

**A NOVEL THERAPY TO REGAIN CONTROL OF SPINAL
MOTONEURONS IN STROKE SURVIVORS**

Behdad Tahayori

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Doctoral committee

David M. Kocaja, Ph.D
Kinesiology Chairman

Nicolas L. Port, Ph.D
Neuroscience Chairman

Georg Rebec, Ph.D
Committee member

Georgia Frey, Ph.D
Committee member

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*Dedicated to my parents and siblings;
For their love and support.
Behdad*

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The purpose of this research was to demonstrate that hemiplegic stroke survivors possess the ability to modulate their H-reflex amplitude through exercise induced operant conditioning. To better understand the changes in the spinal cord associated with hemiplegic stroke, two important inhibitory spinal cord mechanisms, namely post activation depression (PAD) and Group I reciprocal inhibition (RI) were also examined. Examining PAD with conditioning-test intervals between 80 to 300 ms showed a substantial depression in the amplitude of the H-reflex in healthy individuals. In stroke patients there was significantly less inhibition at all intervals, with full recover of the H-reflex at the 300 ms interval.

In healthy individuals conditioning the soleus H-reflex with common peroneal nerve stimulation caused an initial inhibitory phase at about 10 ms interval (D1 inhibition) and a second phase of inhibition at longer intervals (> 100 ms; D2 inhibition). In stroke patients, no statistically significant inhibition was observed, although partial interaction analysis suggested that D1 inhibition followed a pattern similar to that of healthy individuals.

Finally, a three-week exercise induced operant conditioning program was examined in three stroke patients. All patients demonstrated success for down-regulating the amplitude of the soleus H-reflex. More importantly, after training all subjects demonstrated improvements in gait parameters.

It is concluded that spinal cord inhibitory mechanisms are different between healthy controls and stroke patients, and that exercise induced operant conditioning is a promising method for regaining functional control of motoneurons.

David M. Koceja, Ph.D
Kinesiology Chairman

Nicolas L. Port, Ph.D
Neuroscience Chairman

George Rebec, Ph.D
Committee Member

Georgia Frey, Ph.D
Committee Member

Table of Contents

CHAPTER ONE	1
Purpose	4
Specific aims and hypotheses.....	5
Aims	6
Delimitations	7
Independent and Dependent variables	7
Limitations	8
Definition of terms	8
CHAPTER TWO	12
Basic neural circuits	12
Activity dependent plasticity.....	17
Presynaptic inhibition as one method for altering synaptic transmission	18
Functional significance of presynaptic inhibition	20
Goal directed changes in presynaptic inhibition of Ia fiber inputs to the spinal cord.....	23
Practice makes permanence	25
Central vs. peripheral contribution for the induction of plasticity and memory formation in the spinal cord	27
Motor pathologies of adult hemiplegia and changes in the spinal circuitry	29
Operant conditioning as a treatment method	35
CHAPTER THREE	38
Section One	39
Subjects.....	39
Informed consent and subject information	39
Inclusion/Exclusion criteria.....	40
Section Two	41
Equipment.....	41
Section Three	47
Protocols used to study spinal inhibitory pathways	47
Section Four	51
Treatment protocol	51

Section Five.....	53
Data analysis and data reduction	53
Section Six	54
Statistical analysis	54
CHAPTER FOUR	55
Part 1: Examination of spinal cord inhibitory pathways in health and stroke	55
Summary of Part I: Spinal Cord Examination in Healthy Subjects and Stroke Patients	71
Part II: Investigating the training protocol	72
CHAPTER FIVE	79
Part One: Findings of the two presynaptic mechanisms in health and disease.....	80
Part Two: Findings of the treatment protocol	86
Part Three: Review of Study Aims	89
Part Four: General conclusion.....	90
Part Five: Recommendations	92
References.....	94
Vita	

Table of Figures:

Figure 1. Schematic diagram of different inputs to Ia afferents and alpha motoneurons...	21
Figure 2. Flow chart depicting the essential elements of the computer program.....	44
Figure 3. Schematic diagram of the interface.....	46
Figure 4. Variation in the PRD method.....	48
Figure 5. Protocol of conditioning the H-prime.....	50
Figure 6. Changes in PRD in health and stroke.....	56
Figure 7. The relation between various amplitudes of H against a constant H-prime in healthy subjects.....	58
Fig 8. The relation between H and H-prime in healthy group.....	60
Fig 9. The relation between various amplitudes of H against a constant H-prime in stroke group.....	62
Figure 10. Pattern of reciprocal inhibition in healthy and stroke subjects.....	63
Figure 11. Conditioning H-prime by CPN stimulation.....	65
Figure 12. Amplitude of the un-normalized H-reflexes and M-waves.....	67
Figure 13. Interaction of D1 and D2 inhibition on the H-Prime in healthy group.....	68
Figure 14. M-waves and the H-reflexes of PRD with Reciprocal interference protocol in stroke.....	70
Figure 15. An example of the performance in one treatment session.....	74
Figure 16. Success Rate (SR) in down-regulating the amplitude of the H-reflex.....	75
Figure 17. Relative Gait Improvement Index.....	76
Figure 18. Electrophysiological investigation in patient JC.....	78
Figure 19. A hypothetical mechanism of motor improvement due to this intervention.....	91

List of tables

Table 1. Summary of the investigations on inhibitory mechanisms in upper motor neuron pathologies.....	34
Table 2. Demographics of participants.....	72
Table 3. Summary of gait parameters measured before and after the treatment protocol.....	76

CHAPTER ONE

INTRODUCTION

Stroke is one of the leading causes of death worldwide. Each year in the United States approximately 795000 people experience a new or a recurrent cerebrovascular accident. It is estimated that about 6400,000 Americans above the age of 20 have suffered from stroke. For the survivors, the functional impairment caused by such insults can be substantially debilitating and severely affect the quality of life. The Framingham Heart Study showed that 30% of stroke survivors were unable to walk without some assistance and that 26% were co-dependent in their activities of daily living (Kelly-Hayes, Beiser et al. 2003). In the year 2010 an estimated \$73.7 billion was spent for the direct and indirect costs of stroke in the US (Taylor, Davis et al. 1996). After the acute phase of a stroke is past and the patient is medically stabilized, physical rehabilitation is the dominant treatment while surgical and pharmacological interventions are mainly used for controlling the comorbidities and reducing the stroke induced spasticity that is a result of the stroke.

Several schools of thought in terms of the rehabilitation of stroke have been developed over the past century and are still developing. Using therapeutic exercises and movements which can reeducate the cortex and retrieve the functionality of the extremities is one main area of contemporary rehabilitation. Karl and Berta Bobath developed an approach for improving motor function with the core idea of normalizing abnormal muscle tone and suppressing abnormal postural reflexes (Bobath 1990). Brunnstrom developed a movement therapy method in which the emphasis is on encouraging the primitive reflexes or what she referred to as synergies with the hypothesis that the patient should go through the developmental stages to reach the final voluntary movements with coordination. Eventual independence from the synergistic patterns is

expected as the final outcome of this method. The Rood method is based on sensory and reflex stimulation. Facilitatory and inhibitory techniques are used to elicit the desired reflex response. The ultimate goal of this technique is to produce voluntary muscle activity independent from the stimulus (i.e. the reflexes are used as a platform for voluntary movement generation).

Constrained induced therapy is among the newer methods of rehabilitation and is based on the works performed on deafferented monkeys. By limiting the use of the sound limb, the motor performance of the affected limb is shown to improve. Studies suggest that this technique causes reorganization of the cortex and reversal of the neural changes caused by limiting the use of the affected limb.

The introduction of a safe and painless method to stimulate the cortex (known as transcranial magnetic stimulation –TMS), considerably changed our understanding about the motor cortex and sensory-motor organization. TMS has not only been used as a method for investigation, but also has been shown to have potential abilities for treatment, though it is still in its infancy and is not yet clinically approved as a treatment method. Nonetheless, promising results have been reported regarding its effect on reducing the spasticity and function of the upper limb in stroke survivors (Mally and Dinya 2008; Kakuda, Abo et al. 2010; Khedr, Etraby et al. 2010).

While the development of neurorehabilitation approaches such as Bobath's method dates back to the 1970s, new approaches have markedly been changed in different domains. Instead of hands-on methods, new methods are designed as hands-off and coaching (Barnes and Good 2013). The emphasis is directed more toward motor reorganization, motor planning and neural plasticity rather than ideology-based approaches. The neurodevelopmental approach, reflex training of Brunnstrom and similar methods were claimed to be developed based on developmental physiology and focused on movement therapy to reeducate the nervous system.

Extensive research has been conducted to assess the effectiveness of different rehabilitation methods and also to create new methods for regaining function in stroke survivors. However, to date majority of this research has suggested that none of the commonly accepted methods have shown a significant superiority over other methods (Paci 2003; Luke, Dodd et al. 2004; Van Vliet, Lincoln et al. 2005; Lennon, Ashburn et al. 2006; Kollen, Lennon et al. 2009). In the field of stroke rehabilitation, instead of focusing on the issue of “superiority” of a given method, emphasize should be placed on exploring the remaining potentials of the nervous system to restore function, as well as the use of complementary approaches. From a clinical approach, a mixture of all invented methods should be used for the most effective treatment based on the individual needs of patients.

Nonetheless, all the above indicated methods have been developed with the assumption of inducing use-dependent or stimulation-dependent plastic changes in the cortex. Sensory-motor stimulations (through exercise) or electromagnetic stimulation of the cortex might cause cortical reorganization and hence an improvement in motor function. While these methods might very well cause spinal reorganization (which is not well studied), inducing direct plastic changes in the spinal cord is not the main focus of any of these rehabilitation methods.

In the past four decades our understanding about the function of the spinal cord has indeed been revolutionized. Decerebrated animal model studies with parallel studies on human subjects have revealed substantial motor programming and motor learning capacities of the spinal cord. In recent years, basic motor neuroscience knowledge has found its ways to translational experiments to regain function in patients with spinal cord injuries. However, less interest has been shown on developing spinal cord rehabilitation methods for brain insults such as cerebrovascular accidents. Since the alpha motoneurons are gateway for all the descending and ascending commands, any

change in their properties might affect the motor outcome. On the other hand, a plethora of evidence exists to show that the spinal interneurons and motoneurons generate, shape and modify sensory-motor commands.

Purpose

The purpose of the current investigation was to introduce a novel paradigm for inducing plastic changes in the final common pathway for enhancing motor recovery and motor function subsequent to a cerebrovascular accident. Alpha motoneurons are the target of various descending and peripheral connections. The rich interneuronal networks which connect different groups of agonistic and antagonistic motoneurons together, also substantially affect the output of these motoneurons. Therefore, any imbalance in this complex input-output system can lead to a malfunction of the motor system.

In a stroke, the inhibitory control the cortex exerts over the spinal cord is decreased which results in an imbalance between the descending and the sensory inputs to the alpha motoneurons. Due to the loss of several inhibitory mechanisms in the spinal cord, the patient is not able to appropriately activate the alpha motoneurons and therefore, cannot properly perform deliberate and intentional motor tasks.

The reflex arc from the Ia sensory fibers to the alpha motoneurons, while it seems to be simple and monosynaptic, is actually a great source for examining the input-output relations in the alpha motoneurons. The H-reflex is an electrical analogue to stretch reflex which activates the same pathway. This method has enabled researchers to investigate the spinal circuits and has also been used in conditioning protocols in which the effect of one input on the gain of the alpha motoneurons can be investigated. In the early 1980s, voluntary modulation of the gain of the H- and stretch reflex was studied by Jonathon Wolpaw and his group which led to the idea of spinal

memory circuits. Using a protocol known as classical operant condition of the reflexes, they were able to induce permanent change in the gain of the H- and the stretch reflex in both animal models and human subjects. Subsequent studies by the same group revealed a hierarchy of plastic changes throughout the nervous system as a result of this method of conditioning.

However, the functional consequences of such changes were not examined until very recently. Such a potential for inducing functionally meaningful changes at the level of the final common pathway could be another paradigm to complement the other methods currently used for stroke rehabilitation. Patients suffering from various etiologies of brain insult might benefit from the treatments that change the behavior of these final common pathways.

Using another contemporary reflex conditioning method, referred to as “task oriented operant conditioning” we investigated the changes in gait in a small group of stroke survivors to test the plausibility of this novel idea.

Specific aims and hypotheses

This study examined a novel approach for regaining functionality of the spinal neural circuits in stroke survivors. Several studies have shown that a spinal inhibitory mechanism termed presynaptic inhibition (PI) is intact in patients suffering from stroke. We propose that the PI mechanism of stroke patients is trainable and can be used to regain partial control over the spinal circuits and eventually improve motor function. We have developed a computer-patient interface which specifically trains the reflexes of the lower limb during a balance task. The purpose of this research is to carefully document the neural changes as well as motor improvements associated with this intervention in stroke patients. Because the hypothetical framework of this treatment was changes in the presynaptic control of the spinal cord, it was essential to conduct some basic

studies to better understand the normal and pathological status of two main presynaptically mediated inhibitory mechanisms. These mechanisms are long latency antagonistic inhibition and post-activation depression of Ia fibers. To fulfill this aim, we developed new electrophysiological methods and investigated the interaction of these two mechanisms. Briefly, in a new experimental design, we timed both inhibitory mechanisms to arrive at alpha motoneuron pool at the same time. Since these mechanisms do not affect the excitability of alpha motoneuron pool, there could be a possibility that these two mechanisms affect each other (either cancel or exaggerate the effect of each other). Since such an interaction was not introduced in the literature, we had to test it in a group of healthy individuals to provide a norm to compare the results of the patients with.

The Methods and the Results section of this study will, therefore, be presented in two parts: part one will deal with the experimental investigation and part two deals with the treatment findings.

Aims

Aim 1 – The first aim of this study is to investigate the interaction between two inhibitory mechanisms (Reciprocal inhibition and Post activation depression of Ia fibers) in health and pathology. Literature shows that each of these two mechanisms contribute to movement impairment and pathophysiology of spasticity.

Aim 2 – Stroke survivors will demonstrate the ability to down-regulate the lower limb reflexes without increasing co-contraction using a specially designed computer-patient reflex re-training intervention.

Aim 3 – The dynamics of gait will improve in response to the proposed reflex training protocol. In line with our previous work, we hypothesize functional improvements in locomotion after

training. A very recent study has also shown that down regulation of the H-reflex affects the kinematics of walking in rats (Chen, Chen et al. 2011), providing theoretical rationale for our hypothesis.

Delimitations

This study was delimited as follows:

- 1) For Part One of the study (experimental part), eight female and nine male participated in the study. For Part two (Treatment study), two male and 14 female stroke survivors participated in this study. Subjects were recruited from local hospital of Bloomington, and Indiana University Health – La Porte Hospital. Not all the subjects completed the tests.
- 2) The training sessions for each individual subject were scheduled at the same time of the day, as much as that was possible.
- 3) The H-reflex response was used to assess changes in motoneurons of the soleus muscle.
- 4) Prior or contemporary clinical rehabilitation procedures did not interfere with learning this novel task.

Independent and Dependent variables

For Part One of this study, the independent variables are the stimulation intervals and condition (one inhibitory mechanism vs. two inhibitory mechanisms) and the dependent variable is the amplitude of the H-reflex.

For Part Two of the study, the independent variable is the conditioning method and the dependent variables are amplitude of the H-reflex and gait parameters.

Limitations

The limitations of this study were as follows

- 1) Different types of stroke were included in this study.
- 2) Different levels of spasticity and disability was included in this study.
- 3) Subjects with more than one stroke attack were included in this study.

Definition of terms

Definitions of terminologies used in this research report are provided below for the aim of clarity.

Afferent fibers: Sensory fibers originating from muscle spindles which convey information to the central nervous system.

Antagonistic effect: The effect of common peroneal nerve stimulation on the amplitude of the soleus H-reflex. The stimulation of the common peroneal nerve precedes that of posterior tibial nerve.

Background muscle activity: Normal, unperturbed muscle activity during quiet bipedal standing.

Cerebrovascular accident: Stoppage of blood flow to a part of brain either by blockage or a rupture of a blood vessel. This term is equivalent to “stroke”.

Classic operant conditioning: A type of learning method with the aim of down or up-regulating the amplitude of the h-reflex through rewarding the desirable trials immediately after observing the success. No functional task is associated with this learning method.

Conditioning stimulation: A stimulation paired with a test reflex to affect the amplitude of reflex

D1 and D2 inhibition: Two inhibitory phases on the soleus H-reflex caused by the stimulation of common peroneal nerve. The first inhibitory phase (D1) occurs at short latencies (below 20 ms) and is regarded as being disynaptic. D1 is a representation of reciprocal inhibition. The second inhibitory phase (D2) occurs at longer intervals (above 60 ms) and is known to be mediated through presynaptic interneurons.

Gamma motoneuron: A type of lower motoneurons which does not directly adjust the lengthening or shortening of muscle fibers. These motoneurons are involved in adjusting the sensitivity of muscle spindles at various lengths of the muscle.

H-reflex: An artificial reflex elicited by direct electrical stimulation of the Ia afferent fibers which reaches alpha motoneurons. It is an electrical analogue to the stretch reflex.

Interneuron: A neuron that forms a connection between other neurons. Interneurons are neither motor nor sensory.

Interstimulus interval: The time, in milliseconds, elapsed between the conditioning stimulation and the test reflex. It can be a negative number indicating that the test stimulation preceded the conditioning one.

Presynaptic inhibition: An inhibitory mechanism in the spinal cord which affects the efficacy of synaptic transmission between Ia fiber and alpha motoneurons without affecting the excitability level of the alpha motoneurons.

Primary afferent depolarization: A prolonged decrease in membrane potential usually produced by the activation of ionotropic GABAA receptors at the presynaptic terminals of afferent fibers.

Reciprocal inhibition: The inhibitory effect of the Ia afferents of an antagonistic muscle on the alpha motoneurons of the agonist muscle. Reciprocal inhibition is a short latency inhibition (usually below 20 ms).

Spasticity: Velocity-dependent response of a muscle to passive stretching.

Task oriented operant conditioning: A type of learning method with the aim of down regulating the amplitude of H-reflex in while standing on a balance board with emphasis placing on maintaining the stability of the balance board through reducing the amplitude of the H-reflex. Successful trials are those in which the amplitude of the reflex is reduced.

Test reflex: An unconditioned reflex which is regarded as the baseline for the sake of comparison with a conditioned reflex.

CHAPTER TWO

REVIEW OF THE LITERATURE

The purpose of this study was to examine the plasticity of motoneurons of the soleus muscle in stroke survivors. A secondary purpose was to examine the normal and pathological representation of two major spinal inhibitory mechanisms namely reciprocal inhibition and post activation depression. In this chapter, a review of the literature pertinent to this investigation will be presented. For purposes of clarity, this chapter will be divided into the following sections: 1) Basic neural circuits; 2) Activity dependent plasticity; 3) Motor pathologies of adult hemiplegia and changes in the spinal circuitry; 4) Operant conditioning as a treatment method.

Basic neural circuits

The structure and functions of motor neuron axons cannot be separated from their corresponding alpha motor cell bodies. Generally small cell bodies have axons with small diameters. During natural recruitment of motor neuron pools, the small size motor neurons are activated first due to their higher input resistance (Henneman, Somjen et al. 1965). Therefore, axons of small diameter are amongst the first to propagate the signals whereas larger axons are recruited only when more motor neurons of the pool are being activated. However, during cutaneous electrical stimulation, the larger axons can more easily be excited due to their lower threshold. The firing rates of small motor neurons are lower compared to larger ones due to their longer after-hyperpolarization duration. The velocity of nerve impulse propagation is determined largely by the size of the axon; larger axons have faster conduction velocities compared to the smaller ones. Therefore, based on the rate firing of motor neurons and conduction velocity of axons, it can be inferred that

small diameter axons propagate signals with slower velocity (due to their own morphology) with lower frequency capabilities (due to the characteristics of their alpha motor neurons).

The lower motoneurons (referred to as alpha motoneurons) which are located in the anterior horn cells of the spinal cord have been the target of extensive study in terms of their morphology, connections, and their role(s) in motor control. Examining their connections and their roles in motor control was made possible by utilizing two broad methods of investigation: using cutaneous sensory stimulation and using proprioceptive (Ia) sensory stimulation. Cutaneous stimulation is more challenging to study due to its variability and difficulty in controlling. Stimulating the Ia afferents is more commonly used for the study of spinal neural circuits.

The H-reflex is a well-recognized and accepted method for investigating the function of the spinal circuits during various movements. For eliciting an H-reflex, an electrical stimulus (usually a single square-wave pulse with 1 ms duration) is applied to a peripheral nerve (Kukulka 1992). The largest sensory fibers (the Ia fibers), due to their large axonal diameters are the first to be stimulated. These sensory afferents transmit the signal to the spinal cord and synapse both directly and indirectly with alpha motoneurons. The resulting activation of the alpha motoneurons can be detected as a synchronized, coherent biphasic signal in the EMG activity of the corresponding muscle. For this reason, the H-reflex is regarded as an electrical analogue to the stretch reflex (Knikou 2008) (although it must be noted that there is considerable debate about this simple comparison). This reflex arc is nonetheless under the influence of descending drive and input from the periphery as well as other muscle spindles (Pierrot-Deseilligny and Burke 2005).

Applying this technique to any accessible mixed nerve will elicit the H-reflex in the corresponding muscles; however, this technique has been most widely used for examination of

the soleus muscle due to the superficial location of its neural innervation and its clear resultant response due in part to the anatomical distance between the muscle and the spinal cord. More importantly, the soleus is a crucial muscle for the control of posture and gait. Therefore, measuring the H-reflex in the soleus muscle is an appropriate model for studying the role that spinal circuits play in the control and modulation of a motor program. It is important to note that the findings from this type of artificially induced reflex might be different to those of stretch reflexes. There are studies to show that modulations observed in the H-reflex are not present in the stretch reflex (Morita, Petersen et al. 1998). It is assumed that the H- and the stretch reflex are not equally sensitive to inhibitory mechanisms such as presynaptic inhibition. This difference can be partly explained by the fact that the H-reflex is temporally more synchronized than the stretch reflex and therefore, the temporal dispersion associated with the stretch reflex might render the Ia fibers less sensitive to presynaptic inhibition (Enriquez-Denton, Morita et al. 2002). This idea is supported by the fact that repetitive discharge of Ia fibers reduces their susceptibility to presynaptic inhibition (Enriquez-Denton, Morita et al. 2002). Researchers in this field have always appraised the findings with the considerations associated with this technique (Knikou 2008).

The neural component of the motor unit is regarded as final common pathway. This definition was first introduced by Liddell and Sherrington (Liddell and Sherrington 1925). This nomenclature is reasonable both from an anatomical and a functional point of view. A given alpha motoneuron (along with its dendrites and axon) is directly in contact with the muscle fibers through the neuromuscular junction. unit. Therefore, all and any descending signals will merge to this final neuron henceforth directed to the muscle. On the other hand, there are numerous descending neurons, interneurons and internuncial neurons which act on the last neuron in the

pathway and affect its probability and frequency of firing (Jankowska and Hammar 2002) (collectively referred to as excitability). Therefore, this pathway is the final location of many mechanisms such as sensory-motor integration for movement. Receiving different inputs from diverse sources has made this final pathway an extremely versatile point for the regulation of the movement. Different mechanisms and pathways regulate the excitability of motor neurons. Recurrent inhibition, Reciprocal inhibition and Presynaptic inhibition are three examples of such mechanisms.

The activity of alpha motoneurons is being affected by a negative feedback mechanism which induces a reduction in the excitability threshold of the same alpha motoneuron. Renshaw cells, a type of interneuron, are known to have inhibitory connections and cause inhibitory post synaptic potentials (IPSP). These interneurons make connections with different neurons such as the original alpha motoneurons they receive the input from (Renshaw 1941), other Renshaw cells (Ryall 1970) and even indirectly affect antagonistic motoneurons (Hultborn, Jankowska et al. 1971). Since these cells provide a negative feedback to the same motor neurons, this mechanism of inhibition has been termed “recurrent inhibition”. It is important to mention that Renshaw cells also affect the gamma motoneuron because there should be a balance between alpha and gamma drive (Ellaway 1971). During strong voluntary contraction, the activation of these cells can theoretically, reduce the amount of force generated. However, in practice, Renshaw cells will be suppressed, presumably by supraspinal drive to avoid force deterioration. In contrast, during coactivation of muscles (such as during standing) Renshaw cells are facilitated. The result of this facilitation is a reduction in reciprocal inhibition and a reduction in homonymous Ia afferent excitation.

