TECHNISCHE UNIVERSITÄT WIEN
Vienna University of Technology

DIPLOMARBEIT

Conditioning effect of peroneal nerve stimulation on the transcutaneously elicited posterior root-muscle reflex

Ausgeführt am

INSTITUT FÜR ANALYSIS UND SCIENTIFIC COMPUTING

der Technischen Universität Wien

unter der Anleitung von

Ao. univ.Prof. Dipl.-Ing. DDDr. Frank Rattay

und

Dipl.-Ing. Mag. Dr.techn. Simon Danner

durch

Carry Schlaff

Wien, 18.09.2014

Prof DDDr. Frank Rattay

Carry Schlaff

ABSTRACT

The effects of locomotor training and spinal cord stimulation depend on the central excitability of the networks below the injury. Stimulation of the peripheral nerve allows targeting these networks to alter their excitability. We tested the effects of trains of peroneal nerve stimulation. The modifications of monosynaptically evoked lumbosacral motoneuron responses were tested by non-invasive elicitation of posterior root-muscle (PRM) reflexes simultaneously in multiple lower limb muscle groups.

We conducted our measurements on five subjects with intact nervous systems. One-second conditioning trains of peroneal nerve stimulation with a frequency of 15, 30 and 50 Hz were applied at 0.8, 1.2 and 1.5 times the motor threshold. Following 20, 50, 100, 200, 500 and 1000 ms inter-stimulus-intervals, test PRM reflexes were elicited through surface electrodes over T11-T12 vertebrae.

In above-threshold stimulation a general suppression of ipsilateral reflex responses, that increased with increasing conditioning frequency and intensity and lasted at least one second, were observed. This suppression was more prominent in the distal than in the proximal muscle groups. No conclusive

statement can be made regarding the contralateral leg, where both excitatory and inhibitory tendencies were observed.

Peripheral stimulation has an effect on all lumbar segments of the spinal cord, beyond the segments that are stimulated, which can be beneficial for rehabilitation after spinal cord injury (SCI), where the goal is to affect the excitability of the networks below the injury. While we studied only the effects on healthy subjects, they should be further studied in SCI individuals, and additional tests on the altered central state of excitability in SCI should be conducted.

ZUSAMMENFASSUNG

Die Wirkung von Bewegungstraining und Rückenmarkstimulation ist abhängig von der zentralen Erregbarkeit der kaudal der Verletzung liegenden Netzwerke. Die Stimulation peripherer Nerven erlaubt eine gezielte Veränderung der Erregbarkeit dieser Netzwerke. Die Effekte der Peroneusnervstimulation mit Impulszügen ('trains') wurden getestet. Die Änderung der Antworten der monosynaptisch erregten lumbosakralen Motoneuronen wurde durch nicht-invasive Auslösung der PRM-Reflexe simultan in mehreren Beinmuskelgruppen getestet.

Die Messungen wurden an 5 Probanden mit intakten Nervensystemen vorgenommen. Es wurde eine Konditionierung in Form der Peroneusnervstimulation durchgeführt, mit einem Impulszug in der Länge von einer Sekunde und Frequenzen von 15, 30 und 50 Hz, bei Intensitäten von 80, 120 und 150% der Motorschwelle. Im Anschluss an Inter-Stimulus-Intervallen von 20, 50, 100, 200, 500 und 1000 ms wurden Test-PRM-Reflexe durch Oberflächenelektroden auf den T11-T12 Wirbeln ausgelöst.

Bei der überschwelligen Stimulation wurde eine allgemeine Unterdrückung der ipsilateralen Reflexantworten beobachtet, die mit steigender Konditionierungsfrequenz und –intensität zunahm und eine Dauer von zumindest einer Sekunde hatte. Diese Unterdrückung stach in den distalen

Muskelgruppen mehr hervor als in den proximalen. Bezüglich des kontralateralen Beines kann keine schlüssige Aussage getroffen werden, da sowohl erregende also auch hemmende Tendenzen beobachtet wurden.
Periphere Stimulation wirkt über die stimulierten Segmente des Rückenmarks hinaus auf alle lumbalen Segmente, was für die Rehabilitation nach Rückenmarksverletzungen (SCI) vorteilhaft sein kann, da hier das Ziel die Beeinflussung der Erregbarkeit des Netzwerk kaudal der Verletzung ist. In dieser Arbeit wurden die Effekte nur bei gesunden Probanden untersucht. Darüber hinaus sollten auch die Effekte bei Personen mit Rückenmarksverletzungen untersucht, sowie weitere Tests zum veränderten zentralen Stand der Erregbarkeit bei SCI-Individuen durchgeführt werden.

ACKNOWLEDGEMENTS

This thesis could not have been undertaken without the assistance of several people to whom I would like to express my gratitude.

First and foremost, Professor Frank Rattay, whose support and expertise have guided me throughout my studies. His devotion to his students and willingness to make himself available at all times have been greatly appreciated.

I would like to thank Simon Danner for supervising this thesis. He has been an ever-present pillar of support right from the initial development of the study, through its execution and analysis, up until its completion. His constant editing and attention to detail, as well as his willingness and remarkable ability to clarify complex concepts have been crucial to the entire process.

Alongside Simon, Matthias Krenn has been invaluable in the development of the experiment protocol and the conduction of the measurements. He, too, has always been ready to solve theoretical and technical difficulties I encountered along the way.

Karen Minassian and Ursula Hofstötter have been further invaluable sources of knowledge and guidance.

The conduction of the experiments would have been impossible without the generous help of Kevin Beischlager, Julian Martus and Klaus Zeiner.

A special thanks to the subjects who readily volunteered to undergo lengthy electrical stimulation.

Any acknowledgment would be incomplete without mention of my parents, who, from a young age, instilled in me a passion and curiosity for learning, and without whom, this Master's degree and thesis would have remained a mere aspiration.

CONTENTS

ABSTRACT III

ZUSAMMENFASSUNG V

ACKNOWLEDGEMENTS VII

CONTENTS IX

ABBREVIATIONS XIII

1 INTRODUCTION 1

2 BACKGROUND 3

2.1 Nerve cell 3

2.2 The Central Nervous System 6

2.3 The spinal nerves and spinal cord 7

2.4 Spinal Reflexes 9

2.4.1 Flexion-withdrawal reflex 10

2.4.2 Stretch reflex 12

2.4.3 Hoffmann Reflex 15

2.5 Spinal Inhibitory Pathways 16

2.5.1 Reciprocal inhibition 16
2.5.2 Recurrent inhibition 18
2.5.3 Presynaptic inhibition 20
2.5.4 Non-reciprocal (or Ib) inhibition 21
2.6 H-reflex as a tool for the study of the CNS 23
2.6.1 Study of reciprocal Ia inhibition using the H-reflex 23
2.6.2 Limitations 25
2.7 Posterior root-muscle reflex 25
3 METHODS 29
3.1 Subjects 29
3.2 Electrode and stimulation setup 29
3.3 Recording procedure 31
3.4 Stimulation protocol 32
3.5 Data analysis 33
4 RESULTS 35
4.1 Conditioned stimulations 35
4.1.1 PNS 30 Hz 35
4.1.2 PNS 1.2 MT 37
4.1.3 Summary of results 38
4.2 Control measurements 39
5 DISCUSSION 45
5.1 Central state of excitability 45
5.1.1 Excitability of motoneuronal network 45

5.1.2 Excitability of interneuronal network 47

5.2 Central effect of FES 48

5.3 Frequency dependence of SCS 49

5.4 Transition Ia interneurons – motoneurons 50

5.5 Inhibitory pathways 51

5.6 Limitations due to instability of controls 52

6 CONCLUSION 55

BIBLIOGRAPHY 57

SOURCE CODE 67

ABBREVIATIONS

AP Action potential

CNS Central nervous system

CP nerve Common peroneal nerve

EPSP Excitatory postsynaptic potential

FES Functional electrical stimulation

H-reflex Hoffmann reflex

H Hamstring

IPSP Inhibitory postsynaptic potential

ISI Inter-stimulus-interval

MT Motor threshold

PNS Peroneal nerve stimulation

PRM reflex Posterior root-muscle reflex

Q Quadriceps

SCI Spinal cord injury

SCS Spinal cord stimulation

TA Tibialis anterior

TS Triceps surae

tSCS Transcutaneous spinal cord stimulation

CHAPTER 1

INTRODUCTION

“To move things is all that mankind can do, for such the sole executant is muscle, whether in whispering a syllable or in felling a forest” [Charles Sherrington, 1924]. Movement is controlled by the central nervous system. Damages to the motor system have different effects depending on the site of the trauma. Injury of the lower motor system, specifically the complete section of a motor nerve, causes paralysis – loss of movement – of the muscles innervated by the nerve, as well as areflexia, the lack of their spinal reflexes. Paralysis of the legs is known as paraplegia, while the loss of movement in all four limbs is known as quadriplegia [Bear et al., 2007]. With an upper motoneuron dysfunction, brain control of the affected muscles is lost. However, the human spinal circuitry is capable of generating locomotor-like activity even without brain control. Although the spinal cord no longer receives input from the brain to control the muscles, the muscles are still able to contract, and with the right input, the spinal cord can generate the contractions. Dimitrijevic and colleagues have shown that stimulation of the lower spinal cord of humans

results in locomotor-like EMG activity and stepping movement. This movement is initiated by the activation of neurons of the locomotor “central pattern generator” (CPG) with electrical stimulation [Dimitrijevic et al., 1998].

