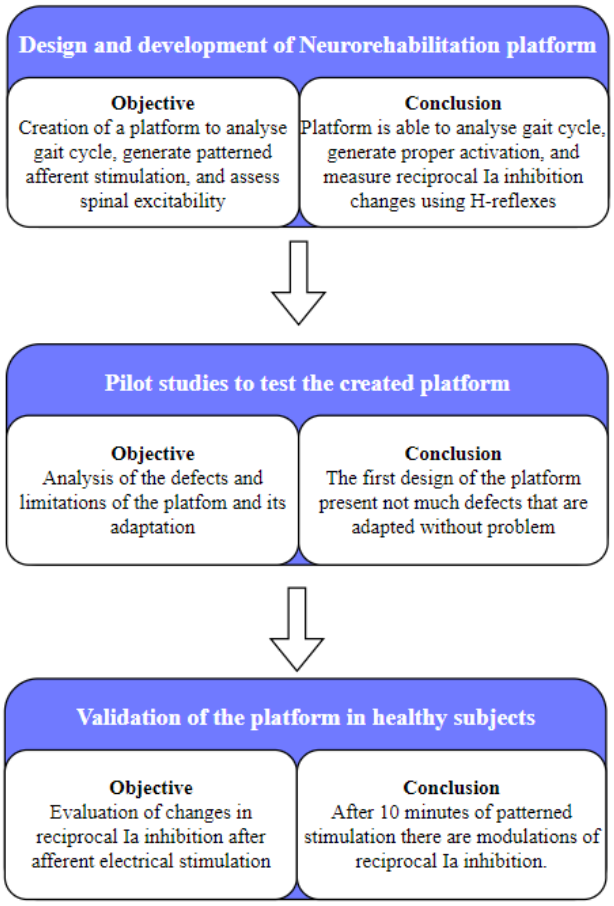

Figure 62: Objectives presented at the start of the project and conclusions obtained at its finalization.

Thanks to the realization of this project, a promising gait neuromodulation platform has been designed and developed. This platform is able to analyse muscle activation at the different phases of GC, to apply afferent electrical stimulation to the participants during different phases of walking, and then to assess the effect of this stimulation on the modulation of the reciprocal Ia inhibition. When applied to voluntary healthy participants, the platform has demonstrated an excellent performance, in spite of some limitation presented in Section 6.1. The main conclusion of this project is that when applying afferent electrical stimulation during 10 minutes in the swing phase of GC, there is an improvement of reciprocal Ia inhibition. This project enforce the idea that when afferent electrical stimulation is applied in a repeated and patterned manner combined with cyclic movements such as walking, the effects of the electrical stimulation are stronger than if stimulation was applied in isolation.

Potential application in neurorehabilitation

The main conclusion obtained in this project, there is an improvement of reciprocal Ia inhibition when afferent electrical stimulation is applied during swing phase of GC, is essential to establish this developed platform as a neurorehabilitation platform.

It is essential to remind that subjects suffering incomplete SCI have a reduction of reciprocal Ia inhibition due to the interruption of fibers in their spinal cord. This reduction generates a co-activation of antagonist muscles such as TA and SOL muscles that should be coordinated to perform movements such as walking. This is the main reason why these patients present severe walking impairments.

For this reason, it is essential to develop a therapy that enhance this reciprocal Ia inhibition in SCI patients enforcing their walking recovery. This project has demonstrated that created platform could be a suitable rehabilitation method to provide this coordination between antagonist muscles when afferent electrical stimulation is delivered to the CPN during the swing phase of GC. However, this therapy could only be applied to those incomplete SCI patients that are able to hold standing by their own or with the aid of a exoskeleton. The reason is that the therapy is based on the idea that stimulation during swing phase of GC produces an improvement of reciprocal Ia inhibition, and therefore, to perform the therapy is necessary to walk producing these swing phases.

One of the advantages of this therapy with respect to other ones such as FES stimulation is that the use of afferent stimulation has a low incidence of adverse reactions due to its lower intensity below the motor threshold [60]. This minimal intensity cannot cause any kind of damage, just a tingling sensation on nerve that can be perfectly tolerated by participants.

Even if a lot of research and validation on this gait neuromodulation platform is still necessary until its therapeutic implementation, it has to be validated with more healthy subjects and posteriorly with pathological subjects, the results obtained in this project are really promising concluding that an improvement of reciprocal Ia inhibition is possible by using afferent electrical stimulation.

Future work

This project demonstrates promising results in the application of afferent electrical stimulation to induce reciprocal Ia inhibition in SCI patients. However, until its establishment as neurological therapy, it is necessary tough future work.