Sherrington (1897) suggested that the Ia of any muscle synapses with an inhibitory interneuron that affects usually the antagonistic muscle. It is believed that each of these inhibitory interneurons project to 20% of the motor neuron pool. It is shown that Ia inhibitory neurons can be activated both prior to the onset of a voluntary contraction and as well as during the contraction. Using the H-reflex protocol, Crone and Nielsen (Crone and Nielsen 1989) showed that 50 ms prior to muscle activation, the H-reflex of the antagonistic muscle depresses. This can be interpreted as a mechanism for the smooth the contraction of muscles. During voluntary contraction motor units with smaller force capacity will be activated first. If the amount of antagonistic activity is not depressed prior to the first initial activation of the motor unites, the results of these fine motor units will be masked by the activity of antagonist muscles. Therefore, supraspinal commands also send input to the reciprocal Ia neurons.

Based on the ground breaking findings of Eccles in the 1960s, it was thought for a few decades that the alpha motoneurons acted as simple “calculators” which add/subtract the inhibitory and excitatory inputs in both spatial and temporal domain and subsequently produce a response. For example, if the sum of the inputs moves the resting potential of the motoneurons above the firing threshold, this will produce a single action potential. However, later studies showed that this is not always the case. It is now known that alpha motoneurons can sustain a long lasting depolarized state (a so called plateau potential) with a single input. This stable potential can sustain for minutes until it gets back to the previous stable potential or by the application of an inhibitory input (Kiehn 1991). The functional significance of these plateau potentials is not clearly understood (Eken, Hultborn et al. 1989). However, studies have shown that this mechanism is impaired in upper motor neuron lesions (Bennett, Hultborn et al. 1998) but can be elicited again with the application of exogenous neurotransmitters (Conway, Hultborn et al.

1988). Indirect evidence have been provided to suggest the existence of plateau potentials in human species (Kiehn and Eken 1997) and their role in motor control (Collins, Burke et al. 2002).

Activity dependent plasticity

In adult humans, monkeys, and rats learning new skills is accompanied by temporary or permanent changes in the spinal cord, and these changes have been extensively studied with both the stretch reflex and the H-reflex (Wolpaw and O'Keefe 1984).

It is well accepted that there exists short-term task-dependent modulation of spinal reflexes and this modulation does not immediately impose any structural or long lasting functional change in spinal circuits. The prevailing notion is that synaptic strength is altered in a task-specific manner. However, practicing the same task or stimulating the same pathway for an extended period of time (e.g., days or years) can result in long-term structural changes in spinal circuits. One example from the athletic area is the reflex regulation in dancers in whom the amplitude of the H-reflex is substantially lower than the normal population (Koceja, Burke et al. 1991; Nielsen, Crone et al. 1993; Ryder, Kitano et al. 2010). Presumably, these long-term changes may in fact weight the contribution of the corticospinal tract in modulating segmental inputs during highly skilled movement, with less weight given to the peripheral input of the muscle spindles.

To examine the induction of such long term change in the adult spinal cord an operant conditioning model of spinal reflexes has routinely been used. In this model of learning, a spinal reflex (stretch or H-reflex) is elicited and the resulting EMG response is recorded. The amplitude of the reflex is presented to the subject as a feedback. A reward is provided if the reflex response is modulated in one particular direction (increase or decrease) as determined by the examiner.

This reward encourages the animal (or human) to purposefully direct its behavior toward the desired reflex response. Operant conditioning has been extensively used for documenting changes in the input-output relationship of both the spinal stretch and the H-reflex. This model has provided a powerful tool for the investigation of spinal circuits as well as any morphological alterations in the motoneurons associated with learning. It is now well established that both animals and humans can similarly increase or decrease the amplitude of the stretch or H-reflex (Wolpaw and Lee 1989). Typically the plasticity in these circuits has consistently been shown to be nearly 150% increases in amplitude for those rewarded for increases, and nearly 50% decreases in reflex amplitude for those rewarded for decreases (Wolpaw, Braitman et al. 1983; Wolpaw 2006).

Presynaptic inhibition as one method for altering synaptic transmission

How do these changes in the reflex pathway occur and how do they become permanent?

For the efficacy of the synaptic transmission to change (either transiently or permanently), there are some mechanisms which act on the presynaptic terminals and some mechanisms which affect the postsynaptic terminal. Collectively, such presynaptic or postsynaptic alterations can increase or decrease the amplitude of EPSPs or inhibitory postsynaptic potentials (IPSPs). There is a variety of these mechanisms throughout the central nervous system that are involved in almost all activities of the CNS from learning and memory (Glanzman 2010), habituation (Krasne and Bryan 1973), gating of pain signals (Melzack and Wall 1965) to the control of movement (Hultborn, Lindström et al. 1979; Hultborn, Meunier et al. 1987; Crone and Nielsen 1989; Hultborn, Illert et al. 1996). At the level of spinal motoneurons, both types of mechanisms exist and have role in the modulation of the H-reflex and stretch reflex in different movements

(Duysens, Tax et al. 1991; Earles, Koceja et al. 2000; Mrachacz-Kersting, Lavoie et al. 2004; Larsen, Mrachacz-Kersting et al. 2006). In general, post-synaptic mechanisms that exert inhibition on alpha motoneurons result in these motoneurons being less responsive to any type of excitatory input. Presynaptic inhibitory mechanisms, on the other hand, can affect the input to the motoneurons without affecting the motoneurons intrinsic properties. This type of inhibition selectively inhibits one input to the motoneurons without affecting other inputs. Likewise, inhibition of the Ia-motoneuron synapses presynaptically can render the reflex gain lower (can reduce the amplitude of the reflex) without affecting the excitability status of the motoneurons. In this case, the normal activity of the muscle will be warranted while its reflexive contraction (and thus its selective control of incoming sensory information) can be independently reduced.

Frank and Fuortes (1957) were among the first to report that sensory inflow can indeed be suppressed without affecting the resting potential of the post-synaptic alpha motoneuron (Frank and Fuortes 1957). However, they did not provide a reasonable explanation on how the monosynaptic transmission can be manipulated without any change in the input level or any change in the resting potential of the postsynaptic cell. Later, Frank (Frank 1959) suggested that there could be what he termed a “remote inhibition” meaning that the site of this inhibition is remote from the soma (Rudomin and Schmidt 1999; Willis 2006). The existence of this phenomenon was confirmed in subsequent research (Eccles 1964) but it was not well understood until the pioneering work of Eccles, who suggested that Ia afferent synaptic strength can be affected through axo-axonic GABAergic inhibitory connections (Eccles, Magni et al. 1962; Eccles 1964). The prevailing hypothesis for the mechanism of presynaptic inhibition of Ia afferents is that the GABAergic receptors in the active zone of the primary afferent terminal (presynaptic Ia terminals) are being activated by interneurons of other sources (refer to figure 1).

Because both sides of this synaptic terminal are axons (Ia afferent and the interneurons), this specific type of synaptic connection was termed axo-axonic to address this phenomenon. These interneurons, while being activated, act GABAergicly on the Ia terminals (Graham and Redman 1994; Miller 1998). Upon the opening of the GABA_A receptors in the Ia terminals, chloride ions leave the presynaptic terminal and thereby cause the active zone to depolarize. It is suggested that this GABAergic mechanism shunts the EPSP through GABA_A receptor activation, or directly affects the Ca²⁺ channels through GABA_B receptors. (Clements, Forsythe et al. 1987; Rudomin and Schmidt 1999). Without an influx of Ca²⁺, vesicle mobilization is impaired, decreasing the probability of neurotransmitter release from the afferent terminals (Rudomin and Schmidt 1999; Rudomin 2009). It was shown in the cat that the interneurons which mediate this primary afferent depolarization (PAD), are under the influence from both peripheral sources such as Ib volleys and Ia input from antagonistic muscles (Eccles, Magni et al. 1962; Eccles 1964; Rudomin, Jimenez et al. 1983) and cutaneous afferents (Seki, Perlmutter et al. 2003), as well as from the descending tracts such as rubrospinal tract (Hongo, Jankowska et al. 1972) and corticospinal tract (Nielsen and Petersen 1994; Meunier and Pierrot-Deseilligny 1998). How the nervous system affects these different pathways to reach the desired level of activity in literally thousands of motoneurons remains a mystery in neuroscience research.

Functional significance of presynaptic inhibition

Presynaptic inhibition of Ia afferents is highly modifiable in response to postural changes (Koceja, Markus et al. 1995) and motor tasks (Capaday and Stein 1986; Stein and Capaday 1988). Presynaptic modulation of Ia inflow could be a physiologic mechanism for adjusting the amount of feedback to the central nervous system.

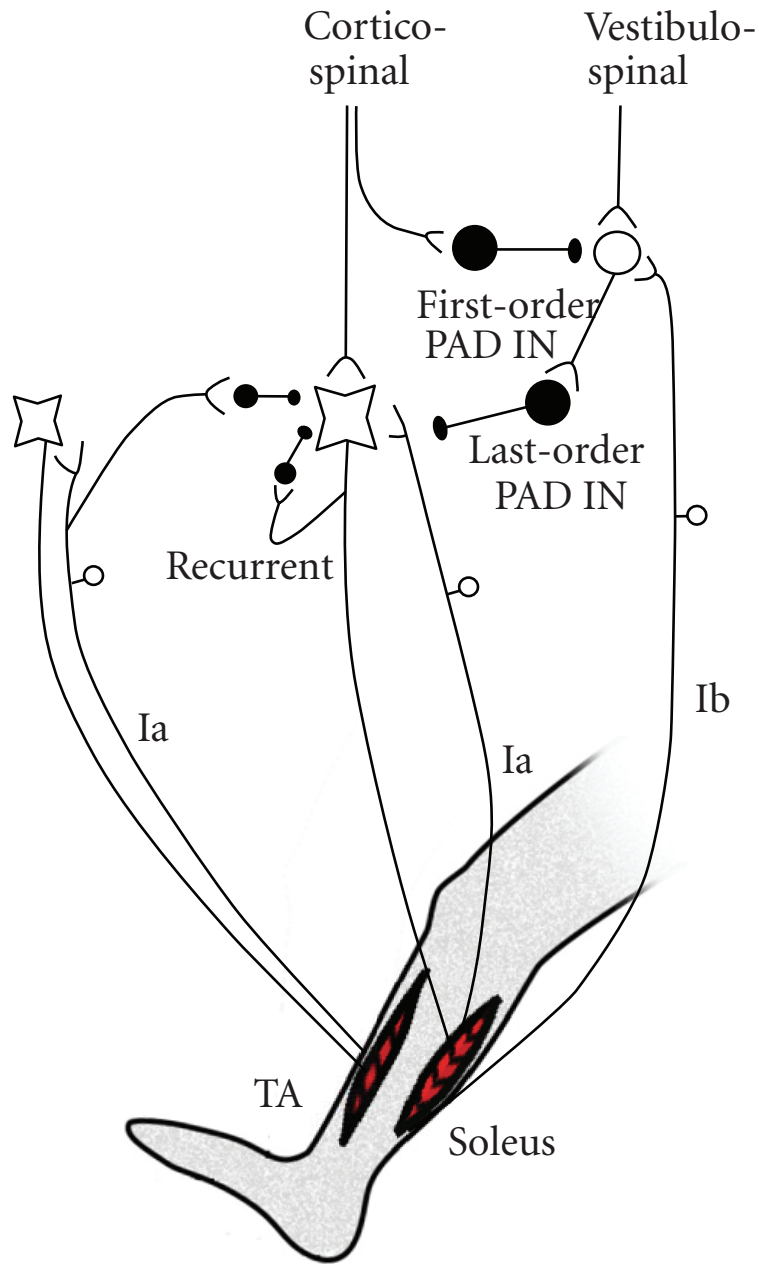


Figure 1. Schematic diagram of different inputs to Ia afferents and alpha motoneurons. Proprioceptive input from Ia fiber can be selectively suppressed by presynaptic inhibition through primary afferent depolarization (PAD) interneurons. The interneuron which makes axoaxonic connection with Ia fiber is GABAergic and regarded as last-order PAD IN. This interneuron is under the influence of an excitatory interneuron which is referred to as first-order PAD IN. This first-order PAD IN receives inputs from both descending tracts and from peripheral afferents. In such a case, different inputs can interact to control the Ia input to motoneurons without affecting the intrinsic properties of motoneurons.

Homonymous (Hultborn, Meunier et al. 1987) as well as heteronymous (Nielsen and Kagamihara 1993) muscle afferents can presynaptically affect the sensory inflow of a given Ia afferent. These sources, due to their origin, are regarded as peripheral sources for presynaptic modulation. There are, on the other hand, centers in the brain (such as the red nucleus and vestibular nuclei) which can also affect the presynaptic terminals through their descending drive. Such a central influence on presynaptic interneurons can be collectively regarded as a central source for presynaptic modulation.

There is evidence to show that peripheral and central drives merge to the same common PAD interneurons (Iles 1996) and therefore, these two sources can interact and integrate at the level of the spinal cord (Nielsen 2004). Such an interaction can modify a reflexive activity that would elicit a large amplitude perturbation.

Taken together, it can be argued that adjusting the amount of presynaptic inhibition through the interaction of central and peripheral inputs has an important role in the execution of voluntary movements. For these reasons it is now difficult to differentiate between reflexive and voluntary movements (Prochazka, Clarac et al. 2000). Whereas data indicate that presynaptic inhibition can significantly influence movement, it is not the only inhibitory mechanism in the spinal cord that has an effect on motor behavior. Other mechanisms such as post-activation depression (Hultborn, Illert et al. 1996), recurrent inhibition (Hultborn and Pierrot-Deseilligny 1979) and reciprocal inhibition (Kasai, Kawanishi et al. 1998; Morita, Crone et al. 2001) all have functional roles in the control and execution of movement. However, prevailing evidence (Hultborn, Meunier et al. 1987; Koceja, Trimble et al. 1993; Trimble and Koceja 1994; Trimble and Koceja 2001; Earles, Dierking et al. 2002; Mynark and Koceja 2002; Seki, Perlmutter et al. 2003; Ung, Imbeault et al.

2005; Tahayori, Port et al. 2012) suggests that presynaptic inhibition has a critical role in the regulation of movement.

On the other hand, presynaptic inhibition has been consistently shown to be modifiable in response to motor practice and learning new skills. In the following sections, we briefly review some key studies which have demonstrated short-term and long-term adaptations of the spinal circuits.

Goal directed changes in presynaptic inhibition of Ia fiber inputs to the spinal cord

During normal movement execution, such as changes in posture (Angulo-Kinzler, Mynark et al. 1998), movement initiation (Eichenberger and Rüegg 1984) and gait (Faist, Dietz et al. 1996), presynaptic inhibition has been shown to be modulated. Besides the naturally occurring task specific modulation of presynaptic inhibition, the amount of presynaptic modulation expressed on spinal circuits is trainable. There is ample evidence in the literature to show that the amount of presynaptic inhibition can be purposefully changed. The experimental methods used to document this inhibition generally fall into operant conditioning of the reflexes and task-related feedback conditioning of the reflexes. It should be emphasized that none of these protocols exclusively target the PI circuits; rather, they exert various changes on spinal and/or even supraspinal circuits including alterations in presynaptic inhibition. However, both protocols can produce short term as well as long term changes in the neural circuits of the reflex pathway.

In operant conditioning protocols, there seems to be a complex interaction of mechanisms involved in the induced plasticity including presynaptic modulation of the Ia terminals, specifically for the short term adaptation phase (Wolpaw 1997). While operant conditioning does not usually involve any specific behavioral task, there are protocols specially designed to

modulate the H-reflex to fulfill some experimentally defined functional task. These task-oriented operant conditioning protocols usually provide feedback to the subject after each trial. Trimble and Koceja were the first to successfully implement such a functional protocol for short term changes in spinal reflexes (1994). Their protocol involved a balance control task in which subjects stood on a tilt board and were instructed to maintain their balance in a highly precarious posture. Applying an electrical stimulation for eliciting the H-reflex to the bilateral soleus muscles during this task produced enough ankle torque to destabilize the subjects during the trial. Over a single session of practice, subjects were able to learn to depress the H-reflex to minimize the destabilizing torque, as a strategy to maintain balance. Subsequent testing of the same subjects on a solid surface (normal upright standing) revealed that the H-reflex amplitude remained depressed for more than 30 minutes after the termination of the training session (Trimble and Koceja 1994). An ensuing study examined the effect of multisession training on the maintenance of the suppressed H-reflex. Two hours of H-reflex suppression training for three days significantly reduced the amplitude of the H-reflex which showed a trend to remain depressed for a longer period of time post-training (Trimble and Koceja 2001).

Such types of training-induced plasticity have also been observed in more complex movements. In a novel locomotion study, subjects were trained to walk backward on a treadmill for several weeks. In untrained subjects a large amplitude H-reflex was observed during the mid-swing phase of walking. Training progressively reduced the amplitude of the reflex. However, these changes in the reflex amplitude were not related to leg muscle motor evoked potentials (MEPs). It was suggested that the plasticity induced in the H-reflex circuits was heavily dependent on the presynaptic control of the inflow of sensory information (Ung, Imbeault et al. 2005). It is interesting that a comparison of the results of studies using task-related feedback conditioning

with those using operant conditioning suggests that the two methods produce relatively the same percentage of change in the H-reflex. What remains to be determined, and may be an important distinction, is whether these two types of feedback result in the same types of functional and/or behavioral consequences. Studies using operant conditioning as a method for functional motor improvement have already been initiated, and thus far have provided promising results (Chen, Chen et al. 2006; Thompson, Stein et al. 2006; Thompson, Chen et al. 2009).

Practice makes permanence

In humans, it has been clearly established that long-term, repetitive activity produces changes in the reflex arc. For example, strength training has been shown to increase H-reflex amplitude; 14 weeks of muscle-specific heavy resistance training can increase the soleus H-reflex amplitude by 20%. Research has also shown that ipsilateral resistance training increases the strength of both limbs, most likely due to neural adaptation, but that the H-reflex amplitude increases only in the trained side (Lagerquist, Zehr et al. 2006). This finding supports the idea that direct increase in sensory inflow is necessary for the induction of plasticity in spinal circuits.

As another example, several studies have shown that the amplitude of the H-reflex is significantly reduced in trained dancers (Koceja, Burke et al. 1991; Nielsen, Crone et al. 1993; Ryder, Kitano et al. 2010). The reduction in the H-reflex amplitude is presumed to be caused by long-term performance of dance specific movements. Co-contraction of the lower limb muscles, which is frequently utilized in ballet dance, induces an increase in presynaptic inhibition and causes a reduction in reciprocal inhibition. This activity-induced change in the H-reflex is most likely a part of the process of acquiring high level skill and maintaining balance for dance-specific techniques. This reduction in response to peripheral sensory input could also be

interpreted as an increase in a cortical role for the control of movement and hence a more precise movement. This permanence may also extend to more complex crossed-spinal reflex pathways, suggesting a much more global cascade of effects in the spinal cord. Preliminary studies from our laboratory have shown that active elderly subjects exhibit crossed-spinal pathways more similar to young subjects than inactive elderly (Ryder, Kitano et al. 2011).

Taken together, these studies provide evidence that neural circuits can undergo long-lasting activity-induced plastic changes. However, these studies cannot unambiguously conclude that the plastic changes were induced solely in the spinal cord circuits. One possibility is that functional changes in these circuits are due to changes in descending drive rather than the spinal cord.

To determine whether the long-term changes occur at the spinal or supraspinal levels, Wolpaw and his colleagues examined the effect of operant conditioning on the stretch or the H-reflex in monkeys and human subjects (Wolpaw, Braitman et al. 1983; Wolpaw and O'Keefe 1984; Feng-Chen and Wolpaw 1996). Wolpaw et al. (1984) demonstrated both in monkeys and humans that the stretch reflex, as well as the H-reflex, can be down- or up-regulated using operant conditioning. Wolpaw and colleagues also demonstrated that plasticity occurs in two distinct phases: an immediate (acute) phase which was observed in the same day of training (approximately 8-10% change) and a long lasting (approximately 1-2%/day for many days) phase. The acute phase was readily observed in the stretch reflex but not the long loop reflexes which are assumed to involve higher centers such as the cortices. This immediate phase was temporary and diminished within a few hours after the termination of the training session. However, by continuing the training sessions for 4-6 months in humans and monkeys respectively, the plasticity became more permanent and the modulation persisted for months after termination of the training sessions. Severing the spinal cord after the reflexes were up or

down-regulated (in two different groups of monkeys) did not diminish the up or down regulated reflex (Wolpaw and O'Keefe 1984), supporting the idea that the plasticity had resided within the spinal circuits.

Central vs. peripheral contribution for the induction of plasticity and memory formation in the spinal cord

Acute changes in spinal pathways are believed to be triggered by descending inputs. However, changes in the descending input over a long period of time can produce permanent changes in the spinal cord which are regarded as spinal fixation. Animals with partial transection of the spinal cord with an intact corticospinal tract are still able to volitionally up- or down-regulate the H-reflex in an operant conditioning protocol (Chen, Carp et al. 2002). However, spinal circuits can undergo plastic changes in response to exercise and skill acquisition which is not dependent on corticospinal drive. Operant conditioning is a specific type of memory formation and due to its nature (volitional alteration of the reflexes based on the feedback and reward) the descending input is an indispensable part of it. While the results of the studies on operant conditioning have provided valuable information and insight about memory formation in spinal circuits, conclusions from these studies should be interpreted with caution. First, it should be considered that during an operant conditioning task, changes in the amplitude of the reflexes are not necessarily the *consequences* of a motor demand. Second, no functional tasks are involved during classical operant conditioning which means that this type of conditioning may not be behaviorally relevant, and these results do not translate to real-life situations.

The question then becomes: does the spinal cord have the ability to acquire new motor skills without the need of the descending drive for this skill acquisition?

Spinalized cats are indeed able to develop functional tasks despite the permanent loss of descending input (Barrière, Frigon et al. 2010; Frigon, Johnson et al. 2011). Such task-dependent modulation in segmental reflexes has also been observed in spinalized human patients as well (Dy, Gerasimenko et al. 2010). Unfortunately there are few studies performed on normal human subjects to parsimoniously demonstrate changes in the spinal circuits, independent from descending drive. One obvious reason for this scarcity of information is the difficulty in differentiating the role of descending and peripheral inputs to the spinal cord. It is possible that a given *pattern* of sensory input (such as that generated by a specific task) may induce plastic changes in spinal circuits without the involvement of descending drive. In an excellent investigation, Meunier and colleagues (2007) examined this possibility by training the subjects to perform two different types of cycling movements. In one group subjects performed a cycling exercise in which the resistance of the pedaling changed every 15 seconds and they were asked to keep the cycling speed constant (e.g., complex task). In a second group, subjects performed the same task under constant pedaling resistance (e.g., simple task). It was shown that homosynaptic depression (the depression in the Ia transmission of sensory information after an immediate preceding stimulation) substantially changed only in the complex task group. Since homosynaptic depression is confined exclusively to the previously activated Ia fibers and there is no anatomical connection from the upper centers, investigators concluded that it was the *pattern of sensory inflow* that produced the change in synaptic efficacy between the Ia afferents and the alpha motoneurons. Again, understanding this discrepancy is extremely important for the improvement of modern rehabilitation techniques for spinal cord injury patients.