Neuroplasticity refers to the changes (adaptive and maladaptive) in the sensorimotor systems caused by spinal cord injury. The training of movement through electrical stimulation can be used to facilitate neuroplasticity [Dietz & Fouad, 2014]. Different factors influence the effectiveness of this training. For locomotion, spastic muscle tone is required in order to induce a locomotor-like EMG-pattern in SCI individuals [Dietz et al., 1995]. Therefore, while spasticity may be undesirable in non-ambulatory SCI patients as it can cause painful spasms, in ambulatory patients it is welcome since it is connected to an increased training effect. Heightened excitability of the motoneurons is one of the main features of spasticity [Dimitrijevic and Nathan, 1967]. Therefore the excitability of the network in SCI is a good indicator of the effectiveness of training. The question remains whether, and how, spasticity, and thus excitability, of motoneurons could be influenced in SCI patients with low spasticity. The goal of this thesis is to learn how the central state of excitability can be influenced by stimulation of a peripheral nerve in healthy subjects.

CHAPTER 2

BACKGROUND

The function of the nervous system is the conduction of electrical signals through the body in order to transmit and process information. It is made up of the central nervous system (CNS) and the peripheral nervous system, which contains the sensory neurons and motoneurons. Sensory neurons, or afferents, send information to the CNS, while motoneurons, or efferents, transmit information from the CNS to the body.

2.1 Nerve cell

The nerve cell, or neuron, comprises the soma (cell body), the dendrites and the axon (nerve fiber), as illustrated in Figure 1. On the terminal region of the axon there are widenings called synapses, which serve as the neuron's contact to other cells. Neural signals can travel through the synaptic cleft to muscle fibres, glands or other neurons by forming a connection to their dendrites. Synapses can be excitatory (when activated by their neuron they promote

excitation of the cell) or inhibitory. Sensory neurons are excited by stimulating the terminal region of the axon with pressure, heat or chemicals. Stimulation of the activated synapses in the input region at the dendritic tree results in a disturbance of the soma's inside potential, which propagates into the axon. However, a reaction will only occur in the terminal region if a certain threshold value is exceeded, in which case a nerve impulse or train of impulses (also known as action potential) is generated and propagates along the whole axon into the output region. The principle of the output region only receiving either a complete impulse or no impulse at all is called the “all-or-nothing law” [Rattay, 1990].

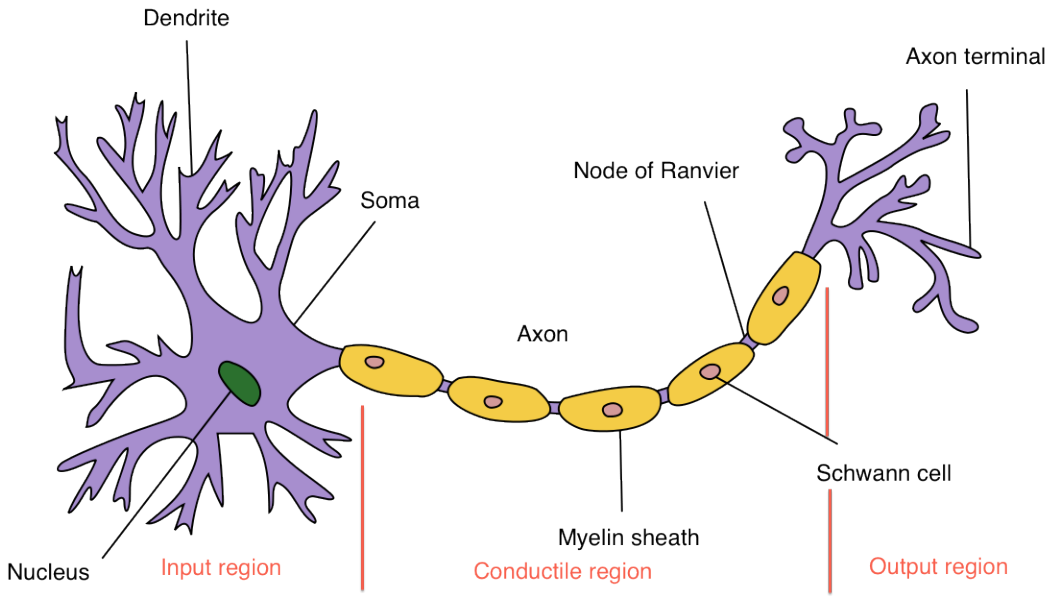

Figure 1: Structure of a neuron. The neuron comprises the soma (cell body), the axon (nerve fiber) and the dendrites. Stimulation of synapses in the input region results in a disturbance of the soma's inside potential. If a certain threshold value is exceeded, an action potential is generated and propagates along the whole axon into the output region [adapted from Wikipedia].

The generation of the action potential originates in the cell membrane, which separates the outside of the cell from its inside. It is a lipid double-layer with pores that allow the passage of ions in certain circumstances. In its non-

excited state, the membrane potential, which results from different ion concentrations in the extra- and intracellular regions, that are maintained by ion pumps, in combination with the membrane's permeability with respect to the specific ions, is around -70 mV. When the membrane potential increases and exceeds the threshold of around -50 mV, sodium gates open to allow the flow of Na^+ ions into the cell and depolarization occurs. With a short delay, potassium gates open to let K^+ ions flow out of the cell and cause repolarisation. Hyperpolarisation also occurs for a short period until the membrane potential reaches its resting potential [Pfützner, 2012].

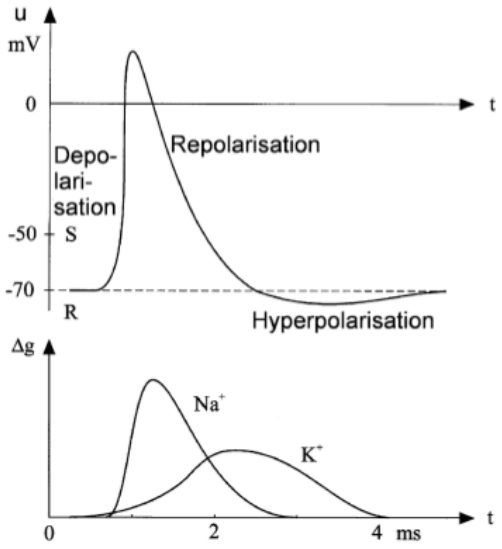

Figure 2: Typical time response of an action potential. R is the resting potential, S the threshold level. g is the permeability of the respective ion gates. When the membrane potential surpasses S, depolarisation occurs and the voltage-gated Na^+ channels open to allow inflow of sodium ions into the cell. With a delay, K^+ channels open to allow potassium ions to flow out of the cell (repolarisation) and Na^+ channels close. After resting potential has been reached again, K^+ channels still remain open, which causes hyperpolarisation [adapted from Pfützner, 2012].

However, it has been shown that at the nodes of Ranvier in myelinated fibres of the rabbit's sciatic nerve, there are barely any, or no, voltage-dependent potassium channels at all [Chiu et al., 1979]. Thus, in a model of the

motor nerve by Sweeney et al., no voltage-dependent potassium channels were incorporated. As a result, no hyperpolarisation occurs. The duration of the AP is therefore not always, as illustrated in Figure 2, 2 ms as in the squid, but can be longer or shorter; in Sweeney's model, for instance, it is only 1/3 ms, as shown in Figure 3 [Sweeney at al., 1987].

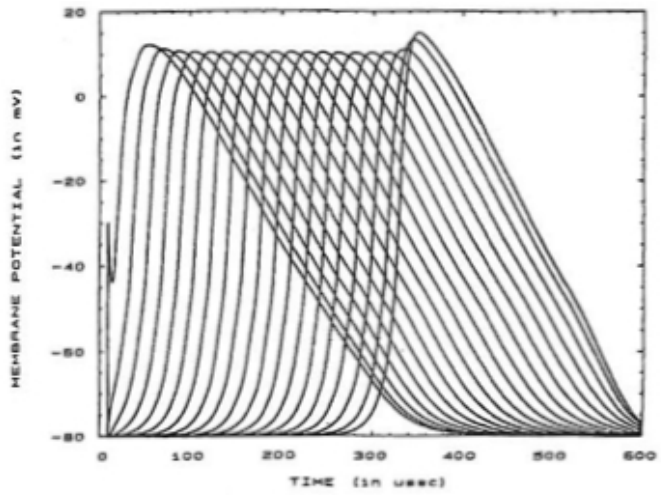

Figure 3: Conduction of action potential. X axis is time in μs, y axis is membrane potential in mV [Sweeney et al., 1987].