First of all, the limitations that have been found in this project and that have been explained in Section 6.1 have to be overcome. To solve the problem with the fragility of footswitch sensors, it is necessary to create a structure such as a special sock or insole that allow the location of sensors as well as its proper connection to the different equipment. In this way, sensors would be more protected being more durable and providing more accurate phase differentiation. Then, in order to reduce the variability of H-reflexes between participants an within the same participant, it would be interesting to generate a simple attachment of the lower limb in order to fix the position of the leg and reduce its mobility when H-reflex assessment is being performed. In addition, to reduce variability between participants, it would be also interesting to incorporate the straps to localize and stimulate properly the PTN in all the participants. Finally, regarding the development of platform, it could be also interesting to reduce the number of cables during the experiment introducing some wireless equipment such as sEMG electrodes or footswitch sensors. This could improve even more the usability and comfort of participants while performing the experiment.

Once these technical aspects and limitations are resolved, it will be necessary to validate the platform with more healthy participants in order to obtain better results in terms of statistical analysis. It would be ideal that these participants had similar anatomical characteristics regarding height, weight and

athletic form in order to improve nerve localization and variability of H-reflex between participants. This increment of healthy participants and the improvement of previous limitations, should improve the statistical analysis, validating even more this gait neuromodulation platform.

After the validation in healthy participants, it will be necessary to perform a validation with pathological subjects suffering from SCI. Probably, this process will be more difficult due to the existing walking impairments in these patients. These impairments will force us to adapt the platform because processes such as the automatic detection of different phases of GC will not be as easily performed in these patients, producing a malfunction of stimulation. Once required adaptations are solved, the platform could be validated on incomplete SCI patients that have standing ability by themselves or with the help of exoskeleton.

If the results provided by this validation on pathological subjects also establish that there is an improvement on reciprocal Ia inhibition when afferent electrical stimulation is applied during the swing phase of GC, this gait neuromodulation platform could be established as a neurological therapy. Obviously, before being determined as a therapy, it must obtain all the required European and Spanish certificates to be apply as a therapy into SCI patients. This therapy would help incomplete SCI patients to recover their walking ability.

Budget and project timeline

Regulatory framework

Ethical issues are inevitable in any project involving human subjects. Therefore, the highest ethical standards have to be complied by means of Ethical Committee approvals. These approvals guarantee that experiments are carried out in accordance with the Helsinki declaration, and that personal data acquired is kept under strict confidentiality based on current Spanish legislation (LOPDCP 15/1999) and European normative (Regulation 2016/679).

The experiments performed during this Master Thesis with healthy subjects have been approved by the Ethical Committee of CSIC. Besides, all the devices were properly certified according to the current Spanish (Spanish Royal Decree 1591/2009) and European legislation (European Directive 93/42/EEC).

The participation of healthy subjects during the project was completely voluntary. All these participants were given the necessary explanations about the nature and purpose of the protocol, benefits, and possible adverse effects, both verbally and in a written information sheet. Once all doubts were cleared, participants signed an informed consent form (Law 14/2007), where they gave their consent to the experiment and to the use and storage of the collected data. This consent could be revoked at any time, before, during, and after the experimental protocol without further explanation.

The collected personal data from the experiments is assigned a unique code that make it not possible to relate the records with each subject. This encoding is performed following the current legislation on the rights and obligations in the field of Biomedical Research (Law 14/2007), Personal Data Protection and Guarantee of Digital Rights (Organic Law 3/2018) and Patient Autonomy and Clinical Information and Documentation (Law 41/2002). The storage of this data is in electronic format on the server of the NRG to ensure its proper conservation. Additionally, it may be uploaded to scientific databases, with the prior consent of the participant, for the purpose of dissemination and scientific exploitation thereof.

Socioeconomic framework

This project has huge economical impact as SCI is one of the conditions generating more direct and indirect costs. The direct medical costs are related to the necessity of patients and their relatives of health care and professionals; while the indirect costs are those resulting from the lost of productivity of SCI patients. The costs assumed by each country varies substantially worldwide.

Figure 63: Average direct acute healthcare costs per case by countries. Modified from [62].

The highest economic impact is presented in USA where total costs during the first year after injury are $334,170 for the minor injuries, rising to more than $1,023,924 for more severe injuries [42]. Then, there is an annual cost of $68,815 after the first year of SCI and a cost of $68543 per patient during the chronic rehabilitation. It is important to highlight that these costs depend on severity and level of injury, patients with ASIA A have a total cost of $2.3 million for thoracic injuries, $3.5 million for C5–C8 injuries and $4.7 million for C1–C4 injuries [6].

All these described costs can be significantly reduced with very small function improvements as the ideally provided by this gait neuromodulation platform.