Motor pathologies of adult hemiplegia and changes in the spinal circuitry

No two patients suffering from a stroke have encountered identical type of lesion and/or present the same type symptoms. Depending on the type of stroke (ischemic or hemorrhagic) and the site of lesion, the symptoms, prognosis and the level of disability can vary greatly. Some common disabling features which are characteristics of corticospinal tract are Babinski response, synkinetic movements, weakness, loss of dexterity, abnormal posture (predominantly extensor synergy of the lower limbs and flexor synergistic posture of upper limbs), increased muscle tone and spasticity. This type of brain insult affects both parts of the central nervous system (brain and the spinal cord). Exaggerated reflex responses (referred to as hyperreflexia) and repeated response to a single reflex stimulation (referred to as clonus), are two of the initial findings associated with a cerebrovascular accident. It makes perfect sense to assume that this hyperreflexia is due to the sensitivity of one or more components involved in the stretch reflex arc of the spinal cord (muscle fiber change, muscle spindle sensitivity change or more intuitively an impairment of inhibitory mechanisms which affect this reflex arc). Spasticity, on the other hand, is another landmark of almost all upper motor neuron lesions, although it has different prevalence in different pathologies. Spasticity is a troublesome term. In clinical medicine, it helps determining a pathological sign and define one of the characteristics of the disease. However, in the scientific society, it is a very vague and etiologically unknown phenomenon. Still the definition provided by Lance is the mostly accepted one for both clinical and scientific literature. He stated: Spasticity is a motor disorder characterized by a velocity-dependent increase in tonic stretch reflex (muscle tone) with exaggerated tendon jerks, resulting from hyperexcitability of the stretch reflex, as one component of the upper motor neuron syndrome” (Lance 1980). Therefore, a long lasting idea about spasticity is that it is due to the enhancement

in synaptic transmission of Ia fibers with the alpha motoneurons. A variety of changes have been observed throughout the neuromotor system (from the cortical motoneurons to muscle fibers) after a spinal or supraspinal damage which are regarded as the potential causes of the development of increased muscle tone and probably spasticity. The following factors can be considered to cause spasticity:

- 1) Increased input from Ia fibers
- 2) Increased efficacy of synaptic input to alpha motoneurons
- 3) Changes in alpha motoneuron excitability
- 4) Intrinsic changes in alpha motoneurons

In the 60s and 70s, one prevailing hypothesis regarding the exaggerated stretch reflex was supersensitivity of muscle spindles. This idea was also backed by the studies which showed that testing the same pathway with the stretch reflex yields a bigger response as compared to the response elicited by the H-reflex (which bypasses the spindle mechanism) (Ashby and Verrier 1975). This difference in the amplitude of the two reflexes (which activate the same pathway), was interpreted as an evidence of spindle over-activity. However subsequent studies suggested that the difference in the amplitude of the two reflexes could be due to fact that the size of the test reflex affects the amount of facilitation or inhibition or could be due to differences that these two types of reflexes have (Nielsen, Crone et al. 2007). Studies on the discharge rate of muscle spindles which used more direct methods such as microneurography also did not support such an idea (Hagbarth, Wallin et al. 1973). It is also shown that during voluntary activity, spastic patients exhibit the same amount of intrafusal activity (aftereffect) as normal subjects (Wilson,

Gandevia et al. 1999; Wilson, Gracies et al. 1999). Therefore, this idea has not received much attention over the past decade (Nielsen, Crone et al. 2007; Burke, Wissel et al. 2013).

Formation of new synapses through sprouting could also account for longer lasting changes in the spinal cord. Morphological changes subsequent to spasticity have been observed (Lieber, Steinman et al. 2004). However, this hypothesis is not backed by objective measurements (Noth 1991).

The gain of the stretch reflex is controlled by different inputs which impinge on the alpha motoneurons or Ia terminals through an axo-axonic synaptic connection. Alteration in these inputs and these inhibitory mechanisms could be, by all their possibilities, the cause of enhancement in synaptic transmission and spasticity. The inhibitory mechanisms can act presynaptically- mainly the two mechanisms of post-activation depression and Ia presynaptic inhibition- or post-synaptically (such as recurrent inhibition, Ib inhibition, reciprocal inhibition). All these mechanisms have been subject to careful investigation by different groups of researcher and in general, a reduction in inhibition has been consistently observed. This is in agreement with the idea of spasticity being due to lack of inhibition on the stretch reflex. However, three important considerations exist which make this simple and general interpretation less persuasive:

- 1) Lack of correlation between the severity of the impairment in most of the inhibitory mechanisms and spasticity, and also the lack of correlation between spasticity and functional impairment (Ada, Vattanasilp et al. 1998; Dietz 2003; Landau 2004)
- 2) Spasticity is a common pathologic finding in many upper motoneurons disorders (Maynard, Karunas et al. 1990; Rizzo, Hadjimichael et al. 2004). Multiple Sclerosis, Amyotrophic Lateral Sclerosis, CVA, spinal cord injury and traumatic brain injury can all cause spasticity. However, research has shown that in each pathologic condition, different

inhibitory mechanisms are more involved. Nonetheless, the manifestation of spasticity is the same for all these pathologies.

- 3) The course of the development of spasticity does not parallel the changes observed in stretch reflex. Immediately after a cerebrovascular accident, an enhancement in the stretch reflex can be observed without any obvious spasticity. Likewise, spasticity can develop in the absence of exaggerated stretch reflex (Sinkjaer, Toft et al. 1993; Schindler-Ivens and Shields 2004). The works of Dietz and his colleagues suggests that in spastic gait, the enhanced stretch reflex does not contribute to the pathophysiology of spasticity (Dietz, Quintern et al. 1981).

The inspiring report of Frank and Fuortes (Frank and Fuortes 1957) provided new insight about the control mechanism of the inflow of proprioceptive information. Subsequent studies on animal models provided a relatively simple method to examine the effect of this mechanism on the stretch reflex. It was shown that vibrating the Achilles tendon of cats presynaptically inhibit the amplitude of the H-reflex (Rudomin and Schmidt 1999). Interestingly enough, the effect of this vibration is less pronounced in stroke patients. The initial interpretation of this observation was that presynaptic inhibition is decreased in stroke patients (Lance, De Gail et al. 1966; Gillies, Lance et al. 1969; Burke and Ashby 1972). However, it was shown that this mechanical method for depression of the H-reflex also activates other mechanisms such as post activation depression. The study of Faist et al. (1994) was among the first to question the idea that lack of vibration induced inhibition in stroke patients is a sign of reduction in presynaptic inhibition of Ia fibers.

Table 1 summarizes the changes in inhibitory mechanisms in stroke and in other pathologies which cause spasticity. Based on the summary provided in Table 1, it can be inferred that

spasticity due to supraspinal lesions might have a different pathophysiology than that of spinal lesions. Multiple sclerosis and many other CNS disorders such as ALS affect both supraspinal and spinal centers and therefore, it is not surprising to observe that these disorders represent similar profiles in terms of the neural circuits' malfunction. Changes in the intrinsic properties of alpha motoneurons have been examined in animal models of spasticity. Initial investigations on the properties of alpha motoneurons suggest the existence of a linear relation between the input summation (spatial and temporal summation) to the membrane and passive transmission of the signal to the spike initiating region (Eccles 1961; Eccles 1964). However, the discovery of plateau potentials showed that the output of motoneurons can be shaped by some intrinsic mechanisms of the membrane and can change the response to the input. Voltage dependent persistent inward current (PIC) is a mechanism by which a prolonged depolarization of the membrane can occur. Plateau potentials occur when a stimulation turns the alpha motor "on" and upon the termination of the stimulation, the alpha motoneuron retains its depolarized status in the absence of the input. This stable depolarized status can continue for a few minutes or until another input causes its termination (Conway, Hultborn et al. 1988; Kiehn 1991). The functional significance of these plateau potentials is not very well understood but few studies have suggested the existence of this mechanism during normal human movement (Kiehn 1991; Kiehn and Eken 1997; Gorassini, Bennett et al. 1998; Collins, Burke et al. 2001; Collins, Burke et al. 2002). After a spinal shock (such as trauma to the spinal cord), all the reflexes disappear in human cases. This is also true in animal models of spinal shock (Bailey, Lieberman et al. 1980). It is shown that plateau potentials disappear in acute spinal shock but can be elicited again in a few cases by using exogenous neurotransmitters. Evidence exists to suggest that these plateau potentials have role in spasticity (Hultborn 2003).

Table 1. Summary of the investigations on inhibitory mechanisms in upper motor neuron pathologies. For purpose of this research, the studies on stroke spasticity have been presented separate from all other pathologies.

Inhibitory mechanism	Stroke spasticity	Other types
Reciprocal inhibition	Reciprocal facilitation (Crone, Johnsen et al. 2003)	Reciprocal facilitation in spinal cord injury cases (Crone, Johnsen et al. 2003).
Recurrent inhibition	Relatively normal RI at rest and reduced RI during voluntary contraction (Katz and Pierrot-Deseilligny 1982)	Decreases in spinal cord injury and is correlated with spasticity (Shefner, Berman et al. 1992) Increases in ALS and is not correlated with spasticity due to ALS (Raynor and Shefner 1994)
Post-activation depression	Upper limb Reduced in hemiplegic patients (Masakado, Kagamihara et al. 2005).	Reduced in paraplegia (Hultborn 2003) PAD due to both stretch and H-reflex repetition was less in MS and spinal cord injured patients (Grey, Klinge et al. 2008).
	Lower limb Reduces in stroke and is correlated with the severity of spasticity (Lamy, Wargon et al. 2009).	
Presynaptic inhibition	Upper limb Decreased in Hemiparesis and hemiplegia on the affected side (Artieda, Quesada et al. 1991)	Decrease in PI through biceps femoris tendon tap on sol H-reflex and an Increase in P Facilitation through stimulating femoral nerve in MS patients (Nielsen, Petersen et al. 1995). Decreased in MS patients (Morita, Crone et al. 2001).
	Lower limb No change in the affected side (Faist, Mazevet et al. 1994; Katz 1999; Aymard, Katz et al. 2000; Pierrot-Deseilligny and Burke 2005)	

In summary, spasticity is a plastic change which has involved different neurological mechanisms and sites. However, the exact definition and mechanisms of spasticity are unknown. Nonetheless, spasticity can be viewed from the perspective of how this plastic change in the spinal cord can be altered from negative (nonfunctional) plasticity to positive (functional) plasticity. This outlook disregards whether spasticity is related to dysfunction. The extent to which these morphological changes affect function in the human model of spasticity is not very well studied.

Operant conditioning as a treatment method

The operant conditioning protocol for purposefully regulating the amplitude of the H- or stretch reflex was successfully tested some 40 years ago. Since then, the group at the Wadsworth Center has conducted numerous studies on the mechanisms associated with operant conditioning and the plastic changes associated with this reflex regulation throughout the nervous system. This method eventually found its way for clinical trials and as a treatment method for functional improvement.

Among the first indirect attempts to show the possibility of changing the spinal response through reflex conditioning in stroke survivors was the work of Veltink et al. (2000), who demonstrated that by stimulating the deep peroneal nerve the amplitude of soleus H-reflex was decreased in these patients. Hyperreflexia and excessive plantar flexion is a barrier for appropriate propulsion in stroke survivors. Potentially, this method can be used to enhance the gait of these patients. What this study did not demonstrate was the possibility of long lasting changes due to such conditioning. It is likely that repetitive performing this method might induce long lasting neural changes and subsequently functional improvements. It should be kept in the mind that the method used by these investigators is a passive method in which the participant is not included. Such conditioning methods use an electrical or mechanical stimulation to activate a certain

pathway which affects the amplitude of the test reflex. Timing of these two stimulations is critical for producing the desired effects and subjects' attention is not involved in these protocols.

However, methods of operant conditioning heavily involve subjects' participation. Two variations of operant conditioning have been reported in the literature. The first one can be regarded as "classic operant conditioning", developed by Wolpaw in the 80s (Wolpaw, Braitman et al. 1983; Wolpaw and Lee 1989) and the second one which was inspired by this method is "task-oriented operant conditioning" which was developed by Koceja in the 90s (Trimble and Koceja 1994). The second method incorporates a balancing task, in which participants balanced on a specially designed balance board. Each electrical stimulation which elicits one H-reflex, will simultaneously causes a perturbation to the participant's balance. Participants, through a problem solving approach, learn that by volitionally decreasing the H-reflex amplitude, they can better manage their balance.

Operant conditioning of the H-reflex as a rehabilitation tool has been successfully tested on patients with partial spinal cord injury. Operant conditioning of the spinal stretch reflex of biceps brachii showed a significant decrease in the amplitude of the reflex after 8 weeks of training (Segal and Wolf 1994). The functional significance of such training was subsequently investigated. Thompson and colleagues (Thompson, Pomerantz et al. 2013) examined the effect of operant conditioning of the H-reflex on locomotion of spinal cord injured patients and found that this treatment improved stepping, increased walking speed and decreased clonus.

Most medications used for reducing spasticity and improving movement usually induce general effects (inhibiting many neurons) and therefore, might affect intact circuits as well. On the other hand, there is considerable debate as whether reducing spasticity is or is not a prerequisite for

improved motor function. Operant conditioning, in contrary, directly targets a selected neural circuit without directly affecting other circuits and/or causing undesirable effect on other neuronal pathways.

As was summarized in table 1, many inhibitory mechanisms of the spinal cord are impaired after an upper motor neuron lesion. This lack of inhibition was classically assumed to be the cause of spasticity. Whether it is or it is not correlated with spasticity, it must be related to impaired function. Therefore, inducing appropriate inhibitory mechanisms (through specific training methods) are new avenues for modern rehabilitation methods. Task oriented operant conditioning is a method which has shown to influence motor function (Trimble and Kocēja 1994). In this method, subjects are prompted to volitionally reduce the amplitude of their reflex. With practice, the spinal circuits will experience permanent or long-lasting plastic changes (Wolpaw and Tennissen 2001; Tahayori and Kocēja 2012).

CHAPTER III

METHODS

The purpose of this study was to investigate alterations in spinal inhibitory mechanisms post stroke and to test the efficacy of operant conditioning of soleus H-reflex in stroke survivors.

Understanding the neural changes associated with operant conditioning and a possible increase in spinal inhibition is the ultimate, long term goal of this study which will be achieved in future investigations.

For the first part of the study a group of healthy young individuals participated in the study. The aim of this part was to establish normative data to compare the pathologic results with. We used two novel protocols for investigating presynaptic inhibitory mechanisms and therefore, obtaining normative data was an essential step.

In the second part of the study, a convenient sample of subjects was selected to be trained with the Human-Computer interface developed as a part of this project. Some electrophysiological and functional tests were performed before and after the treatment to infer any change associated with the training regimen. This chapter provides the details of the methods/protocols used in this study for the aims outlined in chapter one. For the purpose of clarity, this chapter is divided into the following sections: 1) Subjects, 2) Equipment, 3) Protocols used to study spinal inhibitory pathways, 4) Treatment protocol, 5) Data analysis and data reduction and 6) Statistical method.

Section One

Subjects

Eighteen young, healthy individuals (10 male, 8 female, age= 28 ± 3.2 yrs) participated in the first part of the investigation. The first part consisted of a series of experiments and not all subjects participated in all experiments.

Sixteen stroke survivors enrolled in the treatment part of the study. Three participants were recruited from IU Health – La Porte Hospital and completed the first part of the study (investigation of inhibitory mechanisms). Three patients participated in the second part of the study (training the reflex). Data collection of these patients took place in the Motor Control Lab – Bloomington.

Informed consent and subject information. All subjects provided written consent prior to participating in the study. This study was approved by the Institutional Review Board (IRB) of Indiana University and the IRB of La Porte Hospital. Participation in this study was totally voluntarily. Prior to starting the testing sessions and treatment protocols, each patient was individually explained the aim of the study and the procedures. Subjects were provided enough time to read the informed consent form and asked any questions and signed the consent form. As stipulated by the Institutional Review Board of Indiana, the procedures and potential risks of participation in the study were clearly indicated. Participants received monetary reimbursement for each session of participation in this study. The subject Informed Consent Form is shown in Appendix A.

Inclusion/Exclusion criteria. For healthy subjects the inclusion criteria was being between the age of 21 and 50 and the exclusion criteria was having any type of central or peripheral nervous disease.

For the stroke group, certain criteria were set to include the participant into or exclude them from the study. Due to the exploratory nature of this study, it was essential to test this concept on patients who have similar pathology profile.

The inclusion/exclusion criteria are as follows:

Inclusion

- 1) Being above the age of 40
- 2) At least 6 months has passed from their stroke.
- 3) Having a single cerebral vascular accident resulting in motor deficits of the lower extremity.
- 4) Ability to stand independently or with an assistive device such as a cane for at least five minutes.
- 5) Ability to walk for 5 minutes independently or with an assistive device such as a cane or a walker.
- 6) Not having any abnormal muscle tone or sensory-motor deficits in the uninvolved leg.

Exclusion Criteria

- 1) Body mass index $> 31 \text{ kg/m}^2$
- 2) Receptive Aphasia
- 3) Uncontrolled hypertension

- 4) Heart problems, such as a myocardial infarction or congestive heart failure during the past 12 months.
- 5) Peripheral neuropathy, uncontrolled arrhythmia, congestive heart failure.
- 6) Intermittent claudication with symptoms at <150 m of walking.

Section Two

Equipment

The following equipment was used in one or both parts of the study:

Balance Board: A specially designed balance board with an adjustable base was used during the training sessions. The board was equipped with mercury switches to report whether the board is horizontal or inclined in either directions. The information was fed into the computer program.

Biodex body weight support system. This suspension/body support system was used to prevent participants from falling while practicing on the balance board in the training sessions. The harness of the system neither interfered with their performance nor reduced their apparent weight. The sole purpose of using this system was to avoid falling in case they lose their balance.

Therapeutic Unlimited electromyography recording unit. A four channel EMG unit (Therapeutic Unlimited[®]) with bipolar surface electrodes was used to record the EMG activity of the muscles. Electrodes were attached on the skin by using double adhesive tapes.

Grass Stimulator and Digimeter Stimulation units. To elicit the H-reflex, stimulation units were used to produce currents with the duration of 1 ms. Double channel Grass stimulator was used for majority of the participants. For some subjects, the maximum output current of Grass

stimulator was not high enough to stimulate the nerve or to elicit maximum M-response. In these cases, Digitimer Stimulation Unit was used which provides higher currents.

Biometrix electrogoniometer. This device was used to register the ankle angle of participants during practicing on the balance board. A custom made interface was made to directly connect the output of the electrogoniometer to the Analog-Digital Convertor.

Biodex GaitTrainer. Biodex GaitTrainer treadmill was used to evaluate the gait characteristics of the participants in the evaluation sessions. The treadmill is equipped with special belt which registers certain gait parameters.

National Instruments Analog-Digital Convertor. An NI USB-6259 AD board was used for digitizing the data from forceplate, EMG system, balance board and electrogoniometer.

DasyLab (National Instruments). A computer program was written for data acquisition and real-time analysis-interaction with patients. DasyLab was the programming environment.

Human-computer interface. A computer program for data capturing, data monitoring and online processing of the EMG/balance board/goniometer signals was developed in DASYLab 12.00 environment (DASYTec USA a national Instrument Company, Norton, MA, USA). Portions of DASYLab is created using LEADTOOLS© 1991-2006 (LEAD Technologies, Inc). This program was developed to monitor and process the input signals to infer a decision as to whether to elicit an H-reflex. The H-reflex can be modulated by many different factors, such as position and posture(Koceja, Markus et al. 1995), type of sensory input(Stein and Capaday 1988), joint angle(Brooke, Cheng et al. 1995) and the level of muscles EMG activity (referred to as background EMG activity) and co-contraction(Tahayori, Kitano et al. 2010). In this experiment, we aimed to induce a plastic change in the spinal pathways involved in the H-reflex. Various inputs merge into this pathway and can affect its output. To induce the desired plasticity

(volitional down-regulation of the H-reflex through the control of Ia inflow), it was critical to ensure that all other inputs (which can contribute to the modulation of the reflex), were constant and did not interfere in this protocol. Our operant conditioning method, which can be referred to as “task-oriented operant conditioning” is more challenging than classical operant conditioning. In our method, subjects stand in a precarious position and have to maintain their balance. This entails a substantial muscular control, including co-contraction of the soleus (sol) and tibialis anterior (TA). A change in the co-contraction rate can be another confounding factor(Nielsen, Sinkjær et al. 1994). To guard against these confounding factors during the exercise, we created modules in the program to monitor these factors. The following factors were instantaneously monitored by the program: background EMG activity of the soleus and tibialis anterior muscle and their co-contraction ratio, the ankle joint angle and the angle of the balance board. On the other hand, it was necessary to measure the amplitude of the H-reflex and the M-wave while the subjects stood on the board (The H-reflex which was not operantly conditioned). A set of standard values or “criterion values” were, therefore defined to compare the inputs with. These standard values were measured in the first two layers of the program (Figure 2).

To measure the baseline H-reflex, the H-reflex was set to amplitude equal to 15% to 30% of M-max. Five reflexes were elicited with enough interval between each and were averaged by the program to provide a representative value of the H-reflex. If there was more than 20% fluctuation in the amplitude of any of these baseline reflexes, the values would be reset in the program and five new reflexes would be elicited.

Data flow in DASyLab is block-wise. We defined blocks with the length of 400 data points. With the sampling rate of 4k, it would take 100 ms for each block to spread through the program. For EMG signals, the first block was used for offset adjustment and eliminating any DC

component of the signals. EMG signals were subsequently rectified and amplified. Moving root-mean square (RMS) of the signals were measured with the time constant of 0.10 s. In case of data gap, this RMS measurement would restart. In layer two of the program, the RMS of soleus and tibialis anterior were measured for 3 seconds and were saved as standard values. The angle at which the baseline H-reflex was elicited was also saved as the criterion value for joint angle. The balance board needed to be horizontally aligned. Tilting the board in one direction would close a circuit with a +1.5 V potential difference. Tilting the board to the opposite directing would close the second circuit and generate a -1.5 V potential difference. These circuits were connected to the AD board through a BNC cable. The sign of the voltage was a determinant of the direction of tilting. When being balanced both circuits would be open and there was zero potential.

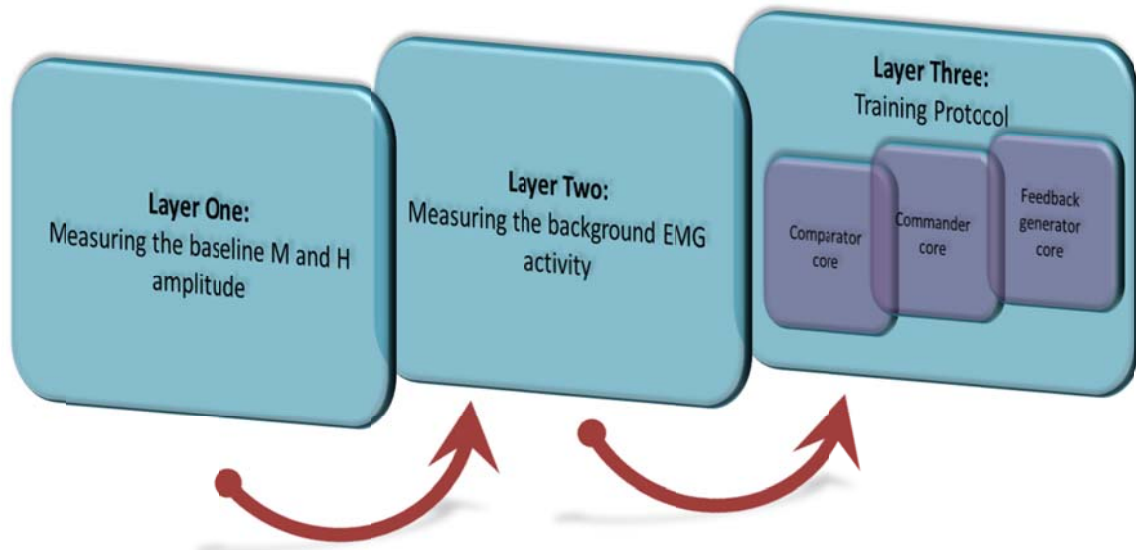


Figure 2. Flow chart depicting the essential elements of the computer program.

By the end of the second layer (step) of the program, all the criterion values were determined and transferred to the third layer. This layer was designed to compare the instantaneous incoming signals with the criterion values. The Comparator Core (CpC) of this layer would compare the incoming magnitude of the instantaneous background EMG activity, the angle of the board and the ankle angle joint with the criterion values. A tolerance range was set for each criterion value: for background EMG activity, a 15% fluctuation in either direction (increase or decrease) and for ankle joint, a total of 5 degree fluctuation was acceptable. The Commander Core (CmC) would receive a logic signal (1 or a 0) from the CpC indicating whether all the incoming signals were within the range or out of the range, respectively. When the CpC sends a logic=1 output to CmC, the CmC sends a Transistor-Transistor Logic (TTL) pulse out to the stimulator every 10 seconds. However, if the output of the CmC is 0, the CmC will stop sending the TTL out until the CmC's output becomes one. This output should be at least "1" for 2 seconds for the CmC to send the TTL out.