2.2 The Central Nervous System

The CNS is made up of the brain and the spinal cord, which both lie within bones. This thesis is concerned with the spinal cord and shall therefore not discuss the brain's function at length. The brain has three parts: the cerebrum, the cerebellum and the brain stem. The cerebellum controls movement and is connected to both the cerebrum and the spinal cord. Attached to the brain stem, the spinal cord lies in the bony vertebral column and receives information from the cerebrum via the brain stem. It also transmits information from the skin, joints and muscles to the brain. A transection of the spinal cord results in a lack of feeling in the skin and paralysis of the muscles below the cut. However, while

this means that these muscles can no longer be controlled by the brain, they can still function [Bear et al., 2007].

This is important for paraplegics, as the human spinal circuitry is capable of generating locomotor-like activity even without brain control. While the intact nerves below the lesion no longer receive any input from the brain, they can transform a different input, for instance electrical stimulation, into functional rhythmic output. This means that the spinal cord can still perform, provided that it is activated externally. It has been shown that stimulation of the lower spinal cord of humans results in locomotor-like EMG activity and stepping movement. This movement is initiated by the activation of neurons of the locomotor "central pattern generator" (CPG) with electrical stimulation [Dimitrijevic et al., 1998].

2.3 The spinal nerves and spinal cord

The spinal nerves are part of the peripheral nervous system. They act as a means of communication between the spinal cord and the body. Via the dorsal and ventral roots axons enter and exit the spinal cord. Together, the dorsal and ventral roots form the spinal nerves (Figure 4). Each spinal nerve exits between two vertebrae of the spinal column, which is made up of 30 vertebrae. The spinal cord, which is part of the CNS, is divided into 4 groups (Figure 5): cervical (C 1–8), thoracic (T 1–12), lumbar (L 1–5), and sacral (S 1–5). Axons carrying information into the spinal cord are located in the dorsal roots, whereas axons transmitting information from the spinal cord to innervate muscles and glands are contained in the ventral roots. "Afferents" or "posterior roots" are another name for dorsal fibres; ventral fibres are also called "efferents" or "anterior roots" [Bear et al., 2007].

Since the dorsal and ventral roots are separate from one another over a long stretch, there is a considerable difference between their respective thresholds – with a lower stimulus it is possible to excite solely the dorsal roots.

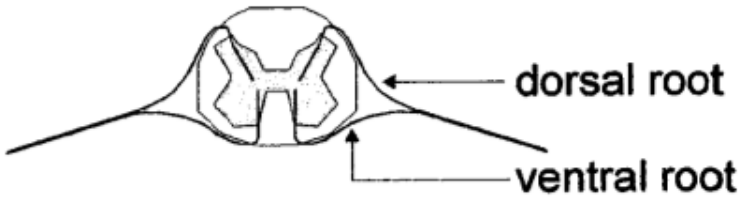

Figure 4: Spinal nerves. The dorsal and ventral roots form the spinal nerves [Rattay et al., 2000].

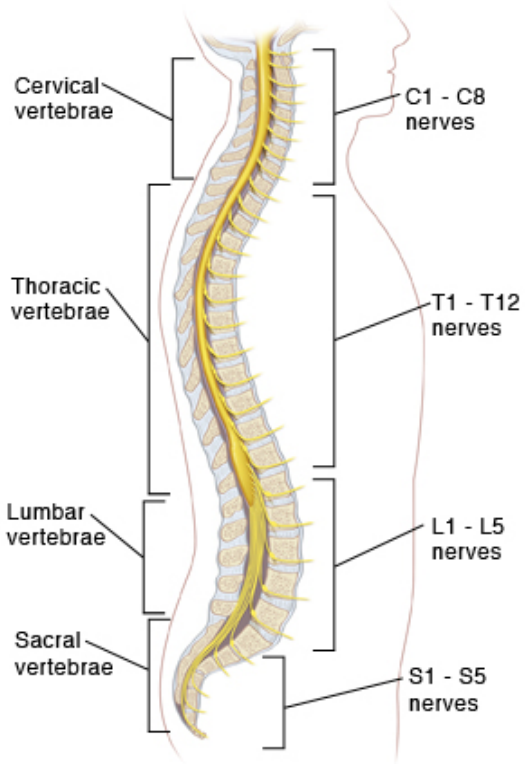

Figure 5: Division of spinal segments. The spinal cord is divided into 4 groups: cervical (C 1–8), thoracic (T 1–12), lumbar (L 1–5), and sacral (S 1–5) [USCD, 2014].

2.4 Spinal Reflexes

In the beginning of the twentieth century, Charles Scott Sherrington delivered a series of lectures at Yale University which made him a pioneer in the field of spinal reflexes. He introduced the concept of reflexes as the basic units for movement. The activation of receptors in skin or muscles elicits stereotyped movements – so-called reflexes. Sherrington suggested that by combining simple reflexes, more complex sequences of movements can be produced [Sherrington, 1906].

Studies of reflexes in animals with central nervous system lesions led to the assumption that reflexes are automatic, stereotyped movements elicited by stimulation of peripheral receptors. However, measurements of reflexes in animals with intact central nervous systems led to today's understanding that reflexes are flexible and adaptable to a motor task, that is, they change as a reaction to the contraction or stretching of a muscle. Thus, the general view today is that centrally generated motor commands integrate reflexes into complex adaptive movements.

This thesis focuses on spinal reflexes, the sensory stimuli for which come from receptors in muscles, joints and skin. While the reflex gain is influenced by the brain, the neural circuitry in control of the execution of the motor response is located in its entirety in the spinal cord.

The following two examples serve to illustrate the adaptability of reflexes to motor tasks. The first example is the stretching of wrist muscles. In kneeling or standing position, the stretched muscles contract, as do muscles in other limbs, in order to prevent a loss of balance. What is interesting is the reflex response of the elbow extensor of the contralateral arm, which can be excitatory or inhibitory, depending on how the arm is used. If the contralateral limb is used to stabilize the body and prevent it from moving forward by holding on to a table, the stretching of the wrist muscles produces an excitatory

response in the contralateral arm grasping the table, in order to prevent the body from moving forward. If, on the other hand, the contralateral arm is used to hold an unsteady object, such as a filled cup, the same stimulus causes an inhibitory response in the elbow extensor muscle, in order to prevent the cup from moving.

The second example of the adaptability of reflexes to motor tasks is the conditioning of the flexion-withdrawal reflex (which will be discussed subsequently). A subject places the palm of his index finger on an electrode and gets mild electrical shocks together with an audible tone. After a short while, the tone alone - even without the electrical shock - will cause the subject to withdraw his finger - the withdrawal reflex. In order to find out what has been conditioned - the contraction of a certain group of muscles or the behavioural act of withdrawing the finger from the painful stimulus - the subject turns his hand over and places the back of his finger on the electrode. In general, the subject will still withdraw his finger when the tone is played without the accompanying stimulus, even though, due to the turning of the hand, the opposite muscles contract in order to remove the finger. This leads to the conclusion that the conditioned response is not just a stereotyped set of muscle contractions, as was widely believed in the 20th century, but rather the elicitation of appropriate behaviour [Kandel et al., 2013].

Two important spinal reflexes are the aforementioned flexion-withdrawal reflex and the stretch reflex.

2.4.1 Flexion-withdrawal reflex

The flexion-withdrawal reflex (Figure 6) is a protective reflex that causes a limb to withdraw from a painful stimulus by contraction of its flexor muscles. When the spinal cord is transected, this reflex does not stop, proving that the flexion-withdrawal reflex is indeed a spinal reflex. Divergent polysynaptic reflex

pathways are activated by the sensory signal; one is excitatory, causing the flexor muscles of the stimulated limb to be innervated by motoneurons, another is inhibitory, hindering the innervation of the extensor muscles by the motoneurons responsible for this task. This is what Sherrington called “reciprocal innervation” - the excitation of one group of muscles and simultaneous inhibition of their antagonists. Hereafter “reciprocal innervation” will alternatively also be referred to as “reciprocal inhibition”. In the contralateral, or opposite limb, the “crossed-extension reflex” causes the opposite reaction - the extensor motoneurons are excited while the flexor motoneurons are inhibited. This helps maintain postural stability, for instance when a foot is withdrawn from a painful stimulus. To summarize: In order to withdraw the limb from a painful stimulus, one excitatory pathway activates motoneurons that innervate ipsilateral flexor muscles. Simultaneously, another pathway excites motoneurons that innervate contralateral extensor muscles for support during the withdrawal of the limb. Motoneurons that innervate antagonist muscles are made inactive during the reflex response with the help of inhibitory interneurons [Kandel et al., 2013].