Environmental impact

The main factors that contribute to the environmental impact of the project are the development of the gait neuromodulation platform and its energy consumption. Regarding the material used for the execution of the project, all the devices were acquired for clinical purposes previous to this project. For this reason the manufacturing and material pollution delivered from these devices is not a direct environmental impact of this project.

During the execution of the project, the main source of polluting waste are the sEMG and stimulation electrodes. These electrodes cannot be used in different patients because they are disposable, which generates important quantities of plastic and silver chloride residues. However, this waste has been minimized to the maximum by using the same electrodes in the same patient at the different trials. Then, regarding the energy consumption, it has also be minimized to the maximum by unplugging the devices each time they were not being used. In conclusion, as the final platform as not any effect on the environment, the environmental impact of this project can be considered low.

Social and Gender equality impact

The elevated impact of SCI on society has been previously demonstrated by analysing the high rates of incidence, mortality, and economic impact associated to this condition. For this reason, the ability of this gait neuromodulation platform to induce neuroplasticity of the spinal cord by delivering peripheral nerve stimulation, contributes to the safe and effective development of rehabilitation therapies. These therapies aim to generate functional recovery in incomplete SCI patients with mobility impairments, improving their quality of life. In addition, these therapies highly benefit the society by reducing costs generated by the lost of productivity of patients with these conditions.

Regarding the gender dimension, this project is completely neutral in terms of gender and its related institutions comply with the European policy of equal gender opportunities. In addition, the influence of the participants' gender in all the experiments performed have been carefully considered during the recruitment of volunteer patients where efforts have been made so that the number of participants of each gender is balanced.

Budget

The budget of the project is divided into technical equipment including software licenses, and personnel budget which are illustrated in following tables correspondingly. The technical equipment expenses are 6,332€ while the personnel expenses are 10,225€, generating a total budget of 16,557€.

This expenditure represents the budget needed if the project was started from zero. However, most of the equipment, software, and personnel were acquired to different and previous clinical purposes. In addition, this internship has been unpaid and the annual licence for MATLAB software is free for students by using the university account.

For this reason, the real cost of this project has been 750€ just considering the purchase of Digitimer DS7A, Arduino UNO microcontroller, and recording/stimulation electrodes.

Table 9: Budget for technical equipment.

Equipment	Cost per unit (€)	Time of use (months)	Depreciation period (months)	Number of units	Total cost (€)
Treadmill	300	8	48	1	50
Footswitch sensors	3	Several Uses	-	20	60
Quattrocento EMG amplifier	27,950	8	72	1	3,105
Additional Quattrocento equipment	550	8	72	-	62
Digitimer DS8R	8,455	8	72	1	940
Digitimer DS7A	3,564	8	72	1	396
Arduino UNO microcontroller	24	8	48	1	4
Arduino Proto Shield	10	8	48	1	2
RehaStim	4,260	8	48	1	710
sEMG electrode	1.5	Single use	-	100	150
Stimulation electrode	2	Single use	-	100	200
Laptop	710	8	72	1	79
MATLAB (annual licence)	860	8	12	1	574

Table 10: Budget for personnel.

Personnel	Salary (€ per hour)	Time (hours)	Total cost (€)
Graduate student	15	540	8100
Postdoctoral researcher	25	75	1875
Senior researcher	25	10	250

Project Timeline: Gantt diagram

The Gantt diagram representing the duration of this Master Thesis, as well as the duration of each of its processes, is shown in Figure 64.