The actual time of stimulation is determined by the Stimulus Monitor Signal from the stimulation machine to the program. This signal was used to find the part of the EMG signal which contains the M and H-reflex signals. The Feedback Generator core measures the peak to peak amplitude of the M and H-reflex and provides feedback to the subject based on the baseline value of the H-reflex transferred from the first layer of the program. A bias was induced in the feedback generator: to guard against normal fluctuation in the H-reflex, the trial H-reflex should be more than 10% depressed to be presented as a successful trial to the participant. A schematic diagram of the interface is shown in figure 3a and a snapshot of the feedback monitor is shown in figure 3b.

The processing time of the program was between 400 to 500 ms. This means that it would take about 500 ms from the instant of receiving EMG signal to send a TTL out and receive the Monitor Signal from the stimulation. The 2 second monitoring buffer time in the CmC was long enough to overcome this delay (it was four times as much as the maximum delay time). This computer interface-setup is currently Patent Pending (Koceja and Tahayori 2013)

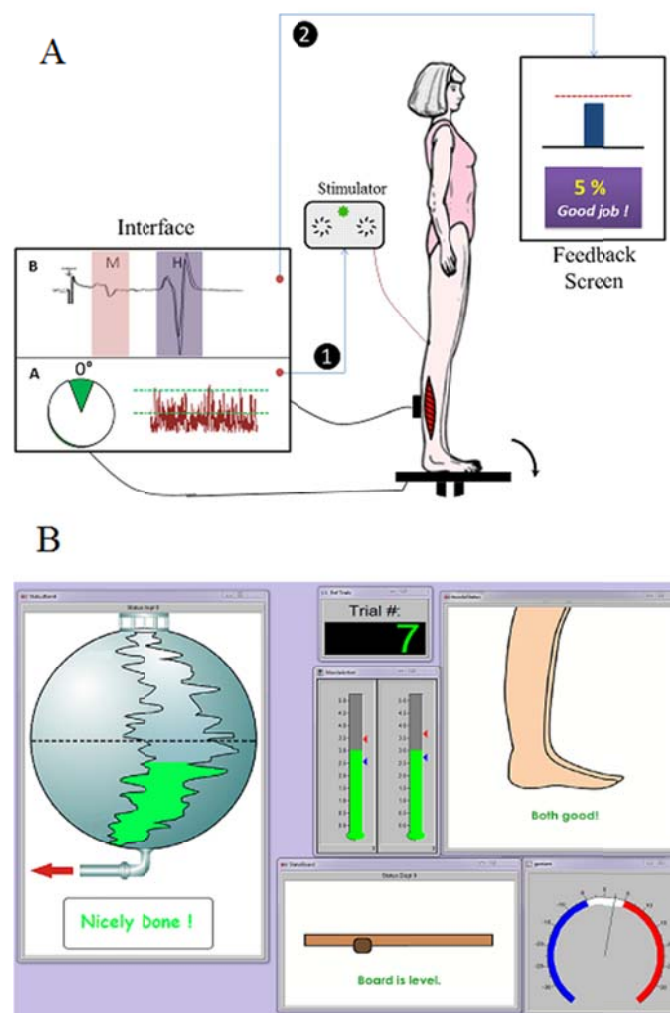


Figure 3. A schematic diagram of the interface and the treatment setup (A) and a snapshot of the patients' screen which provided feedback about their performance and the criterion values (B).

Section Three

Protocols used to study spinal inhibitory pathways

In this section the details of protocols used for investigating the inhibitory mechanisms will be provided. The section will continue with the details of the Human-Computer interface and its methodological considerations.

Protocols used for part one (investigating inhibitory mechanisms). Two main inhibitory mechanisms were investigated in this study: 1- Post-activation Depression of posterior tibial nerve and 2) Reciprocal inhibition from common peroneal to posterior tibial nerve.

Post activation depression. This mechanism is known to act presynaptically. There are several protocols to test this phenomenon. For our study, we used paired reflex depression protocol at various interstimulus intervals. Conventionally, in this method the posterior tibial nerve is stimulated two times with an arbitrary interval of 80 ms or more to produce two H-reflexes. Since the nerve was stimulated with the same current intensity, in the absence of post activation depression the amplitude of the two responses would be statistically equal. Therefore, the ratio of the second response (referred to as H-prime) to the first one, is a measure of post activation depression. As the time interval between the two pulses increases, the ratio becomes closer to 1 (equal responses). For our study, the amplitude of the H-reflex was set to be between 20% to 30% of Maximum muscle response (Mmax). We tested Paired reflex depression at various time intervals ranging from 80 ms to 300 ms. Two to four responses were elicited at each interval to provide a representative response at any given interval.

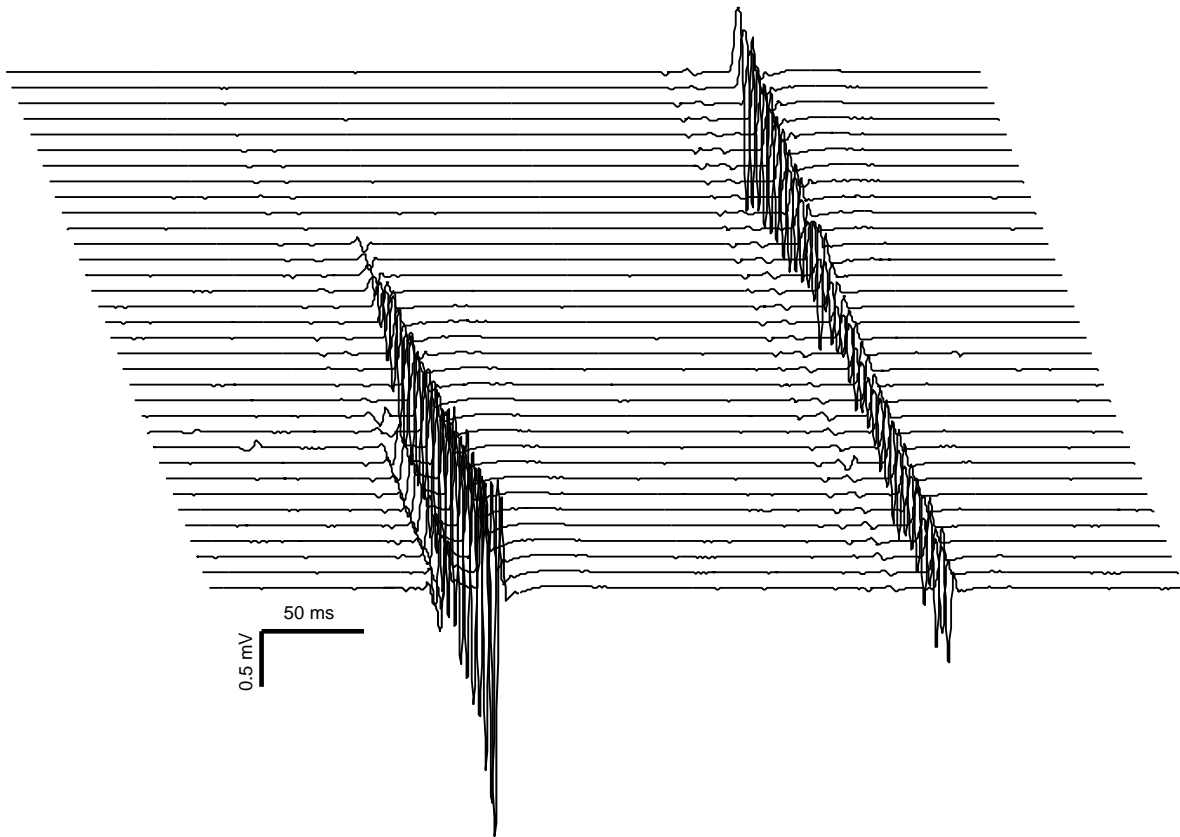


Figure 4. Variation in the PRD method. The amplitude of the first H-reflex is varied, systematically affecting the amplitude of the second response.

We implemented a new variation of this method to better understand the pathologic changes in this mechanism. In this variation, a time interval was selected at which the ratio of H-prime to H was about 0.5. After selecting this interval, the intensity of the first stimulation would be altered, meaning that the stimulation intensity for the first response would vary from the initial value (30% of Mmax) to minimum possible response (no obvious H-reflex). This was possible by delivering each stimulation from a separate channel of the stimulation machine and merge the signals into the same cable by bridging the two channels. We tried to change the intensity of the first H-reflex by a 5% step in order to provide enough data points to determine a relation between the two responses. In this model, the first H-reflex can be regarded as an independent

variable (or input) and the second reflex as the dependent variable (or output of the function established based on the relation of the two reflexes). Figure 4 represents the raw data obtained with this method.

This variation from the conventional method enabled us to observe how conditioning an H-reflex with a preceding H-reflex.

Reciprocal inhibition. In this research the reciprocal inhibition between the tibialis anterior and soleus muscle was examined by stimulating the common peroneal nerve (CPN) prior to eliciting the soleus H-reflex. Using a bar electrode, the CPN was stimulated at the level of caput fibula. We made all our efforts to avoid contaminating the branches innervating the peroneal muscles and/or the posterior tibial nerve. A single 1 ms pulse was used to stimulate the nerve. Motor threshold was measured by determining the minimum palpable/observable twitch in the tendon of the TA. The Stimulation intensity was then set to 1.2x motor threshold of TA in all protocols using CPN conditioning. CPN stimulation preceded that of the posterior tibial nerve (PTN). The effect of CPN stimulation on the soleus H-reflex was examined at different time intervals ranging from 1ms to 300 ms. To ensure that the amplitude of soleus H-reflex was not changing, single pulses (unconditioned H-reflexes) were elicited during the experiment and necessary adjustments of the intensity were made. This ensured that the same amplitude of the H-reflex was conditioned at different time intervals. The unconditioned amplitude of the H-reflex was set to between 20% to 30% of Mmax.

We have introduced a novel variation of the conventional method of reciprocal inhibition. Instead of conditioning the H-reflex, we conditioned an H-prime reflex. Basically, with this method, the reflex pathway (including the motoneuron pool) was affected by two presynaptically

mediated inhibitory mechanisms. To conduct this protocol, we first determined the effect of CPN conditioning at various time intervals and selected a time interval at which the H-reflex was reduced by more than 50% of its unconditioned value. Then, we repeated the paired reflex depression method at a long time interval resulting in about 50% reduction in the second response. Finally, we conducted the new protocol by stimulating the posterior tibial nerve, followed by CPN stimulation followed by the second stimulation posterior tibial nerve. To conduct this method, the H-reflex and H-prime were elicited from one channel of the stimulating machine and the CPN was stimulated by the second channel of the machine. The steps of this protocol are illustrated in figure 5.

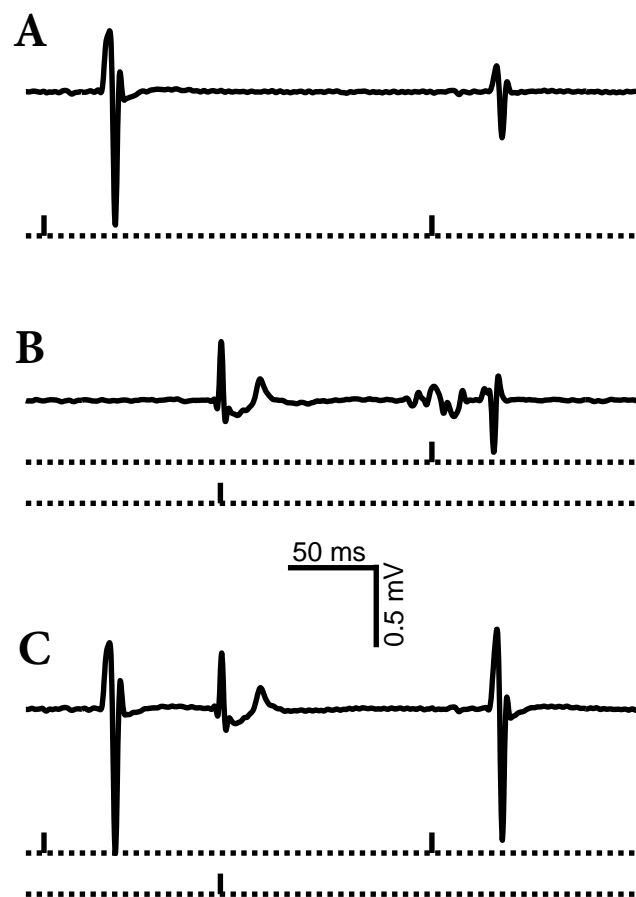


Figure 5. Protocol of conditioning the H-prime. Data from one representative subject. (A) Post activation depression of the H-reflex. (B) presynaptic reciprocal inhibition of the H reflex. (C) stimulation of PTN (eliciting the H reflex) followed by CPN (to affect the succeeding H-prime reflex) and followed by second stimulation of PTN (eliciting the H-prime).

Section Four

Treatment protocol

The purpose of this study was to examine the effects of a task-oriented operant conditioning on the functional outcome in a group of stroke survivors. To achieve this aim, a training protocol was designed.

Training schedule. Participants practiced with the Human-Computer interface for the duration of four weeks. The schedule of this study is illustrated in the following diagram



Prior to the start of the treatment regimen, a functional assessment of the gait of participants was performed. Subjects walked for 4 minutes (or less, if were not able to walk for the total duration) on a GaitTrainer treadmill. The treadmill measured some specific temporal and spatial gait characteristics.

Training protocol. In the training sessions, the surface electrodes were place on the soleus and tibialis anterior of both legs. Subjects stood normally and were encouraged to distribute their weight evenly on both legs. The best position for nerve stimulation was determined by moving the disk electrode at or around the posterior knee line and eliciting the largest h-reflex response without changing the stimulation intensity. Subsequently the M-max was elicited and recorded. An H-reflex between 15% to 30% of the H-max was determined to be used as the baseline H-reflex value. To do so, a few reflexes were being elicited and averaged by the program. If the amplitude of the M was bigger than that of the H, the intensity would be reduced to obtain an H

with a smaller M. The program had the option to reset the recorded values. This was necessary for the occasions that the H-reflex was smaller than M. after determining the values of M and H, subjects were fitted with the harness and stood on the balance board. Normal EMG activity of the designated muscles were being recorded for 4 seconds to determine the baseline EMG activity level of the muscles.

During training, subjects had to learn to control their balance on the board without increasing or decreasing the amount of their muscles activities and to keep the board in a level (horizontal) position. It was possible to adjust the range of deviation from the baseline values. For EMG signal, by default, subjects were allowed to have $\pm 15\%$ deviation in their muscle activity and still being regarded as “within range”. An electrogoniometer was attached to the ankle joint to measure the ankle angle. The range of fluctuation for this angle was set to be ± 5 degrees. All these information were received by the comparator core of the software. If the value of any of these parameters was outside the preset range, the comparator core would stop the commander core from sending triggering signal out and would wait till all the values fell within the range and wait for two more seconds to ensure the stability of the values.

Immediately after each stimulation, the feedback core measured the amplitude of the M and H-reflex and showed the amplitude of the H to the subject as a bar graph. In the program, the value of H reflex had to be more than 10% lower than the baseline to indicate that it was lower. This means that if a given trial yielded an H which was 5% smaller than the baseline, the visual feedback would indicate that it was 5% above the baseline and if it was 10% lower, the feedback would show that it was the same as the baseline. This method was implemented to ensure that any decrease in the amplitude was not by pure change. There is always a fluctuation in the value of the H-reflex even the amplitude of the M and the background EMG activity stay the same. In

each training session, subjects practiced in three blocks of 100 trials. After each block they were given enough time to sit down and rest.

Section Five

Data analysis and data reduction

The custom-written data acquisition programs (DasyLAB) would perform online analysis and plot the data during the testing. However, for actual data analysis, we used the imported the raw data to custom-written Matlab Programs. Offline data analysis provides the opportunity to find any data collection error (such as erroneous triggering of the stimulation machine at a wrong time) and enabled us to do more detailed analysis. Matlab codes are provided in Appendix B.

For the training sessions, the amplitude of M-wave, H-reflex and background EMG activity was measured. For data reduction and presentation, those trials with more than %20 fluctuation of M-wave were omitted. Each group of 5 trials were averaged and a representative M and H were produced. The number of successful trials were subsequently measured. A successful down-regulating of the reflex were determined if the reflex amplitude was at least 10% lower than the baseline H-reflex value.

To assess their locomotion, subjects walked on a Gait Trainer 3TM treadmill at their preferred speed for four minutes. This treadmill measures the Mean Walking Speed, Mean Step Cycle Time, Mean Step Length (SL) and the Coefficient of Variance (CV; the amount of variation occurring between footfalls). A Gait Improvement Index (GII) was defined as follows:

$$GII = 100 - [(CV_{aff} + CV_{un}) + 100 \times \left(\frac{|SL_{aff} - SL_{un}|}{(SL_{aff} + SL_{un})/2} \right)] \quad \text{Eq.1}$$

Where CV_{aff} and SL_{aff} are the CV and SL of the affected side, respectively and CV_{un} and SL_{un} are the CV and SL of the unaffected side, respectively. The difference between the pre and post GII was calculated and used as a Relative Gait Improvement Index (RGII).

Section Six

Statistical analysis

For different parts of the study, different statistical analyses were used. For comparing the control group (healthy subjects) with patient group, a mixed design (Split-plot) was used. For the parts which did not have a comparison between the two groups, Repeated Measure ANOVA was used. For the comparison of simple effects, paired t-test or simple contrasts were used. The alpha level was set to 0.05 and adjustment of alpha was used, if necessary through Bonferroni correction.

CHAPTER IV

RESULTS

This study investigated two inhibitory pathways in healthy subjects and stroke survivors and tested the effect of task-oriented operant conditioning of the H-reflex on the functional improvement in patients suffering from stroke. To better investigate these inhibitory pathways, we developed new electrophysiological protocols. To this end, it was essential to first test a group of healthy subjects to provide a set of normative data and to compare those findings with those gathered from stroke survivors. In this chapter the results of the study are presented. These results will be presented in two parts: 1) Examination of spinal cord inhibitory pathways in health and stroke; and 2) Training-Induced Plasticity.

Part 1: Examination of spinal cord inhibitory pathways in health and stroke

Prior to inducing plasticity in the spinal circuits of stroke survivors, a detailed examination of the characteristics of the spinal pathway mediating the Ia fiber-soleus muscle was performed. In this section of the study, the intrinsic properties of the Ia fiber-motoneuron synapse of the soleus muscle was examined, the inhibitory connections from the antagonist musculature to the Ia fiber-motoneuron synapse of the soleus muscle was examined, and the interaction of these two inhibitory influences on the Ia fiber-motoneuron synapse of the soleus muscle was examined.

Post-activation depression at various time intervals. Paired reflex depression (PRD) is a commonly used method to examine motoneuron excitability in humans, and has been identified as an intrinsic measurement of the Ia fiber-motoneuron synapse. The PRD protocol involves the

elicitation of two H-reflexes separated in time by a variable time interval. We examined PRD with different time intervals in 10 healthy (age = 26.43 yrs) and 10 stroke (age = 71.48 yrs) subjects. We will refer to the time interval between the two stimuli as inter-stimulus interval or ISI for short. PRD was tested with ISI's between 80 ms and 300 ms. Consistent with the literature, in the healthy group, the ratio of the second H-reflex (referred to as H-prime) to the initial H-reflex was below 1.0 (e.g., inhibitory) at all tested ISIs, indicating a pronounced inhibition to the motor pool as a result of a preceding H-reflex stimulus. In this experiment, the stimulation intensity for eliciting both H-reflexes was the same. Therefore, the depression was an indication of post-activation depression in this spinal pathway.

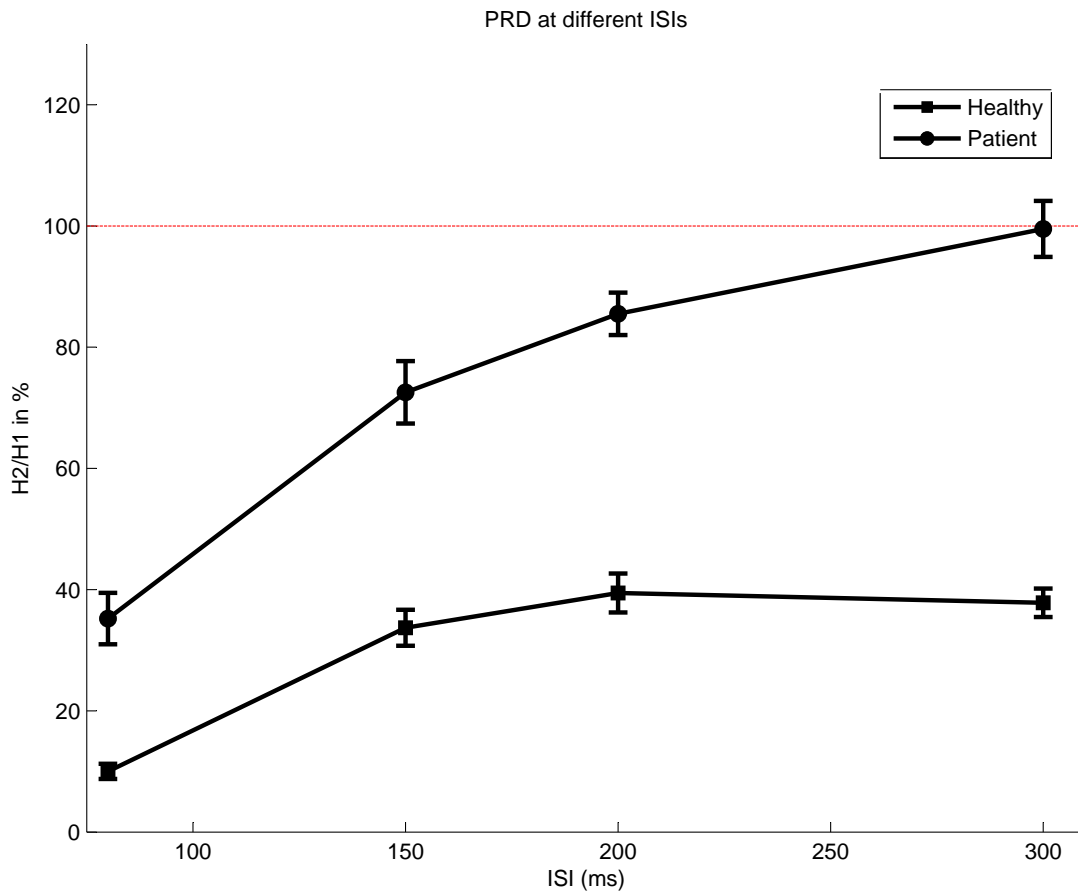


Figure 6. Changes in PRD in health (squares) and stroke (circles).

The same protocol was also tested in the stroke group. In this group, at short ISIs, the ratio was also below 1.0, however, as the time interval was increased, the ratio showed a sharp increasing trend. Statistical analysis between the two groups showed a significant interaction between Group and Interval ($F_{3,54}=3.90$, $p= 0.014$; Fig 6). Pairwise comparisons showed that the two groups were different at all levels of ISIs.

PRD with a variable intensity first H-reflex. To further investigate the mechanism of PRD, we conducted a variation on the traditional PRD method which entailed testing the second H-reflex (H-prime) against different intensities of the first H-reflex (e.g., changing the amplitude of the initial H-reflex and maintaining intensity for the second H-reflex). This method involved a variable first H-reflex and a constant H-prime. Six healthy subjects participated in this portion of the study. To be valid, the second occurring H-reflex needed to be standardized for each subject. To do so, the ISI at which the ratio was close to 0.5 (50% depression) was selected for conducting this new protocol, and then this intensity was kept constant. The electrical stimuli were delivered from different channels of the stimulator and merged to one cable. This enabled us to change the amplitude of the first reflex independent from that of the second reflex. We changed the intensity of the first reflex in approximately 5% increments and elicited a few PRD responses. The results of all 6 healthy participants are summarized in figure 6. It was observed that decreasing the amplitude of the first H-reflex exponentially affected the amplitude of the second response (Fig. 7). The nonlinearity of the change between the two variables was determined by fitting the data points with a linear and an exponential model and measuring the goodness of fit (least squares). Fitting the curves with a linear model yielded an $R^2=0.53\pm 0.09$ while a non-linear exponent yielded $R^2=0.79\pm 0.1$. A statistical comparison between the linear R^2

(IR^2) and exponential R^2 (eR^2) showed that eR^2 significantly explained more of the variability of the data and was a better fit ($F_{1,5}=37.41$ $p=0.002$).