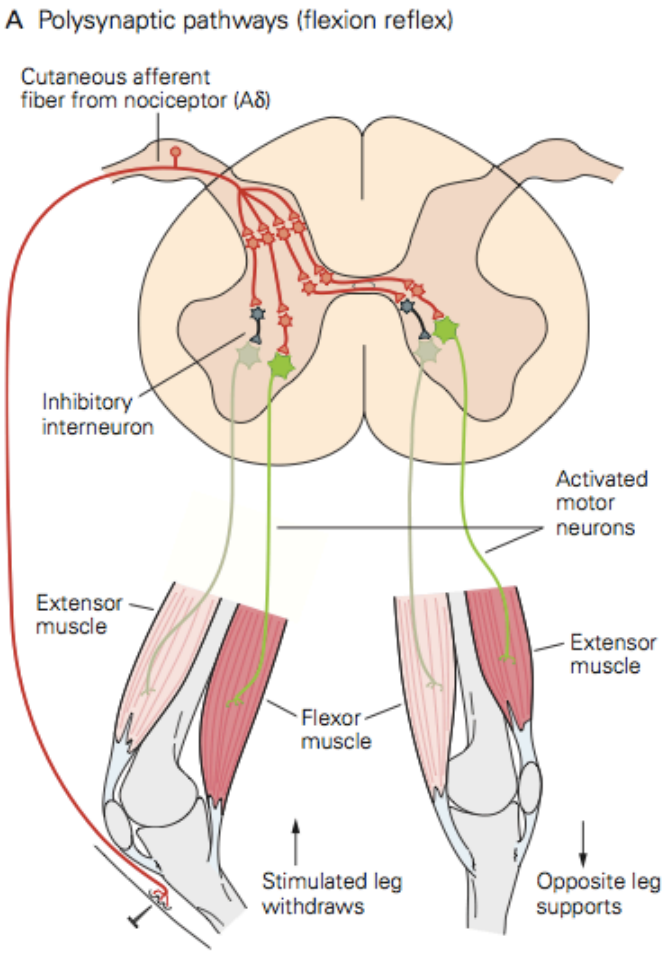

Figure 6: Flexion-withdrawal reflex: one excitatory pathway activates motoneurons that innervate ipsilateral flexor muscles in order to withdraw the limb from a painful stimulus. Simultaneously, another pathway excites motoneurons that innervate contralateral extensor muscles for support during the withdrawal of the limb. Motoneurons that innervate antagonist muscles (extensor muscles in the stimulated limb, flexor muscles in the contralateral limb) are made inactive during the reflex response with the help of inhibitory interneurons [Kandel et al., 2013].

2.4.2 Stretch reflex

The stretch reflex (Figure 7) is a lengthening contraction of the muscle. In contrast to the flexion-withdrawal reflex, the stretch reflex is mediated by monosynaptic pathways. A Ia afferent axon from the muscle spindle (the receptor that senses the change in the length of the muscle) makes a direct excitatory connection to two types of motoneurons; alpha motoneurons that

innervate the same (homonymous) muscle from which they stem and also motoneurons that innervate synergist muscles (synergists perform the same set of joint motions as agonists). The Ia afferent fibre also makes a connection to interneurons that inhibit the motoneurons innervating antagonist muscles (another example of reciprocal innervation). A well-known example of elicitation of the stretch reflex is the tendon tap performed by physicians to test the patellar reflex.

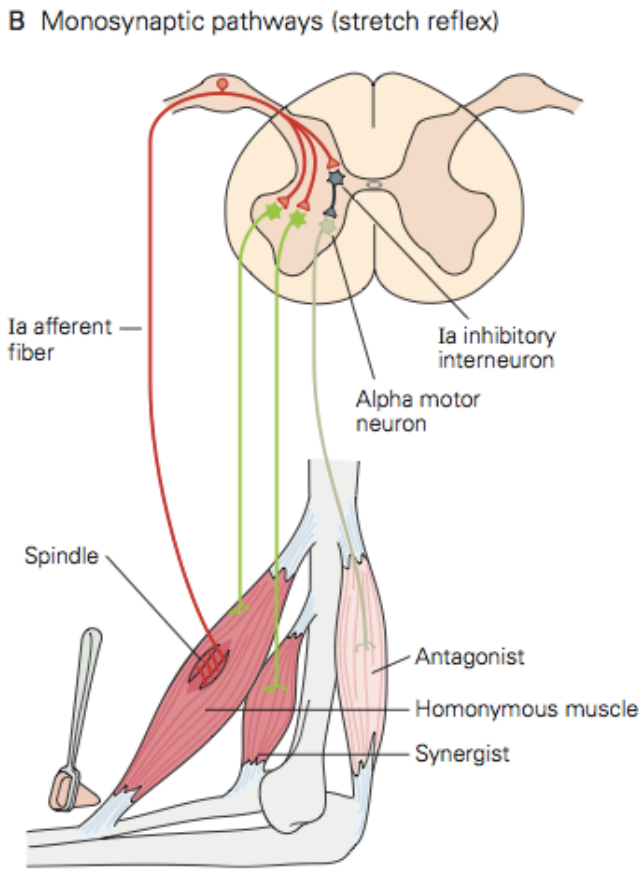

Figure 7: Stretch reflex: a Ia afferent fiber from the muscle spindle makes a direct excitatory connection to 1) alpha motoneurons that innervate the homonymous muscle and 2) motoneurons that innervate synergist muscles. The Ia afferent fiber also makes a connection to 3) interneurons that inhibit the motoneurons innervating antagonist muscles (reciprocal innervation) [Kandel et al., 2013].

The tendon tap (Figure 8) – a sharp tap of the tendon of a muscle with a reflex hammer – is regularly used in clinical settings to elicit stretch reflexes in

different muscles. Although the tendon is tapped, the muscle spindle (a receptor) is stimulated; thus the receptor is not in the tendon, as the name of the response, namely “tendon reflex” or “tendon jerk” would suggest, but rather in the muscle. The elicited action impulses excite motoneurons in the spinal cord, which via one pathway cause the contraction of the extensor fibres. As a reflex response, the lower leg jerks forward. Another pathway, that of the flexor muscle, on the other hand, is inhibited by interneurons. The purpose of the tendon tap is to diagnose conditions mainly in the central nervous system. A decreased (hypoactive) response can suggest a defect in the peripheral reflex pathway: in the sensory or motor axons, in the cell bodies of motoneurons, or in the muscle. However, it can also be caused by lesions of the central nervous system. A hyperactive response, on the other hand, is always the result of a lesion in the central nervous system [Kandel et al., 2013].

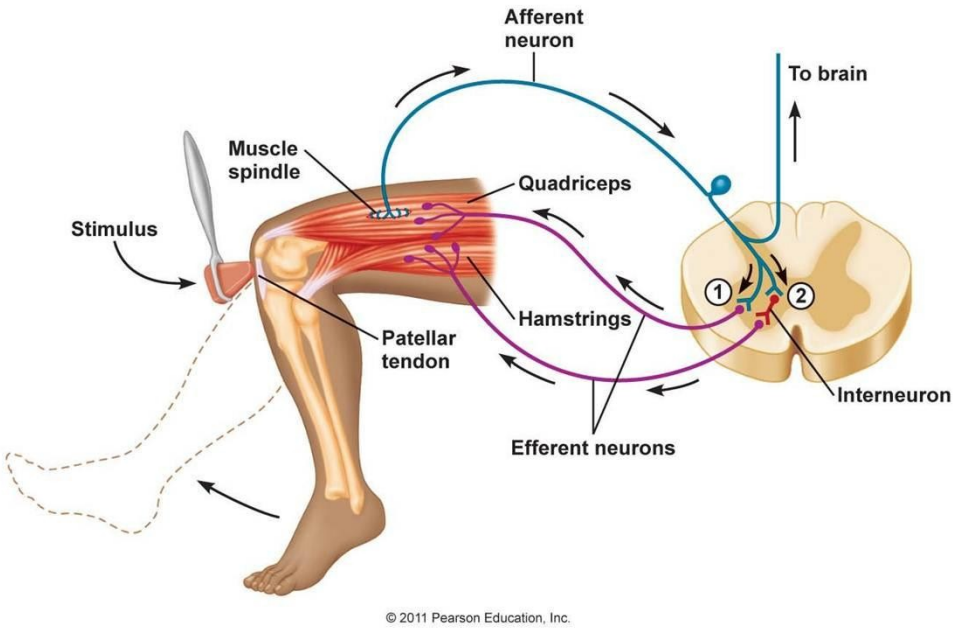

Figure 8: Reflex arc of the tendon reflex. A tap on the tendon of the stretch muscle (extensor) causes the activation of stretch receptors (muscle spindle). The elicited action impulses excite motoneurons in the spinal cord, which via pathway 1 cause the contraction of the extensor fibers. As a reflex response the lower leg jerks forward. Pathway 2, which supplies the flexor muscle, is inhibited by interneurons [Pearson Education, Inc., 2011].