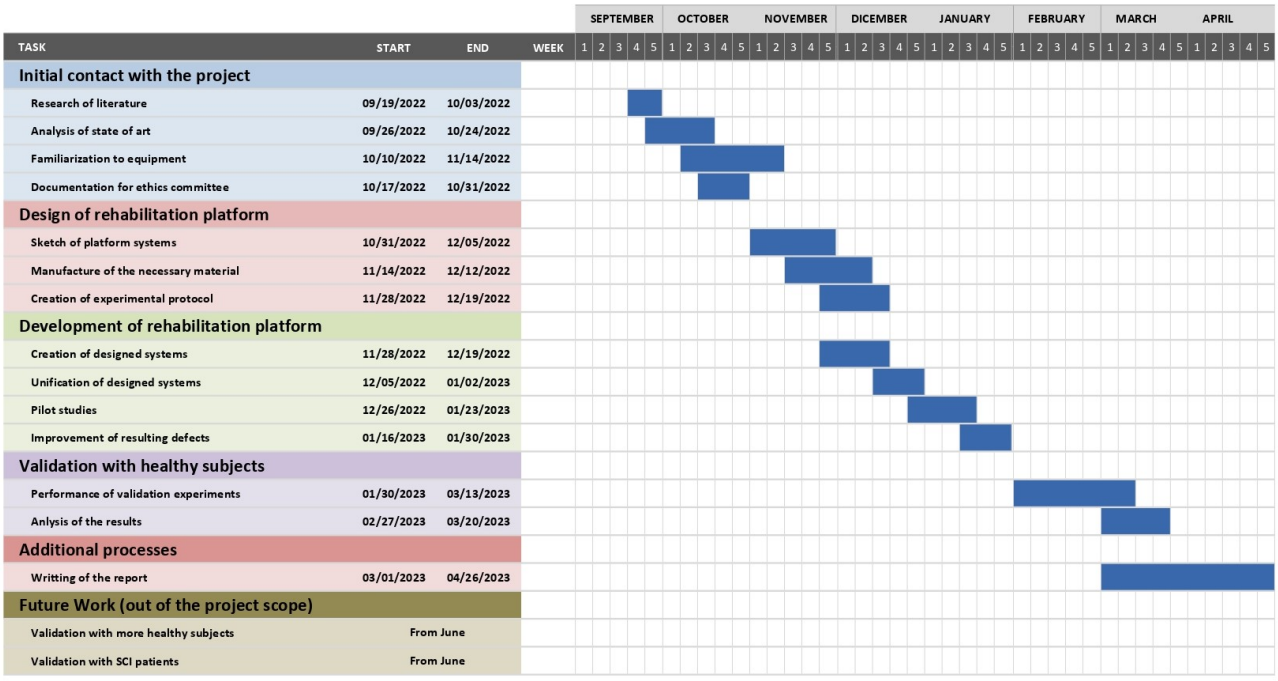

Figure 64: Gantt Diagram representing the project timeline.

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Annex

Several documents that are important for the evaluation of the project but that cannot be included in the report due to its extension are:

- Information Sheet provided to the voluntary participant in order to inform about all the processes, equipment, and time required.
- Experimental protocol followed during the project in order to have an ordered and clear idea of the steps to follow at each time.
- Data collection sheet where all the data related to participant, the parameters of the experiment, and the comfort values were introduced.

In spite of the fact that it is not possible to show these files at this report, this link `https://drive.google.com/drive/folders/1DiFCDF5zKimYQs0miVOewL0N_ffm_XHb?usp=share_link` provides a direct Google Drive link to visualize them.

However, the informed consent form that was signed by all the participants is included in the following page. It is important to highlight that the signature of this document was mandatory for the initialization of the project.

MINISTERIO
DE CIENCIA
E INNOVACIÓN

CSIC
CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS
COMITÉ DE ÉTICA

HOJA DE CONSENTIMIENTO INFORMADO

TÍTULO DEL PROYECTO: "Exploración de la estimulación eléctrica periférica adaptada como herramienta de modulación de inhibición recíproca a través de la activación de fibras aferentes durante la marcha."
PROMOTOR/FINANCIADOR: Investigación no financiada.
INVESTIGADOR/ES RESPONSABLE/S: Juan Camilo Moreno Sastoque, Filipe André Oliveira Barroso.
TELÉFONO: 915 85 47 12
E-MAIL: jc.moreno@csic.es, filipe.barroso@cajal.csic.es
PERSONA QUE PROPORCIONA LA INFORMACIÓN Y CONSENTIMIENTO INFORMADO: Lucía García González
PARTICIPANTE:

D/Dª._____, con **DNI**_____y domicilio en_____.

DECLARO que he leído la *Hoja de Información al participante*, de la cual se me ha entregado una copia, que he contado con el tiempo suficiente, que se me ha ofrecido la oportunidad de hacer preguntas y que he recibido suficiente información por parte del investigador D/Dª. _____ , quien me ha explicado convenientemente las condiciones de mi participación en esta investigación. Asimismo, se me ha asegurado el tratamiento confidencial de mis datos.
Declaro adicionalmente que comprendo que mi participación es voluntaria, por lo que puedo retirarme de la investigación libremente y revocar mi consentimiento, en cualquier momento, y por cualquier razón.

☐ DOY ☐ NO DOY	Mi consentimiento para participar en la investigación propuesta.
☐ DOY ☐ NO DOY	Mi consentimiento para incorporar mis datos a bases científicas.
☐ DOY ☐ NO DOY	Mi consentimiento para la grabación de vídeos (Imágenes y/o sonidos).

Firmo por duplicado y guardo una copia:

Fecha y firma del participante.

D/Dª_____, investigador responsable de la obtención de este consentimiento informado, con **DNI**_____, **DECLARO** que he explicado las características y alcance de la investigación al participante, atendiendo a sus capacidades, (con especial delicadeza si se trata de población sensible) y le he informado de los potenciales riesgos y beneficios directos derivados de la misma. Quien rubrica este documento fechado otorga voluntariamente su consentimiento para participar en la investigación descrita.
Fecha y firma de la persona responsable de la obtención del consentimiento informado.