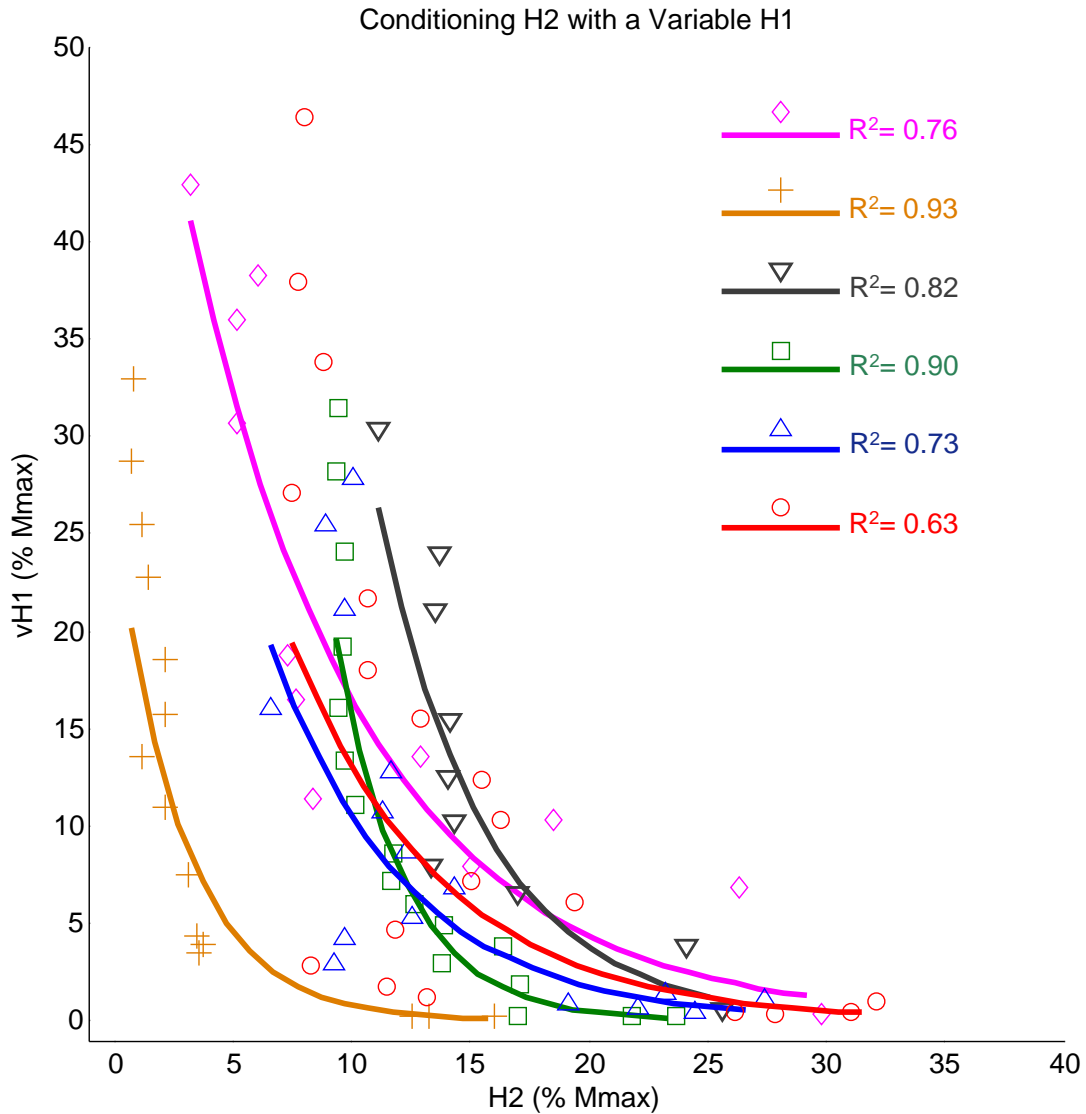


Figure 7. The relation between various amplitudes of H against a constant H-prime in the healthy group. The ordinate shows the amplitude of the test reflex (H1) and the abscissa shows the resultant H2. The amplitude of the unconditioned H2 was set to about 25% of M-max. It can be seen that when the second H-reflex is being conditioned by a smaller first H-reflex, the amount of inhibition exerted on it, nonlinearly changes.

The source of this nonlinear behavior is unknown. The H-reflex is a compound response which is recorded from the target muscle. Therefore, many muscular and neural factors can contribute to its modulation. This interpretation would become more complicated if the ratio of H-prime to H-reflex, obtained in the traditional PRD method, is dependent on the amplitude of the first reflex. For example, if at a given ISI, double stimulation of a given motoneuron pool produces an H-reflex equal to 30% of M-max and a second reflex equal to 15% of M-max (50% reduction in the amplitude of the second reflex), do we expect to observe 50% reduction if the first H-reflex is set to, for example, 20% of M-max? In four participants, we examined the traditional PRD at a given ISI with different amplitudes (keeping in the mind that with the traditional method, the stimulation intensity is kept constant for both reflexes). We observed that when the amplitude of the first reflex is between 10% and 45% of M-max, the amount of depression on the second reflex is fairly constant. This was determined by the existence of a strong linear relation between the two parameters at this level of motor pool stimulation. In figure 8 we have provided the data of one participant to show this linear relation (blue dots) and also the raw data of the nonlinear change in the amplitude of H-prime while being conditioned by different amplitudes of the H-reflex. The mean IR^2 for these data points was 0.84 ± 0.098 . The inner diagram in this figure shows the fitted line for the four subjects.

We conducted the same protocol in 9 stroke subjects. In this group, the results were more variable however the general finding was that the stroke subjects had significantly less PRD (conventional method) than the healthy subjects (presented earlier). As was shown in figure 6, in these patients, at short ISIs (less than 150 ms), the inhibitory profile was similar to that of the healthy group (as there was no statistical interaction at intervals below 150 ms) but at long ISIs the profile was significantly different and there was almost no inhibition for the stroke patients at

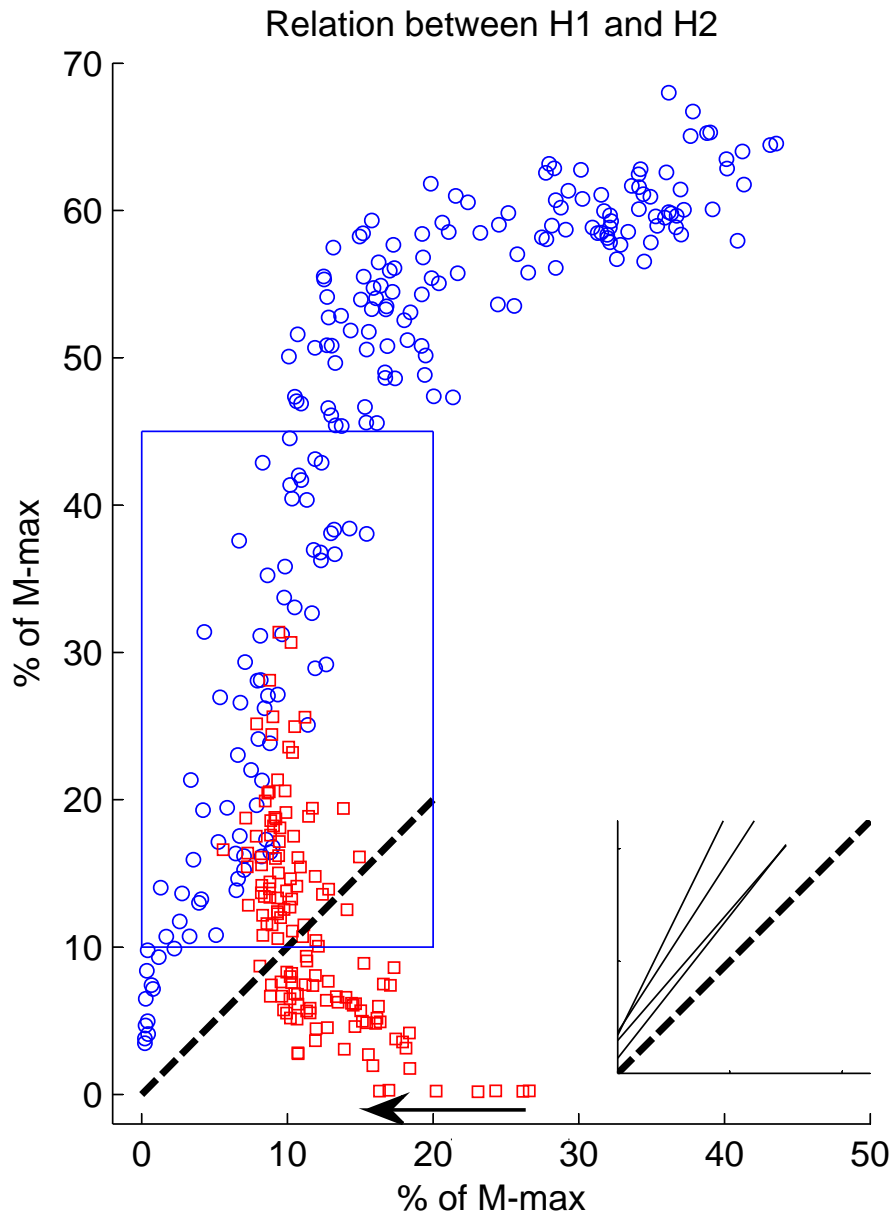


Figure 8. The relation between H and H-prime in healthy group. The blue dots represent the responses elicited with the same intensity. Therefore, a shift to the left and up (above the identity line) shows the depression of the second H reflex. By increasing the intensity, it was observed that when the first H-reflex is between 10% to 40% of M-max a linear relation exists in the amount of depression (delimited by the square). The inset shows the regression lines for the four subjects. All lines fall above the identity line. Red dots show the data from a representative subject in which the amplitude of the first H-reflex was changed. The arrow shows that with a very minor H-reflex, the conditioned reflex shifts to the left.

the 300 ms interval. We conducted our method of conditioning the H-prime with different amplitudes of the H-reflex at two different ISIs in these patients: we chose a short ISI at which maximum inhibition was observed and a longer ISI at which there was no inhibition or even facilitation was observed.

In these patients, testing at short ISIs provided results comparable to those observed in healthy individuals. At longer ISIs, conducting the same protocol resulted in a distorted relation between the two parameters. In many of our subjects, eliciting a small amplitude H-reflex resulted in an exaggerated response of the second reflex. Basically, it seems that a history of activation of motoneuron pool/motor units potentiates the second response at long intervals (as opposed to inhibiting it as observed in healthy individuals). It is important to emphasize that our data showed that this response is time dependent; at short ISIs, the same stimulation intensity partly inhibited the second response. Possible causes of this behavior and its role in the pathology of movement impairment in stroke is discussed in Chapter Five. These individual results are shown in figure 9; due to the complexity of the patterns that were observed group analyses were not conducted.

Inhibition to the soleus motor pool from the antagonist muscle. Inhibition to the soleus motor pool is also controlled in part by inputs from the antagonist musculature. To investigate these connections, we examined common peroneal nerve conditioning to the soleus muscle. The common peroneal nerve (CPN) was stimulated using single 1 ms pulses with the intensities of 1.2 times the motor threshold of the tibialis anterior (TA) muscle. This stimulation preceded that of posterior tibial nerve (PTN) to condition soleus H-reflex. We tested this conditioning effect at

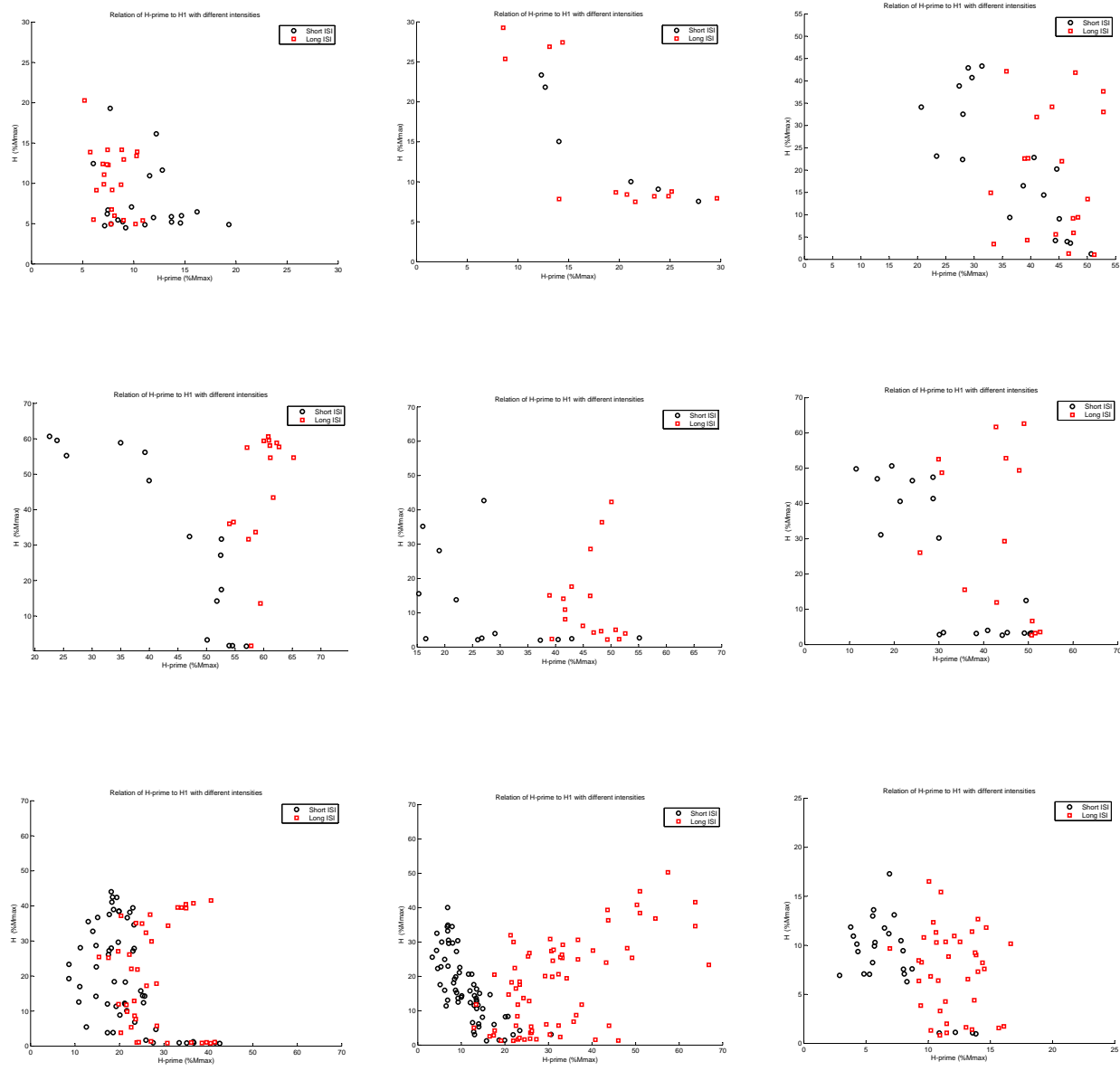


Figure 9. The relation between various amplitudes of H against a constant H-prime in in stroke group

various time intervals ranging from 2 ms to 300 ms (PTN stimulated as early as 2 ms after CPN stimulation to as late as 300 ms).

This protocol was tested in 9 healthy individuals. In this group, an initial depression at an ISI of 10 ms and another substantial depression at ISIs above 100 ms was observed. A facilitatory response was observed at ISIs of 40 ms. Results of all the healthy individuals (n=9) were averaged and are presented in figure 10.

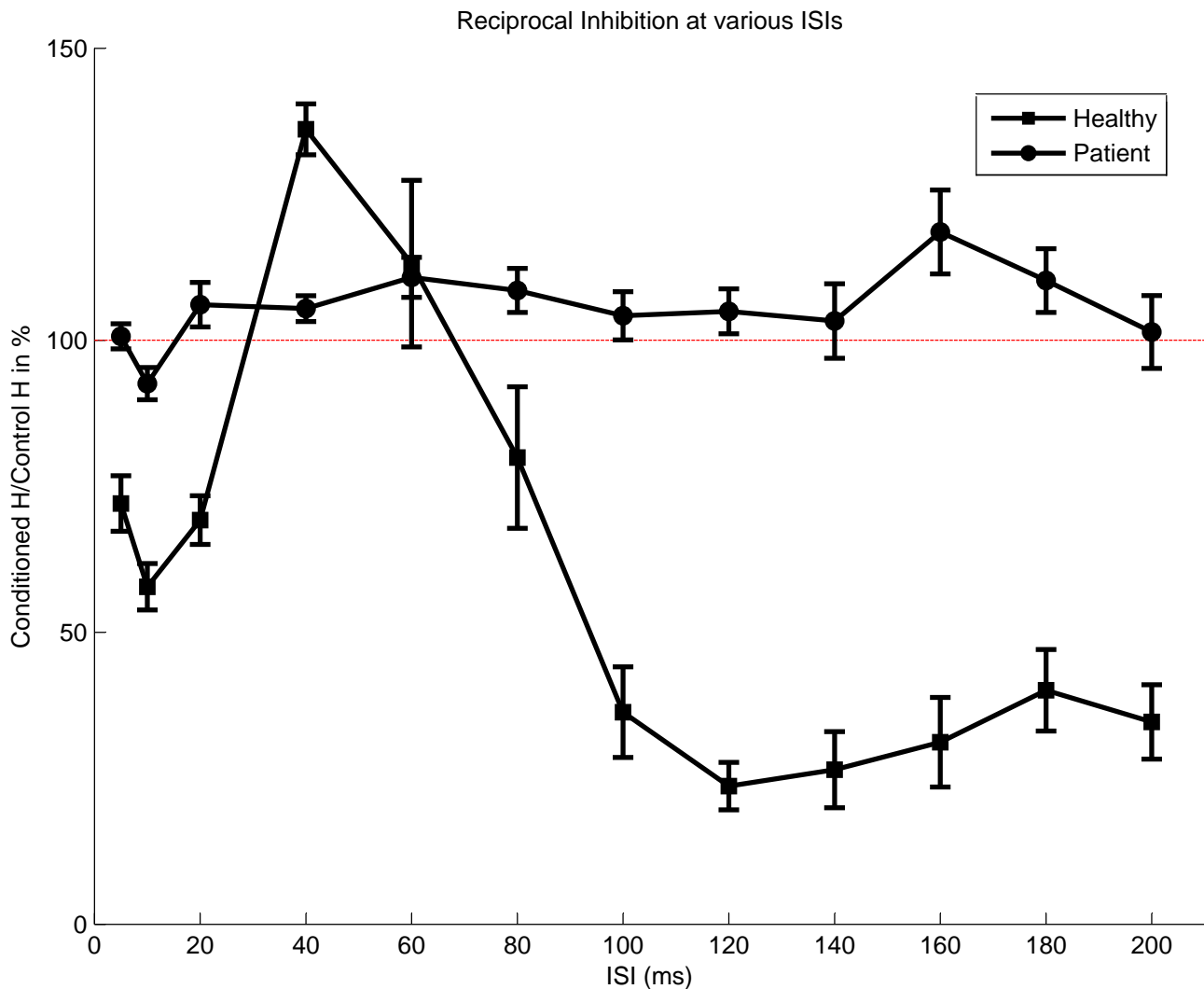


Figure 10. Pattern of reciprocal inhibition in healthy (squares) and stroke (dots) subjects. There is an initial inhibition (at 10 ms interval), followed by a facilitation (at 40 ms interval) and followed by a long lasting inhibition (above 100 ms) in healthy subjects.

The identical protocol was then conducted in stroke patients (n=9). A split-plot design analysis between the two groups showed a significant interaction between the two groups ($F_{11,99}=2.96$, $p=0.002$). To further examine both D1 and D2 inhibition, partial interactions were performed. In this first, the intervals mediating short-latency D1 inhibition were examined. A 2 x 3 (Group x Interval) partial interaction revealed no differences in the pattern of inhibition originated from D1 mechanisms ($F_{2,24} = 0.745$, $p = 0.745$). Conversely the intervals mediating D2 inhibition showed a significant interaction between group and interval ($F_{8,88} = 4.165$, $p < 0.001$).

Combining two inhibitory mechanisms: Conditioning the H-prime by CPN stimulation. In the healthy subjects group, at long ISIs, a substantial depression of soleus H-reflex due to CPN stimulation was observed (for example, ISI =140 in Fig 10). Based on this part of the experiment, an ISI at which more than 50% depression had occurred was selected (e.g., near maximal inhibition in the pathway). The traditional PRD method was repeated, however, in some trials the H-prime was further conditioned by CPN stimulation, to understand whether these two inhibitory influences were additive. We refer to this protocol as “PRD with reciprocal interference”. The ISI between CPN stimulation and H-prime was the one selected from the CPN conditioning experiment. Data of an exemplar healthy subject is provided in figure 11.

In the healthy group, this protocol produced a statistically significant facilitation of the H-prime (reduction in Post-activation depression). Two separate ANOVA tests were performed for the M-waves and the H-reflexes. The statistical test was performed on the raw data (not the ratio of the H_p/H). This was done to examine that the H-reflexes (the first reflex elicited) were not significantly different in Traditional PRD and the PRD with reciprocal interference protocol conditions. Figure 12 summarizes the results of this analysis. In this experiment, none of the M-

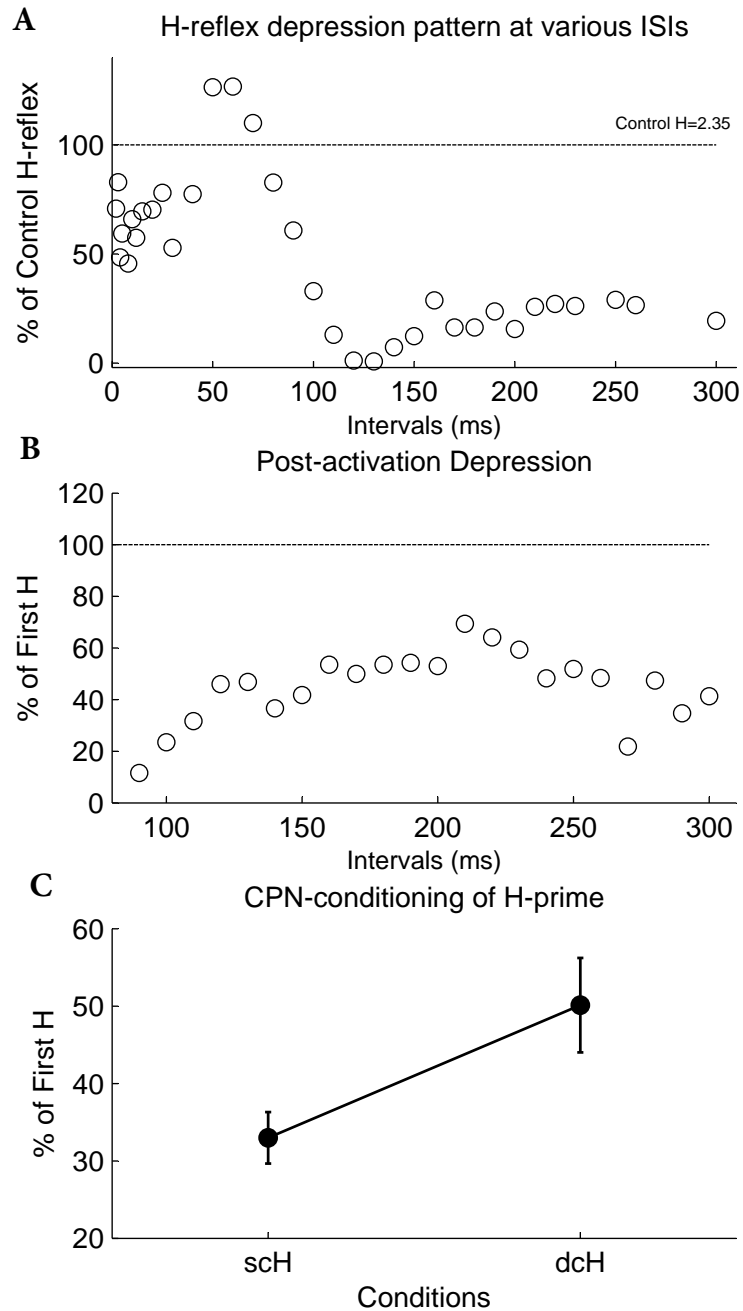


Figure 11. Conditioning H-prime by CPN stimulation. (A) The pattern of inhibition caused by stimulating the CPN at different intervals in one subject. The data are normalized to the control H-reflex. (B) Post activation depression at inter-stimulus intervals ranging from 80 to 300 ms. The data are normalized to the test H-reflex in each trial. (C) Results of conditioning the H-prime with presynaptic reciprocal inhibitory input. scH= single condition H (conventional PRD), normalized to the amplitude of the first H-reflex but without being affected by CPN stimulation, dcH=double conditioned H-reflex (PRD with reciprocal interference). Note the significant release of inhibition in the dcH protocol.

waves was statistically different ($F_{1,17}=3.27$, $p=0.09$). The amplitude of the two H-reflexes (The first H-reflex in PRD and in PRD with reciprocal interference) were not statistically different. Therefore, it is statistically meaningful to compare the H-prime of these two protocols. This analysis showed that the H-prime with the reciprocal interference protocol was statistically larger than the H-prime in the traditional PRD protocol ($F_{1,17}=119.99$ $p<0.001$; pairwise comparison: $p=0.031$).