2.4.3 Hoffmann Reflex

The Hoffmann reflex (H-reflex) is the electrical analogue of the tendon reflex. It is elicited by electrical stimulation of Ia sensory fibres in mixed nerves. As a result, alpha motoneurons are excited and activate the muscle, which can be recorded by the electromyogram (EMG). Two successive responses can be measured (Figure 9); the first is a short-latency direct motor response, which stems from the direct activation of the motor axons and is called the M-wave. The second is the H-wave, which is evoked by stimulation of the Ia fibres. The M-wave precedes the H-wave, because it results from the direct stimulation of the motor axons that innervate the muscles. The H-wave, on the other hand, results from signals to the spinal cord across a synapse and back to the muscle, thus having a longer way to travel and occurring later. At low stimulus strength, only an H-wave can be elicited because the threshold for activation of Ia fibres is lower than that for motor axons. With increasing stimulus strength, the M-wave increases and the H-reflex first increases and then declines, as the action potentials in the motor axons initiated by the electrical stimulus propagate toward the cell body (antidromic conduction) and cancel the orthodromic action potentials generated reflexively by the spindle afferents in the same motor axons. At very high stimulus strengths, only an M-wave can be produced [Kandel et al., 2013].

The elicitation of H-reflexes is possible in nearly all muscles that allow access to their mixed peripheral nerve. It is often used to measure the excitability of interneurons and spinal inhibitory as well as neuronal pathways in man both at rest and during voluntary movement. Studies have shown that whereas it is usually possible to evoke an H-reflex in the soleus muscle, it is rarely seen in the tibialis anterior in healthy subjects at rest [Crone et al., 1987].

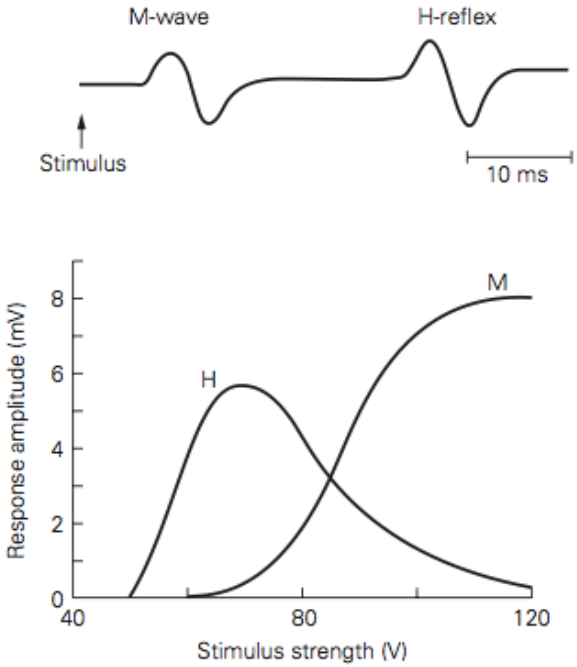

Figure 9: H-reflex and M-wave. The M-wave is a short-latency direct motor response, which stems from the direct activation of the motor axons. The H-wave is evoked by stimulation of the Ia fibres [Kandel et al., 2013; Schieppati, 1987]

2.5 Spinal Inhibitory Pathways

Inhibitory interneurons play an important part in the function of spinal pathways. There are considerably more interneurons than motoneurons and only few are characterised (see Discussion) [Petersen 2014], which suggests that the spinal cord has a larger role than assumed. The interneurons described here are characterised by their function in a defined spinal pathway; however, when stimulated differently, they can presumably have other functions.

2.5.1 Reciprocal inhibition

In humans, the existence of reciprocal inhibition was first shown by Hoffmann, who demonstrated the decrease of the soleus H-reflex when the antagonistic muscles (the pretibial muscles) are contracting [Hoffmann, 1952].

As mentioned above, reciprocal inhibition is used in stretch reflexes, i.e. involuntary movements. However, it is also used in voluntary movements. This creates an efficient mechanism, for antagonist muscles relaxing during movement facilitates the contraction of the agonists. **Ia inhibitory interneurons** (Figure 10) thus coordinate muscle contraction in antagonistic muscles during voluntary movements by way of their direct contact to motoneurons – higher centres have no need to send separate commands to the antagonistic muscles, because the interneurons can receive this input from the motor cortex. While the agonist is innervated by its motoneuron, contraction of the antagonist is regulated by the Ia inhibitory interneuron, which inhibits the motoneuron innervating the antagonist.

Furthermore, Ia inhibitory interneurons also receive excitatory as well as inhibitory inputs from corticospinal and all other major descending pathways. A change in the balance of the supraspinal excitatory and inhibitory inputs results in a reduction of reciprocal inhibition, which allows the interneurons to coordinate co-contraction (simultaneous contraction of prime mover and antagonist) in order to stiffen the joint to an extent appropriate for the current motor act [Kandel et al., 2013].

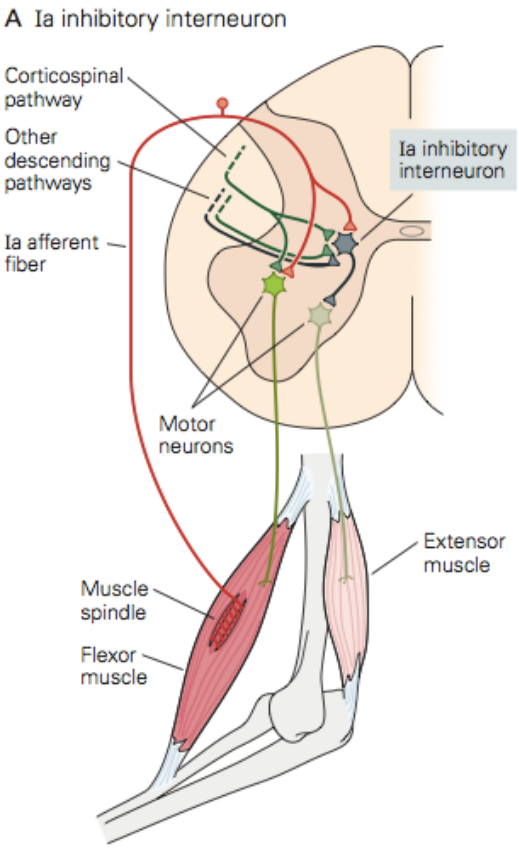

Figure 10: Ia inhibitory interneuron. Stimulation of the flexor muscle results in its activation by a motoneuron. At the same time, contraction in the extensor muscle (antagonist) is regulated by the Ia inhibitory interneuron. It inhibits the motoneuron innervating the antagonist. The interneuron also receives excitatory and inhibitory inputs from corticospinal and other descending pathways [Kandel et al., 2013].

2.5.2 Recurrent inhibition

In 1941 Renshaw showed the inhibition of the monosynaptic reflex in animals whose dorsal roots were sectioned. This inhibition was caused by antidromic impulses in motor axons and had a short latency but long duration [Renshaw, 1941].

Recurrent collaterals that are given off by motor axons release the excitatory transmitter acetylcholine, which activates **Renshaw cells** (interneurons) [Eccles et al. 1954]. The Renshaw cells (Figure 11) then inhibit these same motoneurons.

This forms a negative feedback system that regulates the excitability of motoneurons and can stabilize firing rates of those motoneurons. In addition to the inhibitory connections to the motoneurons that excite them, Renshaw cells also form connections to Ia inhibitory interneurons, which could regulate the amount of inhibition of antagonistic motoneurons. Furthermore, similar to Ia inhibitory interneurons, Renshaw cells receive synaptic inputs from descending pathways and inhibit synergist motoneurons and Ia interneurons that synapse on antagonist motoneurones. As a result, these synaptic inputs regulate the excitability of all motoneurons around a joint by regulating the excitability of the Renshaw cells [Kandel et al., 2013].

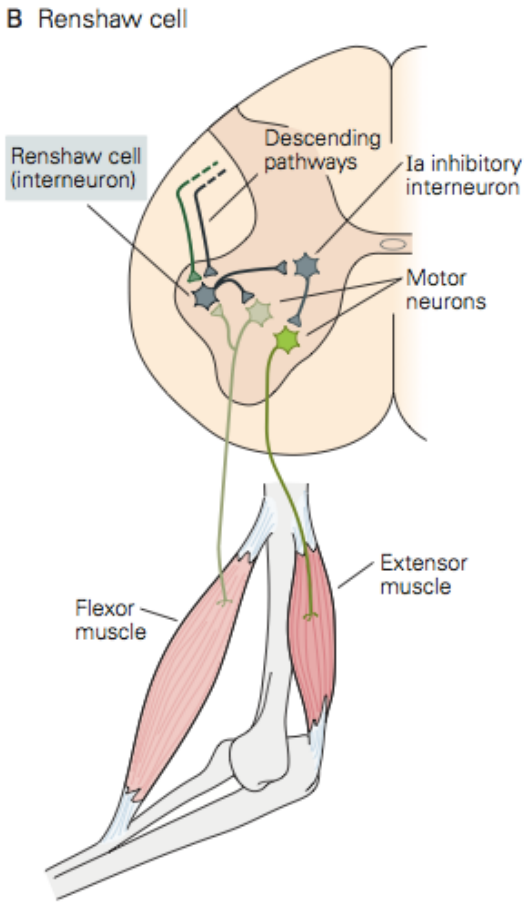

Figure 11: The Renshaw cell produces recurrent inhibition of motoneurons. It is a type of inhibitory interneuron that is excited by collaterals of axons of motoneurons and inhibits these same motoneurons. It also sends collaterals to Ia inhibitory interneurons that inhibit antagonist motoneurons. [Kandel et al., 2013]

From an electrophysiological standpoint, Renshaw cells are discharged repetitively by a single volley in α-motor axons, as a result of a prolonged EPSP from the recurrent collaterals. The recurrent IPSP in motoneurons from a given nerve (elicited by antidromic stimulation of all motor axons of the nerve) has the short central latency of a disynaptic pathway, 1 ms, and a long duration, ~40 ms [Eccles et al., 1954].

2.5.3 Presynaptic inhibition

The spinal cord continuously receives afferent inputs from the skin, muscles, tendons and joints. In order to perform a motor task, this sensory feedback from the periphery has to be managed – one option is its control at the presynaptic level, namely at the presynaptic inhibitory synapses of afferent terminals on α-motoneurones. In the cat, the size of the EPSP was depressed without detectable changes in the resting membrane potential or the excitability of postsynaptic cells [Frank and Fuortes, 1957]. This presynaptic inhibition (Figure 12) resulted in the decrease of the monosynaptic transmission of the Ia excitatory effects. Local modulation of transmitter release is the underlying principle of presynaptic inhibition. This is accomplished by axo-axonal gamma-aminobutyric (GABA) synapses that cause primary afferent depolarisation (PAD) and reduce the size of the presynaptic impulse, resulting in a reduced release of excitatory transmitters and thus in the inhibition of the monosynaptic transmission of the Ia excitatory effects [Rudomin and Schmidt, 1999].

Presynaptic inhibition is triggered by different sources. The modulation of the soleus H-reflex during ipsilateral or bilateral passive leg movements in humans has been linked to changes in the amount of presynaptic inhibition [Brooke et al., 1993; Knikou, 2006; Knikou and Rymer, 2002]. The changes

have also been associated with the soleus H-reflex modulation during passive ankle dorsiflexion [Morita et al., 2001] and standing [Katz et al., 1988].

Moreover, observed differences in the soleus H-reflex amplitude at equivalent EMG levels during walking, standing and running are also thought to be caused partly by presynaptic inhibition [Capaday and Stein, 1987; Morin et al., 1982]. This suggests that presynaptic inhibition is responsible for changing the reflex amplitude during a motor task, no matter how excited the α-motoneurones are.

Presynaptic inhibition acting on Ia afferents in the contracting muscle is decreased at the onset of a voluntary contraction in the human lower limb [Hultborn et al., 1987; Iles and Roberts, 1987]. This is likely due to descending control [Meunier and Pierrot-Deseilligny, 1998; Nielsen and Kagamihara, 1993].

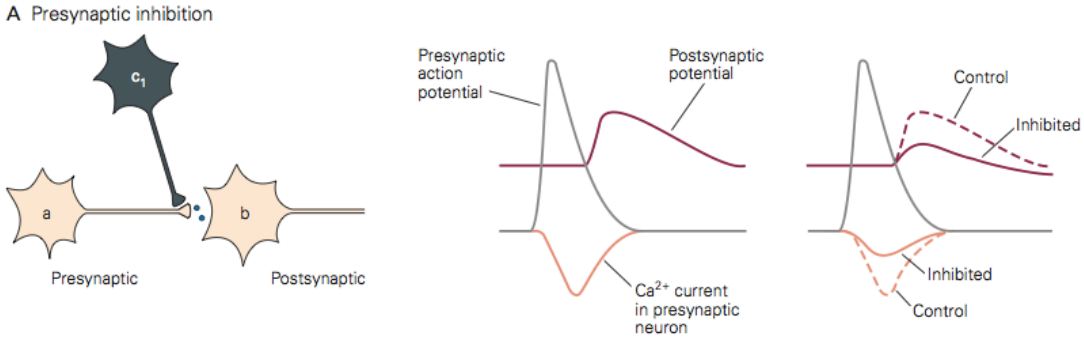

Figure 12: Presynaptic inhibition. The underlying principle is the inhibition of transmitter release. This is done by axo-axonal gamma-aminobutyric (GABA) synapses (c_1) that cause primary afferent depolarisation (PAD) and reduce the size of the presynaptic impulse, inhibiting the Ca^{2+} current and resulting in a reduced release of excitatory transmitters (from a onto b) and thus in the inhibition of the monosynaptic transmission of the Ia excitatory effects [Kandel et al., 2013].

2.5.4 Non-reciprocal (or Ib) inhibition

Golgi tendon organs are situated at the junction between skeletal muscle fibres and tendons. Each is innervated by a single Ib axon. While muscle spindles respond to changes in the length of a muscle, tendon organs respond to

changes in muscle tension, thus they are activated during normal movements. Contraction of a muscle causes a stretching of the Golgi tendon organ, which results in the compression of the Ib afferents and causes them to fire [Kandel et al., 2013].

Interneurons that are activated in reflex pathways from Ib afferents are called ‘Ib interneurons’ (Figure 13), however, they are not excited solely by Ib afferents but also by Ia afferents [Jankowska et al., 1981], as well as group II afferents [Jankowska and Edgley, 2010].

In the spinal cat, the effects of the Ib interneurons are the inhibition of motoneurons that project to synergists and the excitation of motoneurons that project to antagonists [Eccles et al., 1957]

Since the threshold of the Golgi tendon organs to passive stretch is very high, at first the purpose of Ib inhibition was thought to be protection against muscle overloading. The assumption was that the tendon organs always inhibit homonymous motoneurons and only fire when the tension in the muscle is high. However, it has been shown that Golgi tendon organs can be activated by the active contraction of even a single motor unit [Houk and Henneman, 1967]. This suggests that Ib inhibition regulates muscle stiffness [Houk, 1979; Houk and Rymer, 1981]. The combined actions of the Golgi tendon organs and stretch reflex provide the nervous system with information about the force generated by a muscle and thus constant muscle stiffness during movement can be maintained [Rothwell, 1987].

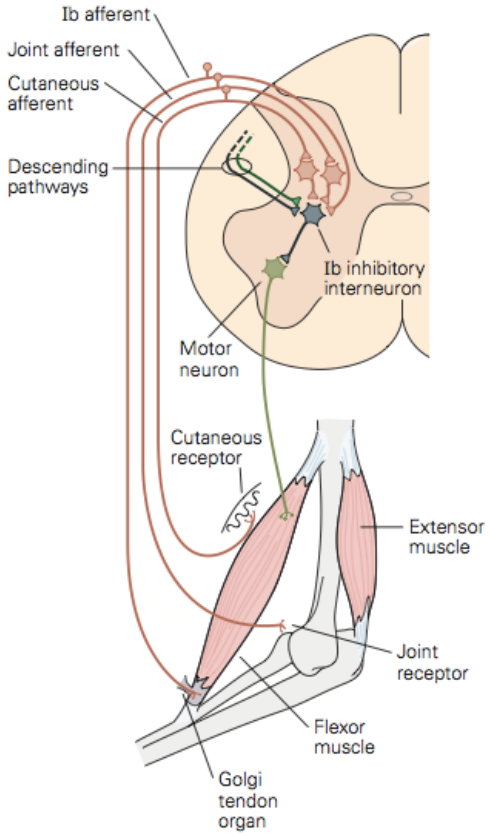

Figure 13: Ib inhibition. Golgi tendon organs, cutaneous receptors, joint receptors and muscle spindles, as well as descending pathways give input to the Ib inhibitory interneuron, which then inhibits the motoneuron that projects to synergists.

2.6 H-reflex as a tool for the study of the CNS

The Hoffmann reflex can be used as a probe for the study of the structure and functions of the human central nervous system, in particular for the study of the excitability of reflexes and interneurons, as well as spinal inhibitory and neuronal pathways.

2.6.1 Study of reciprocal Ia inhibition using the H-reflex

To study reciprocal Ia inhibition in humans, the amplitude of the H-reflex following stimulation of the antagonist peripheral nerve can be measured. In the lower limb, studies usually focus on reciprocal inhibition between ankle flexors

and extensors, as it apparently has a big part in depressing the soleus H-reflex during the swing phase of walking [Ethier et al., 2003]. (However, other studies [Schneider et al., 2000] have credited supraspinal centers with this attenuation.) The protocol for studying reciprocal inhibition between ankle flexors and extensors can be as follows. A single pulse stimulation of the common peroneal nerve of a 1 ms duration is conducted through a bipolar electrode located distal to the fibula head. Intervals of 2–4 ms between conditioning and test pulses are used to detect the presence of reciprocal inhibition of ankle flexors on extensors. It is important that there be activity of the TA without any activity of the peroneal muscles, since the peroneal muscles are not antagonists to the soleus muscle [Meunier et al., 1993]. If the peroneal muscles were active, reciprocal inhibition could be obscured.