This new protocol of PRD with reciprocal interference provides valuable information about the interaction of two presynaptically mediated inhibitory mechanisms. We observed in healthy individuals that the interaction of these two mechanisms results in decrease of inhibition (or facilitation) on H-prime response.

PRD with Reciprocal Interference in Healthy Subjects at Short-Latency Intervals. In 6 healthy subjects we repeated the same method explained above one more time to condition the H-prime not only with the long latency inhibitory effect of CPN nerve, but also with the short latency (disynaptic) inhibitory effect. From the methodological standpoint, the only difference was the time interval between CPN stimulation and the second stimulation of the PTN, which was less than 10 ms in this experiment as opposed to the >100 ms in the previous one. However, from the physiological standpoint, this inhibitory effect is most probably being mediated through a relatively more simplistic disynaptic connection. Statistical analysis showed an overall difference among the conditions ($F_{1,5}= 8.93$, $p=0.03$). Pairwise comparisons showed that the H-prime is significantly lower than the control H-reflex ($p=0.001$) and also with the dcH2 with long ISI ($p=0.03$). However, it was significantly higher than dcH2 with short ISI (reciprocal inhibition; $p=0.01$). The results of this experiment are summarized in figure 13.

M wave and H-reflex in different conditioning protocols

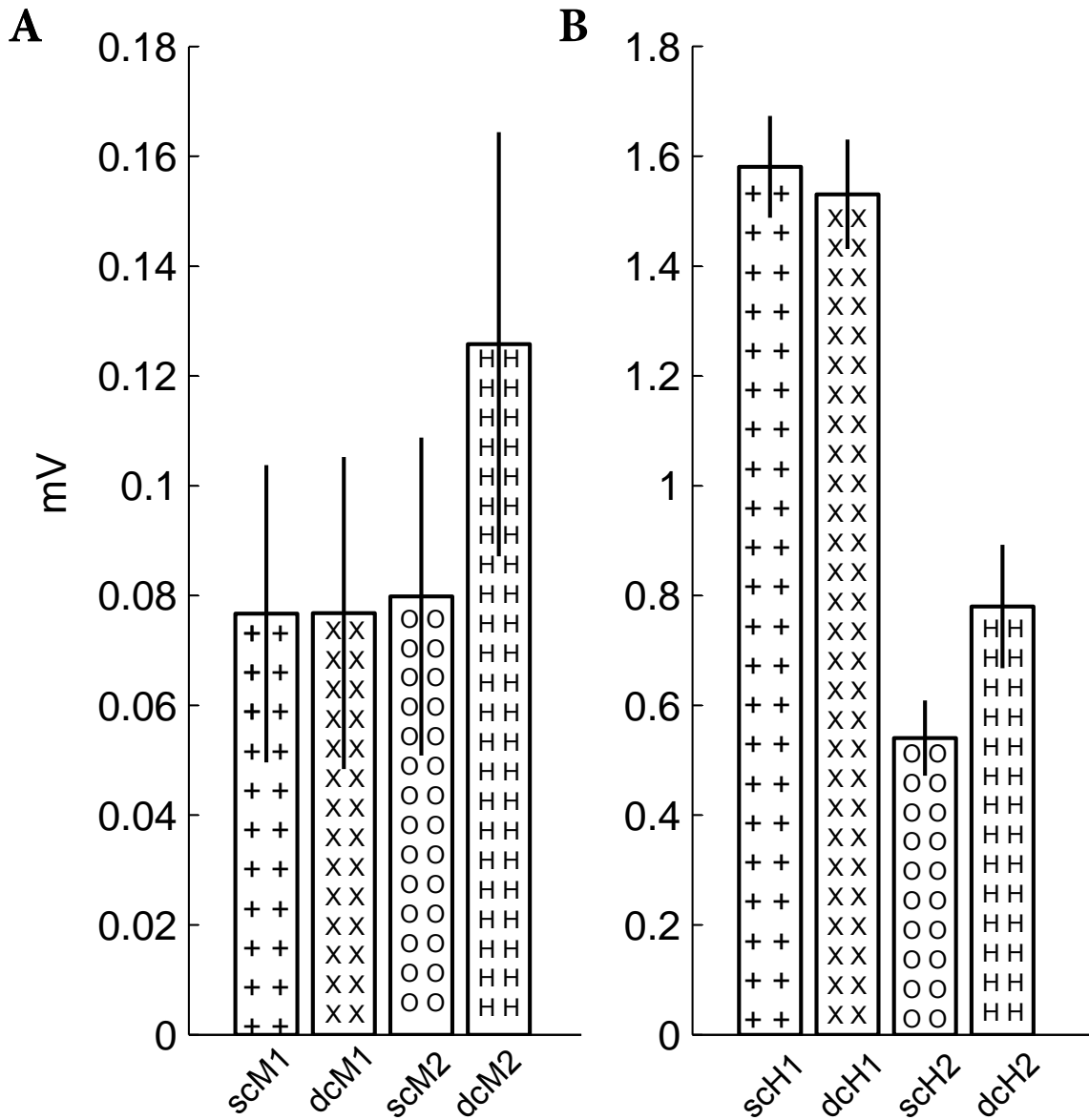


Figure 12. Amplitude of the un-normalized H-reflexes and M-waves. (A) results of the M-waves, (B) results of the H-reflex. scH1 is the H of the first stimulation in the single inhibition trials (only H and H-prime), dcH1 is the first H reflex in double inhibition trials (H-Prime conditioned with CPN). It was expected that these two are not statistically different which ensured that comparing their second responses is meaningful. scH2 is the H-prime not being conditioned by CPN and dcH2 is the H-prime being conditioned by CPN as well. Using ANOVA, it was shown that scH1 and dcH1 were not statistically different; all other pairwise comparisons showed statistically significant differences. For M-waves, no significant differences were observed.

Modulation of H-prime with CPN conditioning

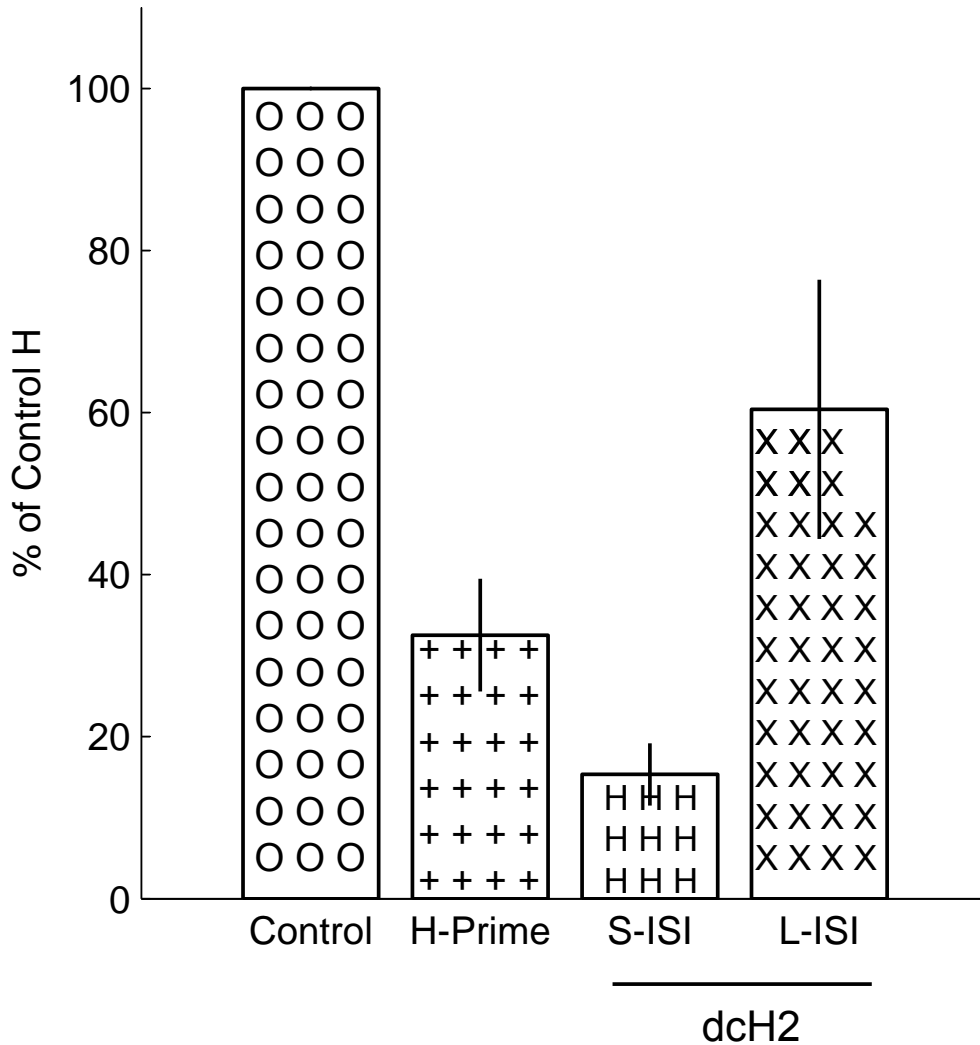


Figure 13. Interaction of D1 (disynaptic reciprocal inhibition) and D2 (presynaptic reciprocal inhibition) on the H-Prime in healthy group. The initial H is regarded as 100%. S-ISI denotes the short ISI which causes disynaptic reciprocal inhibition. The interaction of this with H-Prime caused significantly more inhibition compared to H-prime. L-ISI is the long ISI corresponding to D2. As was observed in the previous experiment, this interaction causes substantial release of inhibition.

PRD with Reciprocal Interference in Stroke Patients. We performed this method on stroke subjects as well. In this group, however, the results did not follow the same trend. We did these tests under the following delimitations. The first delimitation regarded choosing an appropriate time interval for the paired reflex depression protocol (note the differences in inhibition at long-latency intervals for the healthy subjects and stroke patients (Fig 5). Based on the values obtained from healthy participants, it was observed that at ISIs longer than 200 ms, the ratio of the second response to the first one was close to 50%. In some stroke patients, the H-prime response was almost always above 50% of the first H-reflex (e.g., less inhibitory influence). Therefore, in those patients who did not have any inhibition at intervals above 100 ms, we arbitrarily selected the ISI of 300 ms. The second limitation involved the selection of the reciprocal inhibition ISI. In healthy subjects, it was observed that at the ISIs between 100 to 150 ms, the CPN conditioning had the greatest inhibitory effect on the soleus H-reflex. In many stroke subjects, no obvious inhibition was observed at such intervals. Therefore, in testing PRD with reciprocal interference, we made assumption that selecting the *average* of the intervals taken from healthy subjects would provide results which can be confidently compared with the healthy group.

In stroke patients, H-prime in the PRD protocol and that in the PRD with reciprocal interference protocol did not yield any statistically significant change between the H-prime and the H-prime affected by a preceding CPN stimulation ($F_{3,27}=0.16$, $p=0.99$) nor did it show any significant difference among the M-waves ($F_{3,27}=2.33$, $p=0.097$). The results of this experiment are provided in figure 14.

M wave and H-reflex in different conditioning protocols

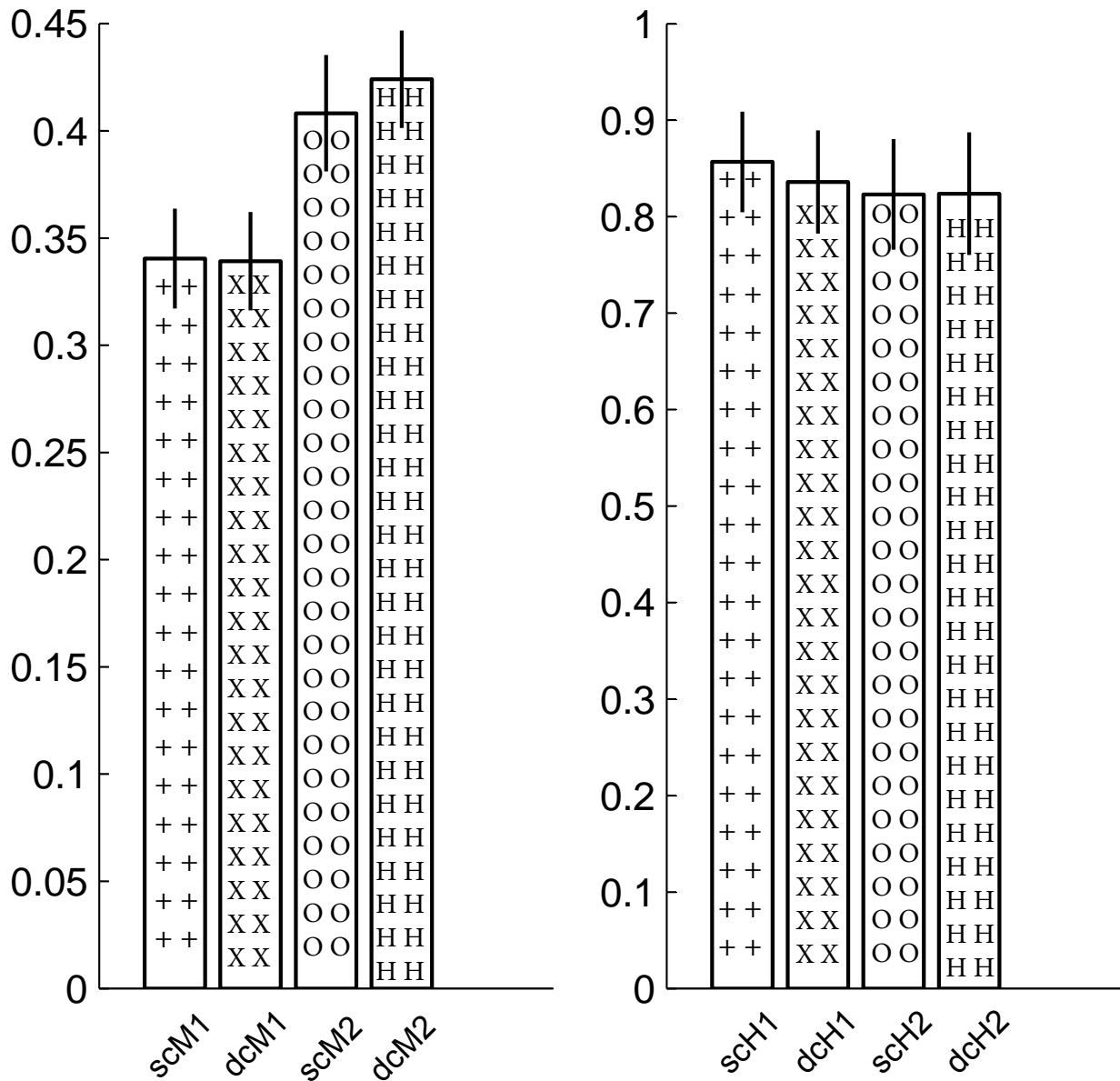


Figure 14. M-waves and the H-reflexes of PRD with Reciprocal interference protocol in stroke. There was no statistical difference among the H-reflexes, suggesting no inhibition due to PRD and no change due to reciprocal inhibition. A non-significant increase in the M-wave of the second stimuli was detected. This could be due to alteration in muscle property after stroke, which has caused muscle potentiation.

Summary of Part I: Spinal Cord Examination in Healthy Subjects and Stroke Patients

The two inhibitory mechanisms of post-activation depression and reciprocal inhibition were tested in health and disease. When examining traditional PRD, in healthy individuals the ratio of H-prime to H-reflex is less than 50% at the tested ISIs of 80 ms and 300 ms, whereas in stroke patients, the ratio of H-prime to H-reflex is less than 50% at short ISIs (below 150 ms) and significantly higher than 50% at ISIs above 150 ms. Thus a statistical interaction was observed between the PRD pattern in healthy subjects and in stroke patients. We further examined the PRD phenomenon by conditioning the second H-reflex with different amplitudes of the initial H-reflex. In the healthy group a non-linear pattern emerges between first amplitude H-reflex and second amplitude H-reflex. In stroke patients, a similar pattern emerged when the two reflexes were separated by a short time interval (less than 100 ms). However, at longer ISIs, the pattern was different from healthy controls. At these longer intervals, by increasing the intensity of the first reflex, the intensity of the second one also increased, shifting the curve to the right.

We also examine traditional reciprocal inhibition pathways between the tibialis anterior muscle and the soleus muscle. In healthy subjects, conditioning the soleus H-reflex with CPN stimulation (tibialis anterior) at different time intervals produced an initial inhibition at 10 ms, a brief facilitation at 40 ms, and a long lasting inhibition at all ISIs above 100 ms. In stroke patients, there was an initial inhibition at 10 ms with no significant modulation of the reflex at any other tested ISIs. When combining both the PRD protocol and the CPN protocol, healthy subjects demonstrated a decrease in inhibition to the motor pool with long latency CPN intervals ($ISI > 100$ ms). Contrary to this, at short-latency intervals, the healthy subjects demonstrated more inhibition on this reflex. Conversely, in stroke patients conditioning the second H-reflex in the

PRD method with a preceding long latency CPN stimulation (ISI>100 ms) did not cause any significant modulation of the H-prime.

Part II: Investigating the training protocol for down-regulating the H-reflex in stroke subjects

The purpose of this section was to examine the efficacy of inducing spinal cord plasticity in a small cohort of stroke survivors. Spinal cord plasticity was induced with a patent-pending neurobehavioral biofeedback system. Three stroke patients completed the training part of this study. The training consisted of nine sessions of exercise induced operant conditioning of soleus H-reflex. Before and after the treatment protocol, some functional and neurological measurements were taken. This section will be summarized in the following sections: 1) subject description; 2) Training Protocol; 3) Demonstration of Spinal Cord Plasticity; and 3) behavioral Consequences of Neural Plasticity.

Subject description

Three of these participants (83±13.12 y/o and 5.17±5.92 y/post stroke) completed both the first part (investigation) and the second part (treatment).

Table 2. Demographics of participants.

Subject	Gender	Age	Affected Side	Patho-mechanism	Time since stroke (y)	Pre-training Ashworth Score	Post-training Ashworth Score
DH	F	59	Right	Ischemic	15	1	1
JT	M	69	Left	Ischemic	2	1+	1+
JC	F	85	Left	Ischemic	1.5	0	0

Patient participants had at least one incidence of a cerebrovascular accident in the past 15 years. The demographics of the participants are provided in table 2.

Training protocol: In each session, subjects practiced in three consecutive blocks. As the sessions progressed, they were able to better control their reflex amplitude. Figure 15 shows the three blocks of one of the final sessions of subject DH. As can be seen, the amplitude of the reflex was substantially depressed in all three blocks. However, as the blocks proceeded, successful down-regulation of the reflex occurred at earlier trials (as is indicated by arrows in fig 14, which are shifted to the left). The consistency of the M-wave ensures that the modulation of the H-reflex was not influenced by a biased input to the spinal circuits.

The rate of success of down-regulating the reflex of each session was calculated. The success rates of DH, JT and JC at their first week of training were 49.00, 51.69 and 57.40, respectively. These values were 86.67, 68.58 and 84.85, respectively for the last week of their training. The success rate for all sessions and all weeks are presented in figure 16.

Behavioral Consequences of Neural Plasticity. These results of gait evaluation are summarized in table 3. To better clarify that these improvements were related to the reflex conditioning, the Relative Success Rate (RSR) and Relative Gait Improvement Index (RGII) were calculated by subtracting SR and GII of the post-treatment from those of the pre-treatment, respectively. These are presented in figure 17. It can be seen that the improvement in function had a strong agreement with the success in down-regulating the H-reflex (filled symbols). This was also the case between walking velocity improvement and the success in down-regulation (open symbols in fig 16)

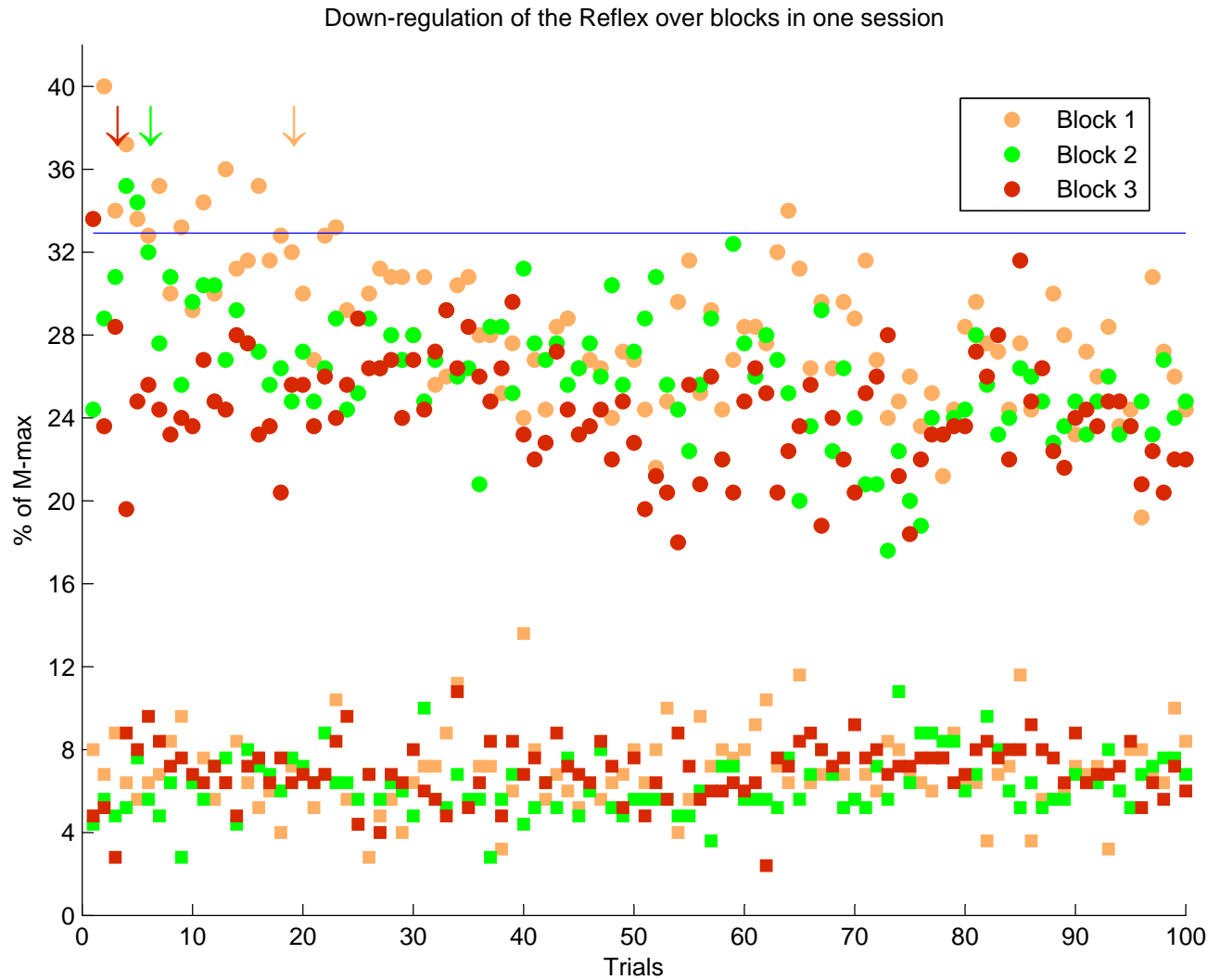


Figure 15. An example of the performance in one treatment session. Each session consisted of three blocks. Circles show the peak to peak amplitude of the H-reflex, normalized to M_{\max} . Squares represent the corresponding M-waves at each trial. The M-wave values were kept constant throughout the trials and among the blocks. Arrows show the trials from which a substantial down-regulation in the H-reflex occurred. The leftward shift in the arrows depicts an earlier learning (compare 19 trials in block 1 to 3 trials in block 3), as the blocks progress.

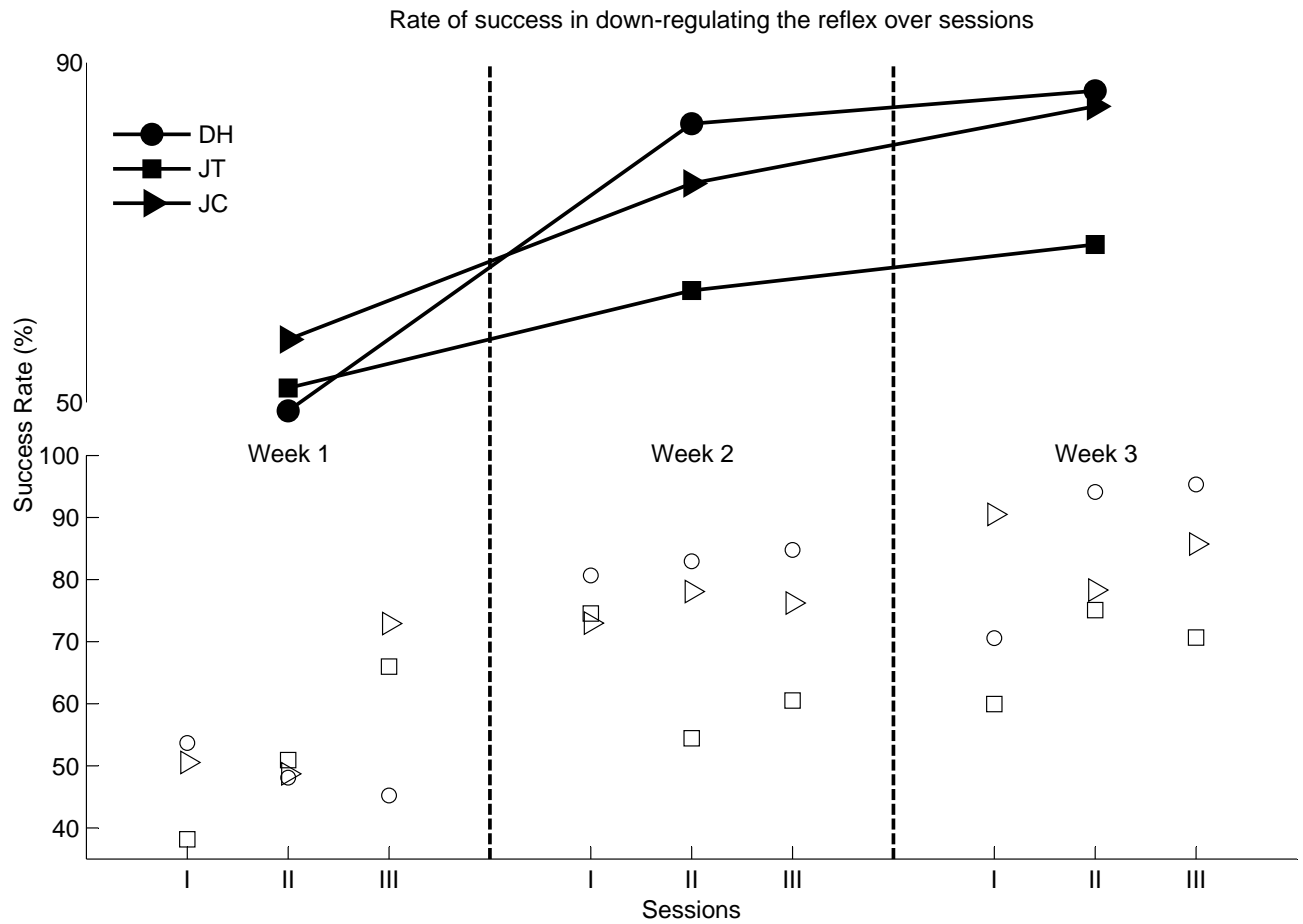


Figure 16. Success Rate (SR) in down-regulating the amplitude of the H-reflex in the 9 sessions (three weeks) of the treatment. Lower panel shows the SR in each session and the upper panel shows the SR in each week (the average of the three sessions).

Table 3. Summary of gait parameters measured before and after the treatment protocol.

		DH		JT		JC	
		Pre	Post	Pre	Post	Pre	Post
Mean Walk Speed (m/s)		0.22	0.44	0.4	0.54	0.28	0.48
Mean Step Cycle Time (cycles/s)		0.62	0.61	0.79	0.80	0.43	0.72
Mean Step Length (cm)	Left	9.0	23.00	32	36.00	23.00	42.00
	Right	18.00	26.00	25	38.00	15.00	33.00
Coefficient of variation (%)	Left	39.00	13.00	13	11.00	17.00	12.00
	Right	18.00	10.00	19	9.00	23.00	11.00

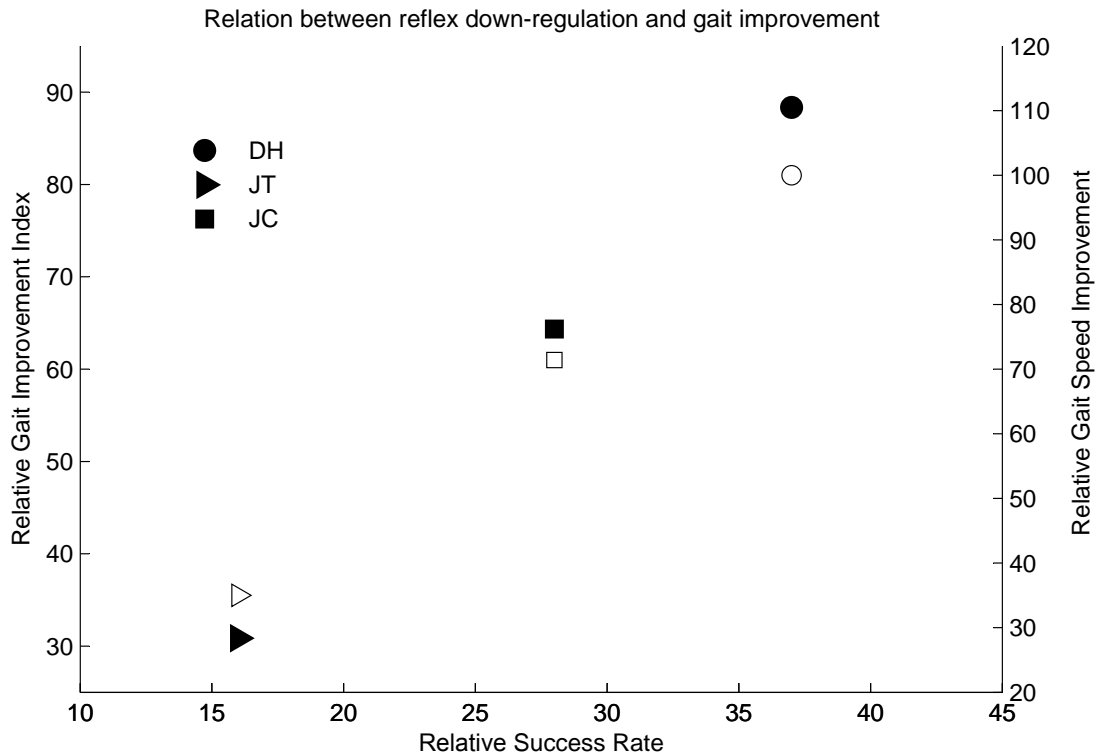


Figure 17. Relative Gait Improvement Index (left ordinate, filled symbols) is in agreement with Relative Success Rate. Relative Gait Speed Improvement (Right ordinate, open symbols), provides the same trend.

Evidence of changes in the presynaptic inhibition profile after training

The main purpose of this investigation was to examine the ability of hemiplegic patients to down-regulate the H-reflex and to observe its functional consequences. However, in one subject (JC), we examined two presynaptic inhibitory mechanisms (Post-activation depression and reciprocal inhibition) as well as measuring the change in H-max. The results of these examinations are summarized in figure 18.

This subject demonstrated 11.03% reduction in the H_{\max}/M_{\max} ratio after the treatment (from $H_{\max}/M_{\max} = 0.56/2$ of pre-treatment to that of $0.34/1.97$ post treatment). At long intervals of reciprocal inhibition, an average of 5.6% decrease in the conditioned reflex was observed. This inhibition pattern more closely agrees with what is seen in the normal population (right panel of fig 17A), although the normal population demonstrates substantial decreases in the conditioned H-reflex. In the pre-treatment evaluation, a reduction in post-activation depression was observed which did not show any change after the treatment (fig 17B).

Summary of the main findings of the training protocol

- 1) A Human-Computer interface was developed for this study and successfully tested in three stroke survivors. Through the preliminary data obtained from these three patients, it was shown that stroke patients are able to down-regulate the amplitude of the soleus H-reflex.
- 2) It was observed that this down regulation of the soleus H-reflex parallels with an improvement in gait index in the three stroke survivors.
- 3) Preliminary data suggest a change in the presynaptic inhibitory profile (D2 inhibition) after the treatment protocol.

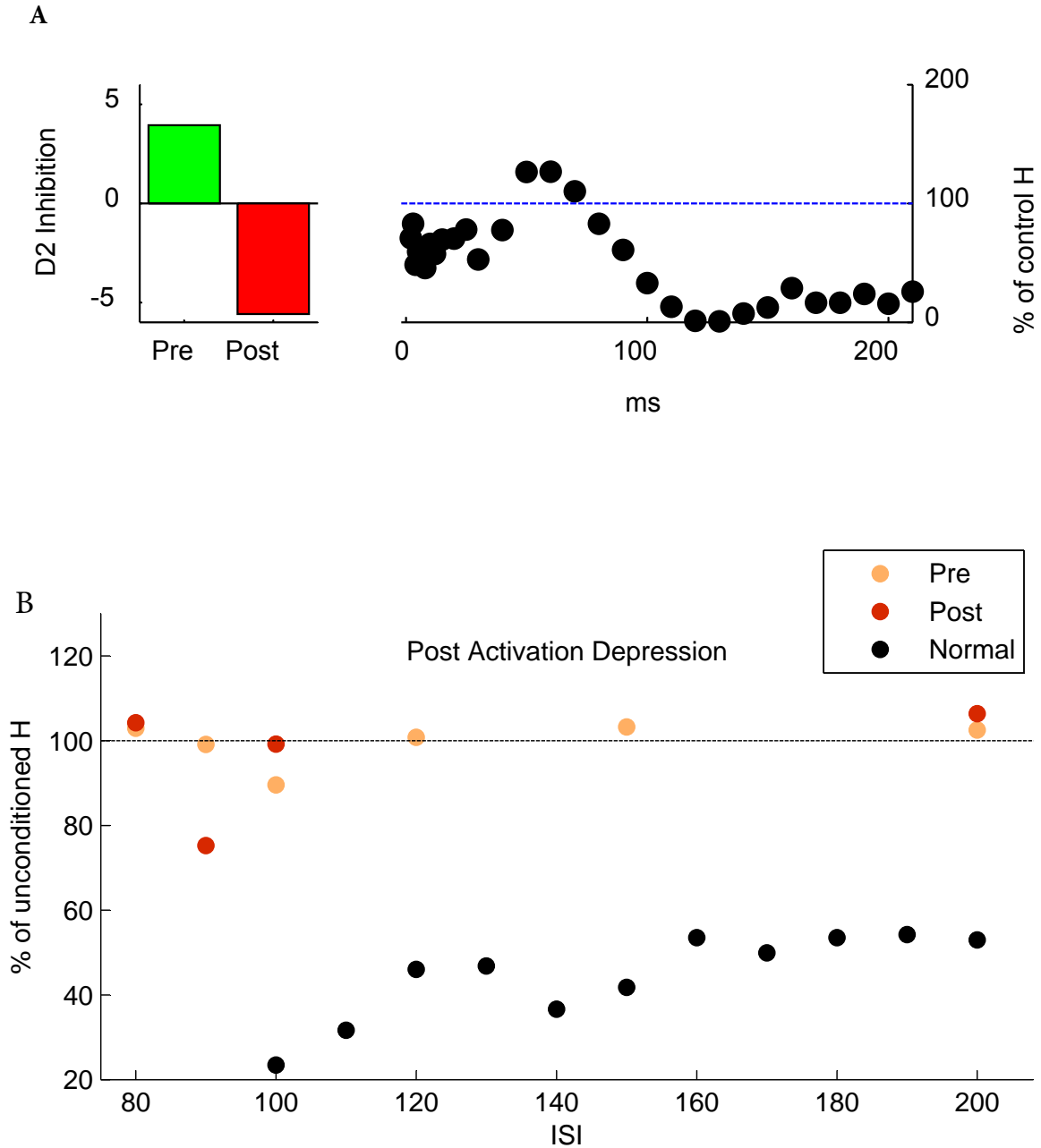


Figure 18. Electrophysiological investigation in patient JC. The post-activation depression was tested over the intervals up to 200 ms. (A) shows the change in reciprocal inhibition at different ISI. The left side panel shows the changes in presynaptic reciprocal inhibition at long ISIs in the patient. The Right panel shows a representative healthy individual. Panel B shows the results of post activation depression in a healthy individual (filled black) and the pre/post treatment values of the patient. No substantial change is observed. In Stroke patients a slight depression might be observed at ISIs<100, as is the case in this patient.

CHAPTER FIVE

DISCUSSION

This study consisted of two interrelated parts. The goal of this study was to examine the possibility of inducing plastic changes in the spinal circuits of stroke patients and to test the hypothesis that the plastic changes are induced through presynaptic modulations of Ia-alpha motoneuron connections. Achieving all these goals transcends one PhD dissertation with its time and funding limitations.

In this study, we 1) developed new methods for investigating presynaptic mechanisms of the spinal cord, 2) provided normative data based on these new approaches, 3) tested these protocols on our patient population and compared the results with those of healthy subjects, 4) developed a Human-Computer interface to down-regulate the soleus H-reflex in stroke subjects, 5) provided evidence that this patient population possesses the ability to volitionally down-regulate the reflex and 6) provided preliminary data to support the hypothesis that this down-regulation is accompanied by changes in presynaptic control of Ia-inflow.

This chapter is organized in the following sections: 1) A discussion on the findings of the two presynaptic mechanisms in health and disease, 2) A discussion on the findings of the treatment protocol, 3) Review of the study aims, 4) General conclusion, 5) Recommendations.

Part One

Findings of the two presynaptic mechanisms in health and disease

Spinal inhibitory mechanism in health and stroke

Magledary and McDougal (1950) were the first to systematically demonstrate the long lasting depression of the H-reflex following a preceding activation of the reflex loop. This observation has since been repeatedly reported with different methods such as repeated electrical stimulation of the nerve (Teasdall, Languth et al. 1952; Clair, Anderson-Reid et al. 2011), mechanical tendon tap (Katz, Morin et al. 1977) or passive stretching of the target muscle (Nielsen, Petersen et al. 1993; Robertson and Koceja 2003). This inhibitory phenomenon is widely known as post-activation depression. Different methods have been developed and validated to elicit this mechanism: conditioning the H-reflex with a preceding H-reflex (Trimble, Du et al. 2000; Sefton, Hicks-Little et al. 2008), eliciting a train of reflexes with fixed intervals and measure the frequency dependent depression of the reflex (Schindler-Ivens and Shields 2000; Schindler-Ivens and Shields 2004), conditioning the H-reflex with a preceding mechanical stretch (Morita, Petersen et al. 1998) or tendon tap (Crone and Nielsen 1989; Morita, Shindo et al. 1993). Because all these methods are based on the activation of the Ia-fibers, it is commonly agreed that they all follow the same mechanism. This idea is further strengthened by the fact that causing ischemia on the sensory nerve abolishes post activation depression, regardless of the method used to elicit it (Rossi, Mazzocchio et al. 1988; Hultborn, Illert et al. 1996). However, the nature of this neural mechanism is a matter of debate. Initial studies suggested the involvement of supraspinal loops (Eccles 1966; Taborikova and Sax 1969; Chofflon, Lachat et al. 1982). The site of inhibition on alpha motoneurons has also been questioned. Rossi et al. (1988) argued that

the inhibition acts postsynaptically. They also suggested that it is not due to the depletion of neurotransmitters. However, the studies of Hultborn and colleagues in the 1990s (1996) provided evidence on the presynaptic nature of this inhibitory mechanism. For example, stimulating the cortical neurons by transcranial magnetic stimulation after eliciting a conditioning reflex did not affect the amplitude of these motor evoked potentials. Parallel experiments on animal models using more direct methods confirmed that the membrane potentials were not being affected by a preceding activation of Ia fibers. Taken together, these findings provided strong evidence to suggest that the depression of the test H-reflex was not occurring after the synapse and probably was due to the reduction in neurotransmitter release and was confined solely to the activated Ia fibers.

Our results on paired reflex depression at various ISIs is consistent with the past studies using equal conditioning and test reflexes (Olsen and Diamantopoulos 1967), however, we did not observe any facilitatory phase while conditioning the H-reflex with a preceding H-reflex with smaller amplitude. This could be partly due to methodological difference. For example Taborikova and Sax (1969) conditioned the H-reflex with a subliminal preceding H-reflex at various time intervals, whereas we used a fixed interval and conditioned the reflex with different amplitudes of a preceding reflex.

Our findings on post-activation depression are not in full agreement with the current understanding of the mechanism. 1) We observed that the relation between conditioned H-reflex with the amplitude of the preceding H-reflex follows a nonlinear pattern. This could be due to the following reasons: The first possibility is that by increasing the amplitude of the first H-reflex, more bigger sized motor units are being inhibited and therefore, a more substantial decrease in the amplitude of the H-reflex results. The second possibility is that the inhibition

caused by the first stimulation spreads to other branches and therefore, causes more substantial effect. Such a thing can only be observed with our method and not the traditional method. 2) Our studies on the patient population showed that the inter-stimulus intervals plays a significant role in the pathology of the mechanism. If the inhibition is confined to those fibers being activated, we would expect to see similar results in testing the mechanism at short and long ISIs in the patient population. This finding suggests that other sources such as supraspinal (Eccles 1966; Taborikova and Sax 1969) play a role in this mechanism. Also our finding regarding the interference of CPN stimulation on the release of PRD inhibition (discussed later) could be regarded as another evidence to suggest that the mechanism is more complicated than being confined to the activated fibers. It is suggested by Voigt and Sinkjaer (1998) that the short term effect of post activation depression might be due to a spinal mechanism and the long term depression due to depletion of neurotransmitters. In line with this hypothesis, an explanation of the current observations is that such an inhibition affects the interneuronal system such as Ia interneurons.

Post activation depression is shown to be strongly correlated with the impairment and spasticity (Masakado, Kagamihara et al. 2005; Grey, Klinge et al. 2008; Lamy, Wargon et al. 2009). It is also reported that robotic training for 4 weeks can partially restore this inhibitory mechanism in stroke patients (Trompetto, Marinelli et al. 2013). Our sample was too small to establish a correlation between PRD and severity of stroke. However, we expect to see changes in the post-activation profile of patients after the training method we have introduced here. In the one patient that we tested these two inhibitory mechanisms pre and post training, we observed changes in the reciprocal profile but no substantial change in the PRD results. A large scale study is warranted to observe any changes in PRD after this training method.

Reciprocal inhibition

CPN conditioning at different intervals prior to eliciting the H-reflex produced a complex response. It was first accompanied by an initial depression which maximized at 20 ms interval in our data. This initial depression was referred to as D1 by Tanaka and Mizuno (Mizuno, Tanaka et al. 1971; Tanaka 1974). This D1 inhibition is now known to be disynaptically mediated (Crone, Hultborn et al. 1987) and is one of the best studied pathways (Crone, Nielsen et al. 1994). Our data also clearly showed this D1 inhibition in healthy individuals, and to a smaller extent, in stroke subjects. In their investigation, Mizuno and Tanaka (Mizuno, Tanaka et al. 1971) reported a second depression occurring at 60 ms, maximizing at 130 ms and lasting for a long duration (500 – 1000 ms). We observed the second depression beginning at about 70 ms, maximizing at 120-140 ms and lasting for a long duration (up to 300 ms interval- the longest interval we tested). Given that the stimulation intensity of the CPN substantially affected the shape of this pattern, our results are in good agreement with what Mizuno and Tanaka first reported. Our results also match those reported by Geertsen and colleagues (2011) and Iles and Pisini (1992). But there are other reports with smaller intervals (Meunier and Pierrot-Deseilligny 1998). However, we have observed a facilitatory response peaking at 40 ms which has not been consistently reported in other studies. Levin and Chapman (Levin and Chapman 1987) have observed a facilitatory effect of CPN conditioning at 130 ms.

It is generally agreed that the D2 inhibition is mediated presynaptically (Tanaka 1974; Crone, Hultborn et al. 1985; Crone, Hultborn et al. 1987). However, the D1 could be due to presynaptic inhibition or post synaptic Ib inhibition (Mizuno, Tanaka et al. 1971; Tanaka 1974).

We observed that our patient population did not have any D2 inhibition, but demonstrated a weak D1 inhibition. In the one subject that we tested pre and post training, we observed the emergence of a 5% increase in D2 inhibition (induction of inhibition after training).

This is not the first report to show that presynaptic reciprocal inhibition has an interaction with other inhibitory sources. Indeed, D1 and D2 inhibition have been shown to be modulated by descending drive. Illes and Pisini (1992) showed that cortical stimulation can inhibit the H-reflex and D1 and D2 inhibition also decrease the amplitude of the reflex. However, with appropriate timing and intensity of the D1/D2 influence, a release of inhibition due to pairing them with cortical stimulation was observed. This cortical control of this long-lasting reciprocal inhibition was confirmed in a well-controlled investigation (Meunier and Pierrot-Deseilligny 1998). Recurrent inhibition also influences disynaptic reciprocal inhibition. Recurrent inhibition influences the Ia interneuron and decreases its inhibitory effect on the antagonistic motoneuron pool. This interaction was first observed in the cat (Hultborn, Jankowska et al. 1971) and indirect observations suggested its existence in man as well (Baret, Katz et al. 2003). Here, we have findings which strongly suggest that post-activation depression is influenced by the heteronymous presynaptic inhibition. From the results of this study, it cannot be inferred where this interaction takes place. However, because the interaction causes a reduction in inhibition, it is in all likelihood happening prior to alpha motoneurons and most probably affecting the inhibitory interneurons.