The elicitation of reciprocal inhibition is possible at conditioning stimulus intensities below, at, or above MT [Crone and Nielsen, 1989; Crone et al., 1985, 1987; Kido et al., 2004].

With stimulation below MT it is probable that only TA Ia afferents are excited, but without a TA M-wave it is hardly possible to ensure the stability of the conditioning stimulus during the experiment. While the TA H-reflex could theoretically be used for this purpose, it is rarely seen in healthy subjects at rest [Crone et al., 1987].

With a conditioning stimulus above MT (1.1–1.5 x MT) reciprocal Ia inhibition could be contaminated by Ib afferent discharges [Pierrot-Deseilligny et al., 1981] and stimulating the CP nerve (the conditioning stimulus) could excite not only group II and cutaneous afferents but also motor efferents, which could result in the activation of Renshaw cells. In this case, recurrent inhibition would affect the amount of reciprocal inhibition [Baret et al., 2003; Hultborn et al., 1971; Katz et al., 1991]. This means that even though the soleus H-reflex depression may be stronger at 1.5 x MT of the TA, it cannot be ascribed entirely

to the TA Ia afferents, because there is a possibility that other neuronal pathways are involved as well.

Reciprocal inhibition depends on the size of the test soleus H-reflex [Crone et al., 1985] – when the control soleus H-reflex is in a range of 5 to 15% of the Mmax, maximal reciprocal inhibition can be observed.

With the above method for the study of reciprocal inhibition, involvement of other interneurons at a post- or presynaptic level (other than Ia interneurons) cannot be excluded entirely. As a result, the depression of the antagonists could be due, not only to reciprocal inhibition alone, but also presynaptic and even recurrent inhibition [Lavoie et al., 1997]. Since it is therefore not simple to differentiate between the involvement of these different mechanisms on the depression of the observed reflex (in particular during movement), it has been recommended to study the conditioning effects of CP nerve stimulation, not on the H-reflex, but rather on the EMG-activity of the soleus [Stein and Thompson, 2006].

2.6.2 Limitations

A drawback of the use of the H-reflex as a probe is that only the reflex pathways are studied, not more. On the other hand, it is not possible to study solely the excitability of motoneurons, since, as has been shown, the spinal inhibitory pathways can be influenced by various factors. As mentioned above, when studying reciprocal inhibition, it is possible that the observed depression of the antagonist muscle is also caused by presynaptic and recurrent inhibition in addition to reciprocal inhibition.

2.7 Posterior root-muscle reflex

In 1943 Lloyd reported the elicitation of “dorsal root-ventral root reflexes” in cats, after recording a reflex discharge from an anterior root following single-

shock stimulation of the posterior root of the same spinal cord segment [Lloyd, 1943]. Correspondingly, in humans the “posterior root-muscle reflexes” (PRM reflexes) are recorded electromyographically as compound muscle action potentials (CMAPs) from the muscle to which the motoneuron discharge is directed as a monosynaptic reflex. The PRM reflexes are the basic components of the lower-limb muscle responses evoked by epidural stimulation of posterior lumbar cord structures [Minassian et al., 2011]. PRM reflexes can be compared to the H-reflex: both have constant latencies, waveforms and amplitudes of the surface-recorded CMAPs during constant conditions. Furthermore, the excitability changes for several seconds when there is a prior conditioning stimulus; and the response amplitudes decrease during Achilles tendon vibration [Mao et al., 1984; Pierrot-Deseilligny & Burke, 2012; Minassian et al., 2007a]. However, the elicitation of the reflexes differs (Figure 14). While both are evoked by stimulation of the same type of sensory axons, the PRM reflex is elicited at proximal sites close to the spinal cord. For example, to elicit the H-reflex of the triceps surae muscle, transcutaneous electrical stimulation of the posterior tibial nerve in the popliteal fossa will be used while the PRM reflex is elicited in the same group of sensory fibers, but close to the spinal cord.

Due to the short afferent limb of the PRM reflex arc – i.e. the signal has a shorter way to travel, as its starting point is closer to the spinal cord – the latency of the triceps surae PRM reflex is shorter than that of the H-reflex (63.2 ± 1.2% of the H-reflex delay) [Minassian et al., 2007a].

Another important difference is that with increasing stimulus intensities, the stimulation of mixed peripheral nerves will not only activate sensory fibers, but also motor axons, thus causing an M-wave in addition to the H-wave, as well as antidromic action potentials that cancel the orthodromic action potentials generated reflexively by the spindle afferents in the same motor axons [Schieppati, 1987]. This is unfavourable when studying the excitation of reflex pathways. However, with transcutaneous stimulation of the spinal cord and

cauda equina, it is possible to selectively recruit only the sensory fibers in the posterior roots, which are separated from the motor fibers in the anterior roots [Minassian et al., 2007a; Kitano & Koceja, 2009; Ladenbauer et al., 2010].

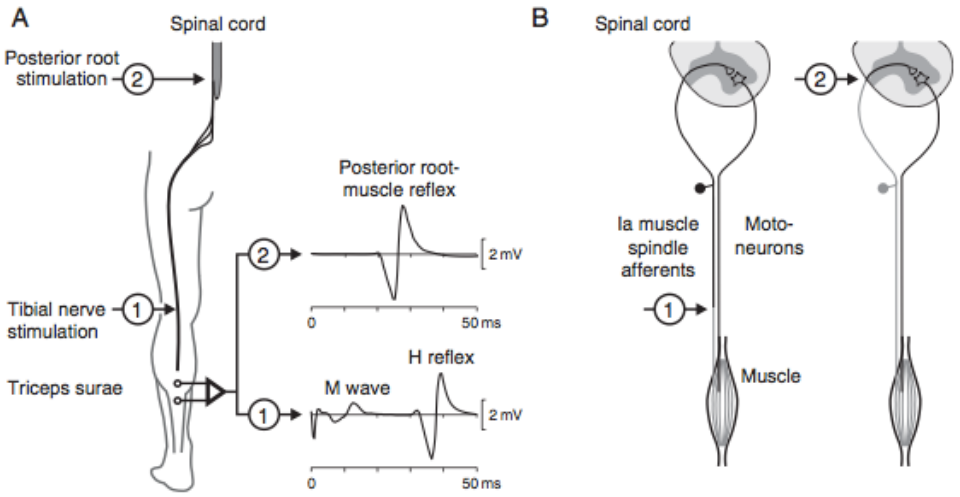

Figure 14: A. Elicitation and recording of H-reflex (1) and PRM reflex (2) of triceps surae. The H-reflex is elicited by stimulating the posterior tibial nerve in the popliteal fossa, while the PRM reflex is elicited at proximal sites close to the spinal cord. B. Reflex arcs. The latency of PRM reflex is shorter than that of the H-reflex due to the short afferent limb of the PRM reflex arc – the signal's starting point is closed to the spinal cord, thus it has a shorter way to travel [Minassian et al., 2011].

Furthermore, peripheral stimulation allows the elicitation of a monosynaptic reflex in one single muscle or muscle group, while stimulation of multiple posterior roots allows for the activation of afferents that are involved in the reflex arcs of both agonists and antagonists simultaneously, which means that PRM reflexes of different muscles elicited in series and in close succession can affect each other [Delwaide et al., 1976]. However, it is also possible that a single pulse is affected by another. There is evidence that Ib inhibitory interneurons that have been activated by a conditioning volley produce disynaptic inhibition which cancels out the monosynaptic Ia excitation that has been provided for the quadriceps [Marchand-Pauvert et al., 2002], thus suppressing the H-reflex. This can decrease the size of the H reflex and create

a problem for H-reflex studies - the reflex cannot be considered entirely monosynaptic, as the inhibition comes not only from the Ia transition but also from the activated Ib interneurons.

CHAPTER 3

METHODS

The purpose of these measurements was the investigation of the conditioning effects of peroneal nerve stimulation on the transcutaneously elicited posterior root-muscle reflex.

3.1 Subjects

The measurements were conducted on eight healthy subjects, four male, four female, between the ages of 22 and 26.

3.2 Electrode and stimulation setup

Electrical stimulation of the spinal cord (tSCS) was performed using commercially available self-adhesive transcutaneous electrical neural stimulation electrodes (Schwa-medico GmbH, Ehringshausen, Germany). A pair of round electrodes with a diameter of 5 cm was placed over the paravertebral skin on each side of the spine at the T11-T12 interspinous space. Either 2 or 3

self-adhesive rectangular electrodes (8 cm x 13 cm), depending on the size of the subject and the electrodes, were placed longitudinally over the abdomen, symmetrically around the umbilicus. These electrodes were connected to function as a single reference electrode (Figure 15). A constant-voltage stimulator was used to deliver symmetric, biphasic rectangular pulses of 2 ms (1 ms + 1 ms) width. The way the electrodes were connected to the stimulator ensured that the paravertebral electrodes acted as the cathode and the abdominal electrodes were the anode in the second phase of the biphasic stimulus, when the polarity of stimulation is changed. This abrupt change of polarity causes action potentials to be elicited.