It is shown that repetitive stimulation of Ia fibers render them less sensitive to presynaptic inhibition when they are activated by a stretch reflex. This hypothesis was used to explain why the H-reflex is substantially depressed with a conditioning stimulation from Biceps Femoris or from CPN stimulation (Morita, Petersen et al. 1998). Subsequent intracellular studies confirmed

this hypothesis (Enríquez-Denton, Morita et al. 2002). However, it is very unlikely that this was the case in our observation. The insensitivity of Ia fibers to presynaptic inhibition was shown only with mechanical stimulation and not the H-reflex. Here we used solely the H-reflex which does not cause any dorsiflexion torque. The initial H-reflex might cause a twitch and stretch on the soleus muscle. A stretch is itself a factor for depressing the second H-reflex (Pierrot-Deseilligny and Mazevet 2000). Therefore, the observation of decrease in inhibition of the second H-reflex cannot be attributed to insensitivity of the Ia fibers to presynaptic inhibition or due to any mechanical stretch due to the first stimulation.

We propose that homonymous Ia afferents (Ia fibers from the soleus muscle), make presynaptic connections with Ia interneurons. While no axo-axonic connection has been yet observed at the terminals of these interneurons, the possibility of presynaptic control of Ia interneurons has been suggested (Jankowska 1992).

While the current findings call for more in-depth investigations and require more direct methods to confirm our findings and interpretations, it can be suggested that activating Ia fibers is a regulatory mechanism which interacts with other presynaptic mechanism and might affect the interneuronal system. There could be functional implications for the interaction. During co-contraction of antagonists, the feedback from the agonist muscle can modify the inhibitory effect of the antagonistic and vice versa. This possibility needs more investigation in a well-controlled study.

Aging and changes in spinal inhibitory and reflex profile

The effect of aging on spinal cord function in general and reflex pathways in particular has been extensively studied over the past several years (Burke, Schutzen et al. 1996; Robertson and Koceja 2003; Scaglioni, Narici et al. 2003). Indeed, aging affects the postural modulation of the

H-reflex (Koceja, Markus et al. 1995). It is suggested that differences in spinal modulation of the reflexes might be due to difference in presynaptic control of Ia afferents (Tsuruike, Koceja et al. 2003). In the investigation of the inhibitory mechanisms of spinal cord, we used a sample group of young healthy individuals, which were not age-matched to our stroke sample, and we investigated two new spinal cord conditioning methods in this research. Our aim was not to identify age-related changes in these spinal pathways, but to examine the effects of hemiplegic stroke on these pathways. We chose to examine spinal control of these pathways in young individuals, as investigating these methods in age-matched elderly would have provided mixed results since normal aging provides a cascade of changes as a result of a variety of behavioral and environmental factors throughout the lifespan (e.g., physical activity levels, nutrition, socio-economic factors). This study examined the interaction of spinal cord pathways between young subjects and stroke survivors. However, it remains that one limitation of this study is that when comparing the results of young control subjects with the stroke group, it is unknown the degree to which the observed differences are attributed to age differences rather than the pathologic condition.

Part Two:
Findings of the treatment protocol

Disturbances of the inhibitory mechanisms due to stroke have been a subject of meticulous investigations over the past few decades. The impairment of these mechanisms can contribute to the pathophysiology of spasticity (Nielsen, Crone et al. 2007) and movement disorders observed after stroke. Abnormalities in recurrent inhibition (Katz and Pierrot-Deseilligny 1982), post activation depression (Aymard, Katz et al. 2000) and reciprocal inhibition (Crone, Johnsen et al.

2003) have been reported in stroke patients. Part of these abnormalities is due to the release of spinal pathways from descending drive. In a stroke, the inhibitory control that the cortex exerts over the spinal cord is decreased which results in an imbalance between the descending and the sensory inputs to the alpha motoneurons. The loss of several inhibitory mechanisms in the spinal cord is an important contributing factor to the pathophysiology of movement disorder. Inducing a positive plastic change in these pathways seems to be promising avenue for rehabilitation. Here, we showed that inducing this plastic change is possible in stroke survivors. More importantly, this plastic change was accompanied by improvement in motor performance as was observed in their walking speed and RGII. Patients reported “feeling better in daily activities” after the second week of training. This was independent from spasticity as the comparison of pre and post-treatment Ashworth scores did not suggest any change in the severity of spasticity. This observation is crucially important and impactful for researchers who work on rehabilitation methods for stroke survivors. There is a long lasting debate as to whether reducing spasticity is a key point in success for rehabilitation and should be emphasized or spasticity is not a debilitating factor and does not necessarily need to be reduced (Burke, Wissel et al. 2013). On the other hand, spasticity is, by many clinicians, defined as “hyperreflexia” and “hypertonia”. We showed that depressing the amplitude of the reflex does not affect hyperreflexia but does affect function. Modern views on spasticity are that it is not necessarily needed to be treated (Burke, Wissel et al. 2013). Here, we provided neurological evidence to support this notion. This notion is in line with studies which suggest (through outcome measures) that the degree of spasticity is not correlated with the disabilities of the patients (Sommerfeld, Eek et al. 2004).

The duration of treatment in our protocol falls within the first phase of plastic changes associated with reflex down-regulation.(Wolpaw and O'Keefe 1984; Wolpaw, Maniccia et al. 1994) During

this phase, plastic changes are reversible and do not cause a long lasting depression in the amplitude of the reflex. Nonetheless, functional improvements were observed in this first phase. Further studies with larger sample sizes and longer durations are warranted to investigate the possibility of permanent changes in the reflex pathways and their functional consequences.

Placebo effect

One aspect of the training phase of this study which must be considered is the placebo effect. Sometimes patients given a placebo (sham) treatment will have a perceived or actual improvement in performance, a phenomenon commonly called the placebo effect. The placebo effect has been observed in different types of settings; from psychological therapy (Quitkin, Rabkin et al. 2000) to physical therapy (Marchand, Charest et al. 1993).. The placebo effect is beyond a psychological reaction and is shown to be accompanied by physiological responses similar to those accompanied by real treatment (Mayberg, Silva et al. 2002).

It is important to note that in our treatment study we did not investigate the placebo effect. Conditioning the H-reflex (up-regulation or down-regulation) is an objective and measurable physiological response. However, the consequences of operant condition (kinematic or functional changes), can be subject to placebo effect. There is a possibility that in the absence of a successful conditioning of the H-reflex some degrees of functional improvement would be observed. The main goal of our study was to provide initial evidence as a proof of concept that operant conditioning is indeed possible in hemiplegic stroke patients. Measuring the pure outcome of H-reflex conditioning on function will entail a strictly controlled randomized clinical trial study, in which a sham treatment condition is introduced. This was beyond the scope of this study; however, its importance should not be undermined.

Part Three:

Review of Study Aims

In this concluding section, the original aims of the study are revisited, and a summary of the aim achievement is presented:

Aim 1 – The first aim of this study is to investigate the interaction between two inhibitory mechanisms (Reciprocal inhibition and Post activation depression of Ia fibers) in health and pathology. Literature shows that each of these two mechanisms contribute to movement impairment and pathophysiology of spasticity.

Our study provided new information regarding the inhibitory profile of stroke patients. We showed that the time interval in post activation depression is an important contributing factor of the impairment of this mechanism. We observed that the second response in PRD method can either be normal or exaggerated depending on the timing between the two stimuli. We also showed that in stroke subjects the D2 inhibition is largely absent while D1 inhibition is partially and weakly present. Finally, we observed that the interaction between these two mechanisms (which is a decrease in inhibition), is affected by stroke. Basically, both mechanisms should be intact for the interaction to provide release of inhibition.

Aim 2 – Stroke survivors will demonstrate the ability to down-regulate the lower limb reflexes without increasing co-contraction using a specially designed computer-patient reflex re-training intervention.

We successfully trained 3 stroke participants with the Human-Computer Interface we developed for this study. These three subjects demonstrated significant improvement in the success rate of down regulating their H-reflexes. This down-regulation took place while the EMG activity of tibialis anterior and soleus muscles were being monitored. Subjects received electrical

stimulation only when the muscles were within 30% of their normal activity level. Therefore, the changes in H-reflex were independent from muscle co-contraction.

Aim 3 – The dynamics of gait will improve in response to the proposed reflex training protocol.

One of the striking findings of this study was an improvement in gait parameters after a successful down-regulation of the H-reflex. Providing a statistical correlation between the success rate and improvement in gait was not possible due to the limited sample size that we had, but the preliminary data suggest that these two are correlated. We have also provided preliminary data to suggest that this training is accompanied by an increase in presynaptic inhibition in the spinal cord.

Part Four:

General conclusion

Inducing plastic changes at the level of spinal cord for the rehabilitation of neurological disorders such as spinal cord injury and stroke has gained attention over the past few years (Motta-Oishi, Magalhães et al. 2013). Changes in the inhibitory mechanisms as a result of a specific training have also been examined. For example, robotic training has shown to improve post activation depression in stroke survivors (Trompetto, Marinelli et al. 2013). Also, patterned stimulation of peripheral nerves have also been successful for inducing changes in the inhibitory pathways of patients with spinal cord injury (Perez, Field-Fote et al. 2003). It is also shown that operant conditioning of the H-reflex affects the kinematics of motion of spinal cord injured patients (Thompson, Pomerantz et al. 2013; Thompson and Wolpaw 2013). The findings of this study provided another evidence to support this notion and also extended it for stroke patients. We

have shown, in a limited sample of subjects, that stroke patients also have the ability to volitionally modulate their reflexes. Through our preliminary data, we also showed that this training affects the inhibitory profile of the spinal cord.

This study did not directly investigate the mechanisms involved in the improvement of function. We only have some preliminary data for this part to propose a possible mechanism. Sensory inflow can also be regulated through the complicated mechanism of presynaptic inhibition of Ia afferents which acts to regulate the amount of neurotransmitter release. Surprisingly, it has been repeatedly demonstrated that this mechanism is not substantially affected in stroke patients. (Burke and Ashby 1972; Faist, Mazevet et al. 1994; Katz 1999; Aymard, Katz et al. 2000; Pierrot-Deseilligny and Burke 2005; Lamy, Wargon et al. 2009). In our study, we tested

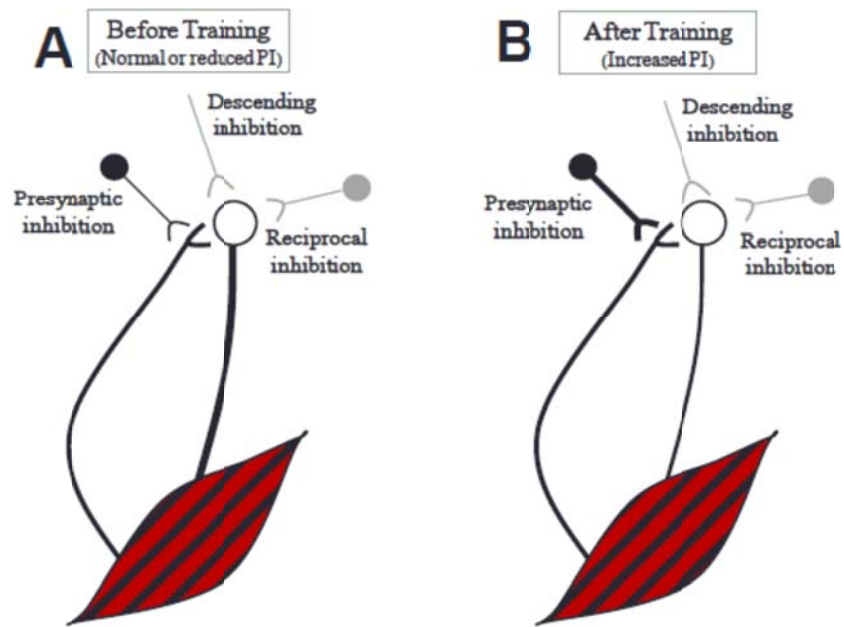


Figure 19. A hypothetical mechanism of motor improvement due to this intervention. After stroke, many inhibitory mechanisms are weakened (shown by gray connections). Presynaptic inhibition is reported to be intact (A). After this intervention, an increase in the presynaptic inhibition of Ia afferent can improve motor performance by decreasing the interruption of cortical commands.

two of the many presynaptic inhibitory pathways. One other important pathway which presynaptically affects the soleus H-reflex is through the femoral nerve. Most of the above mentioned studies have used femoral conditioning for the investigation of presynaptic inhibition in stroke subjects. Nonetheless, our study provided good evidence to show that presynaptic mechanism is trainable in these patients. Figure 19 illustrates a proposed mechanism/pathway which can cause these changes.

Presynaptic control of Ia afferent is shown to regulate movement in normal conditions (Tahayori and Koceja 2012; Tahayori, Port et al. 2012). An increase in presynaptic control of Ia afferent could prevent the interruption of cortical drive and hence encourage volitional motor commands.

Part Five:

Recommendations

Based on the results from this study, the following recommendations are made for future research investigating the inhibitory mechanisms in health and disease and the impact of operant conditioning for stroke rehabilitation.

- 1) In this study, new observations were made regarding the mechanism of post activation depression. It was shown that the interstimulus interval plays a significant role in the impairment of this mechanism. We examined the effect of various intensities of soleus H-reflex on the H-prime at short and long ISIs. We also examined the effect of same intensity H-reflex on the H-prime. However, we did not examine the effect of a low-intensity H-reflex on the H-prime at different ISIs. Based on the current findings, we expect to observe an increase in the abnormality of the pattern with the increase in the

ISI. This study, along with our current findings, will better investigate the interaction between the intensity of Ia activation and the timing of the activation.

- 2) Inducing two presynaptic inhibitory methods (PRD + D2) on soleus alpha motoneurons caused a reduction in inhibition in healthy individuals. We observed that it happens only when the two methods act presynaptically (since there was more inhibition with PRD+D1). The mechanism of action of these two inhibitions is not the same; D1 is accompanied by primary afferent depolarization while PRD is not. However, they cancel/reduce each other's effect. Our results make us conclude that there is an unknown mechanism responsible for this phenomenon. It might be as simple as sharing a common pathway. Regardless of the mechanism, to further strengthen this conclusion, it is recommended to repeat this method with another presynaptic inhibitory source. For example, conditioning the H-prime with femoral nerve stimulation. This can provide us with a more generalized conclusion that presynaptic mechanisms interact with each other.
- 3) Our results of the effect of operant conditioning on functional improvement in stroke survivors were very promising. It showed that stroke patients can down regulate their soleus H-reflex and this is accompanied by an improvement in function. However, at this stage, the results are still preliminary and cannot be generalized. It is not known whether this method is effective on all types of stroke. It is also not known for sure that this method is effective through increasing the presynaptic control of Ia fibers. Conducting this study (training stroke patients and examining their inhibitory profile before and after the treatment) in a large scale, as a randomized-control trial, is essential to provide higher level of certainty about the current findings.

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CV

Behdad Tahayori (PT, PhD)

Licensed PT (License # 05011194A)

btahayor@indiana.edu

behdadt@gmail.com

Education

2009-2014 PhD. Double PhD Program in Kinesiology and Neural Sciences. Indiana University.

2008-2009 M.S. Kinesiology. Louisiana University, Baton Rouge, LA, USA.

GPA: 4.00

2003-2005 M.S. Physical therapy. Iran University of Medical Sciences, Tehran, Iran

GPA 18.69 (Equal to 3.74), (Ranked 1st among 4 students).

1999-2003 B.Sc. Physical therapy. Shiraz University of Medical Sciences, Shiraz, Iran

GPA 17.8/20 (equal to 3.56), (Ranked 3rd among 40 students).

Academic Honors, Scholarships and Awards

Recipient of Siefker scholarship from La Porte Hospital Foundation, \$1000, June 2014.

Recipient of American Heart Association Pre-doctoral Fellowship, \$26000, Dec 2012.

Recipient of ACSM doctoral dissertation grant, \$5000, July 2012.

Recipient of the Cooper Research Scholarship.\$3400, September 2012.

Recipient of the Gallahue-Morris Research Scholarship, \$800, September 2011.

Ranked 1st in MSc. program, Iran University of Medical Sciences 2006.

Ranked 3rd in BSc. program, Shiraz University of Medical Sciences 2004.

Grants

1- **Behdad Tahayori (PI)**, David Kocaja. A novel therapy to regain control of spinal motoneurons in stroke survivors. American College of Sports Medicine Doctoral Dissertation Grant, 2012 [\$5000, Funded].

2- **Behdad Tahayori (PI)**, David Kocaja. A novel therapy to regain control of spinal motoneurons in stroke survivors. HPER Student Research Grant-in-Aid, 2012. [Funded \$900].

3- **Behdad Tahayori (PI)**. Travel Grant-in-Aid, 2010,2011, 2012. [\$400, Funded].

Patent

Method and System for Training an Individual. US Patent Pending (Co-Inventor - 61/766,739)

Teaching Experiences

Motor learning (Lab sessions). Indiana University, Fall 2009 and Spring 2010, Fall 2010, Spring 2011.

Anatomy Labs. Louisiana State University, Spring 2008, Fall 2008, Spring 2009.

Biomechanics. Louisiana State University, Summer 2009; Indiana University, Fall 2012.

Hand Therapy. Shiraz University of Medical Sciences. Spring 2007.

Professional Membership

American Physical Therapy Association (APTA)

American College of Sports Medicine (ACSM)

American Heart Association (AHA)

Society for Neuroscience. (SfN)

Iranian Physiotherapy Association (IPTA)

Physical therapy work experience

Indiana University Health La Porte Hospital, Outpatient physical therapist. Full time job. March 2014 – January 2015.

Home visit physiotherapist. Shiraz, IRAN 2006-2007

Physiotherapist. Shayamehr Hospital for reconstructive surgery. (Post ACL reconstruction surgery therapist). Tehran, IRAN 2004-2006

Clinical Instructor HFZ Hospital for reconstructive surgery. (Hand Therapy). Tehran, IRAN 2005-2006.

Volunteer physiotherapy assistant. Aliasghar Hospital. Shiraz, IRAN. 2000-2002.

Volunteer physiotherapy assistant. Private clinic. Shiraz, IRAN. Summer 1999.

Publications

Manuscripts under review:

[1] **Behdad Tahayori,** David Koceja. Group I antagonistic modulation of soleus H-reflex in healthy individuals and hemiplegic stroke patients. *Submitted to Neuroscience Letters.* November 2014.

[2] **Behdad Tahayori,** David Koceja. Exercise induced operant conditioning of the H-reflex in stroke patients: hopes for improving motor function through inducing plastic changes in the spinal pathways. A preliminary study. *Submitted to Disease and Disability.* November 2014.

Published papers:

- [1] Xenofondos A, Patikas D, Koceja D, **Tahayori B**, Bassa E, Kellis E, Kotzamanidis. Post activation potentiation: on the neural effects of post activation depression. *Muscle & Nerve*. Accepted for publication. November 2014.
- [2] **Tahayori B**, Koceja D, Nelson A. A biomechanical analysis of the contributing factors to increases in vertical jump height following exercise with weighted vests. *J Rehabilitation Science and Research*.1(1),2014:1-8.
- [3] **Tahayori B**, Port L, Koceja D. The inflow of sensory information for the control of standing is graded and bidirectional. *Experimental Brain Research*. 218(1), 2012: 111-118.
- [4] **Tahayori B**. Koceja D. Invited Review: Activity dependent plasticity during developmental stages and in a mature spinal cord. *J Neural Plasticity*. 2012:2012:1:12.
- [5] **Tahayori B**, Mahmoudian A, Koceja D, Hong L. Rambling and Trembling in response to body loading. *Motor Control*. 16:2012:144-157.
- [6] **Tahayori B**, Kitano K, Hong L, Koceja D. Amplification of Background EMG activity affects the interpretation of H-reflex gain. *Journal of Electromyography and Kinesiology*. 2010,20: 1219-1222.

Journal Reviewer

- 1- Journal of Sports Medicine and Physical Fitness (Since 2013)
- 2- Journal of Rehabilitation Sciences and Research (Since 2014)

Conference Presentations

- [1] **Tahayori B**, Koceja D. Inducing plastic changes in lower motoneurons and functional improvement in stroke survivors. SfN conference 2014, Washington DC.
- [2] Koceja D, Tahayori. The interaction of Post activation depression and Presynaptic inhibition in the spinal circuits. SfN conference 2014, Washington DC.
- [3] **Tahayori B**, Koceja D. Does activity-dependent induced plasticity in spinal circuits improve motor function in stroke survivors? Fifth annual reflex plasticity meeting, 2012, New Orleans, LA, USA.
- [4] **Tahayori B**, Acikada C, Chapman R, Koceja D. Fatiguing to exhaustion affects spinal inhibitory mechanisms. SfN conference, 2012, New Orleans, LA, USA.
- [5] Kitano K, Ryder R, Tahayori B, Koceja D. Crossed spinal influence on the human soleus H-reflex and efficiency of postural recovery from perturbation in young and elderly subjects. SfN conference, 2012, New Orleans, LA, USA.
- [6] **Tahayori B**, Port L, , Hong L, Koceja D. The inflow of sensory information for the control of standing is graded and bidirectional. SfN conference, 2011, Washington, DC,USA.
- [7] **Tahayori B**, Kitano K, Hong L, Koceja D. Amplification of Background EMG activity affects the interpretation of H-reflex gain. SfN 2010. San Diego, CA.

- [8] Kitano K, **Tahayori B**, Tsuruike M, Koceja DM. Effects of conditioning stimulation of post-activation depression in human soleus H-reflex. SfN 2010. San Diego, CA.
- [9] **Tahayori B**, Hong S.L, Nelson, A.G. Practicing with weighted vests induces neuromechanical changes in countermovement jump. ACSM 2010, Baltimore, USA.
- [10] Li L, Kwon T, **Tahayori B**. Timing of expert and novice taekwondo athletes during front kick motion. Capetown, ISBN conference, July 2009.
- [11] **Tahayori B**, M.A Sanjari, F Bahrami, M Razeghi. Sagittal Extremum Technique: A Simple Method for Event Detection During Treadmill Walking. Salford's 4th International Biomechanics Conference. Salford, UK Sep 2007.
- [12] **Tahayori B**, M.A Sanjari, F Bahrami, M Razeghi. Introducing a protocol to determine treadmill walking events using phase plane analysis. Salford's 4th International Biomechanics Conference. Salford, UK, Sep 2007.
- [13] Hoseini N, Emrani A, **Tahayori B**, Ebrahimi I. The effect of chronic anterior knee pain on the timing of plantar pressure distribution. Salford's 4th International Biomechanics Conference. Salford, UK Sep 2007.
- [14] **Tahayori B**, M.A Sanjari, F Bahrami. Introducing a new method to evaluate treadmill walking for patients with lower limb pathologies. 1st Iranian International Student Congress on Medical Research.
- [15] **Tahayori B**, M. Razeghi, M.E Batt, B Tahayori. "Biomechanical comparison of stance phase of treadmill walking and running," 14th annual meeting of ESMAC, Barcelona, Spain, 22-24 September 2005. Gait & Posture 22S (2005) S1–S53.
- [16] **Tahayori B**, M.E. Batt, M. Razeghi. "Does the imposed reduction of foot pronation affect knee sagittal motion in running? Proceedings of 6th Congress of the Italian society of movement analysis in clinics. 26-29 October 2005. Gait & Posture.
- [17] **Tahayori B**, H. Tahayori. "Introducing a Method to create an artificially intelligent analyzer to analyze and understand the physiotherapy records. 14th annual meeting of the Iranian Society of physiotherapy. Tehran, Iran 13-15 May 2003.
- [18] **Tahayori B**, "The review of the history of gait determinants" 2nd Iranian Students' Seminar of Physiotherapy. Tehran, Iran. 9-10 December 2003.

Book

B. Tahayori. Multiple Choice Questions of Manual Muscle Testing. Zar Publication. Shiraz, Iran. 2003.

Laboratory Skills

Kinesiologic Electromyography.

H-reflex (Different conditioning protocols) in functional tasks.

Posturagraphy.

Marker-based 3-dimensional human motion capturing and analysis.

Software Programming

Matlab programming

DasyLab National Instrument programming

Language Proficiency

Fluent in English (Written and Spoken)

Fluent in Farsi (Native Language)

References

Dr. David Koceja

Professor and Chair

Indiana University

Bloomington, IN, 47405

Phone: (812) 855-7302

koceja@indiana.edu

Dr. George Rebec

Chancellor's Professor

Indiana University

Bloomington

rebec@indiana.edu

phone: (812) 855-5351

D. Nicholas Port

Assistant Professor

Indiana University

Bloomington

nport@indiana.edu

phone: (812) 856-0124