A pair of ECG electrodes was placed lateral to the fibula head for peroneal nerve stimulation, with a self-adhesive rectangular electrode (8 cm x 13 cm) over the patella as the reference electrode. The same constant-voltage stimulator was used to deliver a train of biphasic rectangular stimuli with pulse widths of 2 ms to the peroneal nerve.

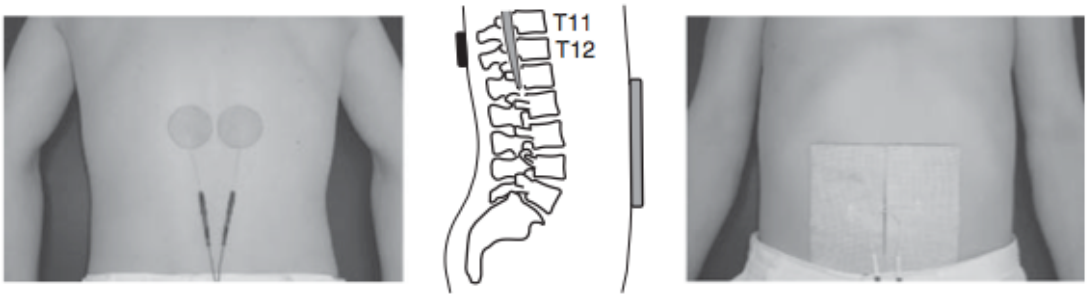

Figure 15: Electrode placement for elicitation of PRM reflex by tSCS. A pair of round electrodes is placed over the paravertebral skin on each side of the spine at the T11-T12 interspinous space. 2 self-adhesive rectangular electrodes are placed longitudinally over the abdomen, symmetrically around the umbilicus and are connected to function as a single reference electrode.

3.3 Recording procedure

The electromyographic (EMG) activity of the stimulus-evoked compound muscle action potentials (CMAPs) of the left and right quadriceps, hamstrings, tibialis anterior and triceps surae was recorded using pairs of silver-silver chloride surface electrodes of red or blue colour (see Figure 16, Figure 17). After preparing the skin by rubbing off its upper layer in order to reduce the impedance, each electrode pair was placed centrally over the corresponding muscle belly (Figure 17).

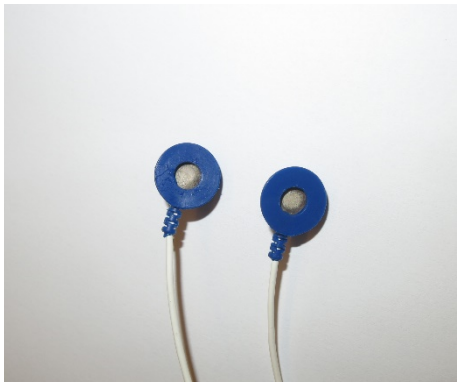

Figure 16: Silver-silver chloride surface electrodes (recording electrodes).

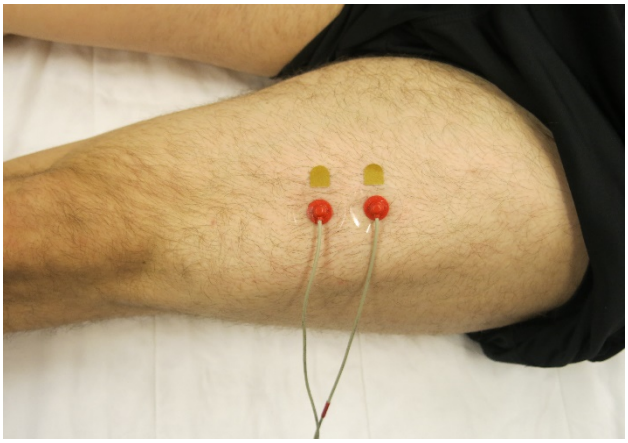

Figure 17: Recording electrodes placed over left quadriceps muscle belly.

3.4 Stimulation protocol

The experiments were performed with subjects in a relaxed, supine position. Moreover, the subjects were encouraged to listen to calm music during the stimulation, in order to avoid distractions and tedium. First, the maximal M-wave (Mmax) in the triceps surae was found by stimulating the posterior tibial nerve in the popliteal fossa. 25% of Mmax was the desired peak-to-peak amplitude of the EMG of the triceps surae for the peroneal nerve stimulation, with an acceptable range of 20% to 40% of Mmax. Next, the motor threshold (MT) for the conditioning stimulus was found by stimulating the peroneal nerve, such that the tibialis anterior showed activity, while the triceps surae was inactive. For the conditioning stimuli – a train of one second - we used 80%, 120% and 150% of MT for the respective experiments. The last step of the preparation was finding the threshold for tSCS, such that the EMG of the triceps surae was around 25% of Mmax. The position of the paravertebral electrodes could be changed until the desired value was reached.

A series of experiments was performed on each subject according to the following protocol:

- We began with one minute rest in which there was no stimulation and the subject was able to reach a fully relaxed state which was monitored on the EMG.
- Next we performed a series of 5 or 10 unconditioned control stimuli, with 15-second pauses between each two pulses.
- This was followed by a series of conditioned stimuli, such that a train of conditioning stimuli to the peroneal nerve was followed by a test stimulus to the spinal cord. There were six conditioning-test (C-T) intervals, namely 20, 50, 100, 200, 500 and 1000ms, which we called “inter-stimulus-intervals” (ISIs). For each ISI, the conditioning-test stimulation was performed three times.

- Lastly, after the last series of conditioned stimulations, there was one minute of rest, followed by 5 or 10 unconditioned control stimuli in 15 second intervals.

Table 1: Stimulation protocol

	Control	**C-T**	**C-T**	**C-T**	**C-T**	**C-T**	**C-T**	**Control**
	1 min rest							1 min rest
ISI	15 s	20 ms	50 ms	100 ms	200 ms	500 ms	1000 ms	15 s
# stimuli	5 x	3 x	3 x	3 x	3 x	3 x	3 x	5 x

In accordance with this protocol, in each experiment carried out, the frequency and intensity of the peroneal nerve stimulation were modified. As mentioned above, the PNS intensities were 80%, 120% and 150% of MT, respectively, while the frequencies of the trains were 30 Hz, 15 Hz and 50 Hz, respectively, with the burst duration remaining one second in each experiment. The relevant experiments for this thesis are the following:

1. PNS 30 Hz, 30 p (pulses), 120% MT
2. PNS 30 Hz, 30 p, 150% MT
3. PNS 30 Hz, 30 p, 80% MT
4. PNS 15 Hz, 15 p, 120% MT
5. PNS 50 Hz, 50 p, 120% MT

Relevant data were collected from up to 5 subjects per experiment.

3.5 Data analysis

The data were processed and analysed using Matlab (see Source Code). For each experiment the means of the peak-to-peak amplitudes of the PRM reflexes before (at ISI 20 ms) and after C-T (at ISI 1000 ms) were interpolated linearly using the Matlab function *interp1*. The C-T values for each ISI were divided by

the interpolated control values for the corresponding ISIs and the mean and standard errors (standard deviation divided by the square root of the sample size) were computed and then plotted to display the size of the conditioned PRM reflex (as the percentage of the unconditioned control value) as a function of the ISI between conditioning and test stimuli.

Since during the measurements the controls seemed to fluctuate, they were also analysed separately. For each muscle, the control values before and after the conditioned tests were plotted, and the minimum, maximum, mean value and standard deviation of the absolute value of the difference of the responses before and after the conditioned tests were found. The statistical values were also found for all muscles combined.

In addition, the coefficients of variation of the control responses were found by dividing each control of a test series by the mean of all controls of that series. Then the mean of the coefficients of variation was plotted for each muscle, subject and experiment, before and after the conditioned test. The minimum, maximum, mean and standard deviation were found for each muscle and for all muscles combined, with ‘before’ and ‘after’ values put together.

CHAPTER 4

RESULTS

4.1 Conditioned stimulations

Figure 18 and Figure 19 display the means of the sizes of the conditioned PRM reflexes as a percentage of the unconditioned control values for each ISI between the conditioning and the test stimuli. The errorbars represent the standard error, which reflects the distribution of the mean, or the probability that the mean is in the range of the samples. Figure 18 illustrates the results of the three 30 Hz stimulations, while Figure 19 shows those of the three stimulations at 120% MT.

4.1.1 PNS 30 Hz

As is illustrated in Figure 18, the subthreshold stimulation at 80% MT and 30 Hz elicited a suppressed response in the ipsilateral quadriceps and hamstring and increased responses in all other muscles.

Stimulation intensities above threshold, namely at 120% MT and 150% MT respectively, resulted in the suppression of all ipsilateral muscle responses for the duration of up to 1 second, for all stimulation frequencies, with the effect being stronger in the distal than in the proximal lower limb muscle groups.

At 120% MT no suppression of the contralateral muscle responses can be concluded.

The 30 Hz, 150% MT, stimulation resulted in the attenuation of both ipsilateral and contralateral reflexes, with the ipsilateral suppression being greater.

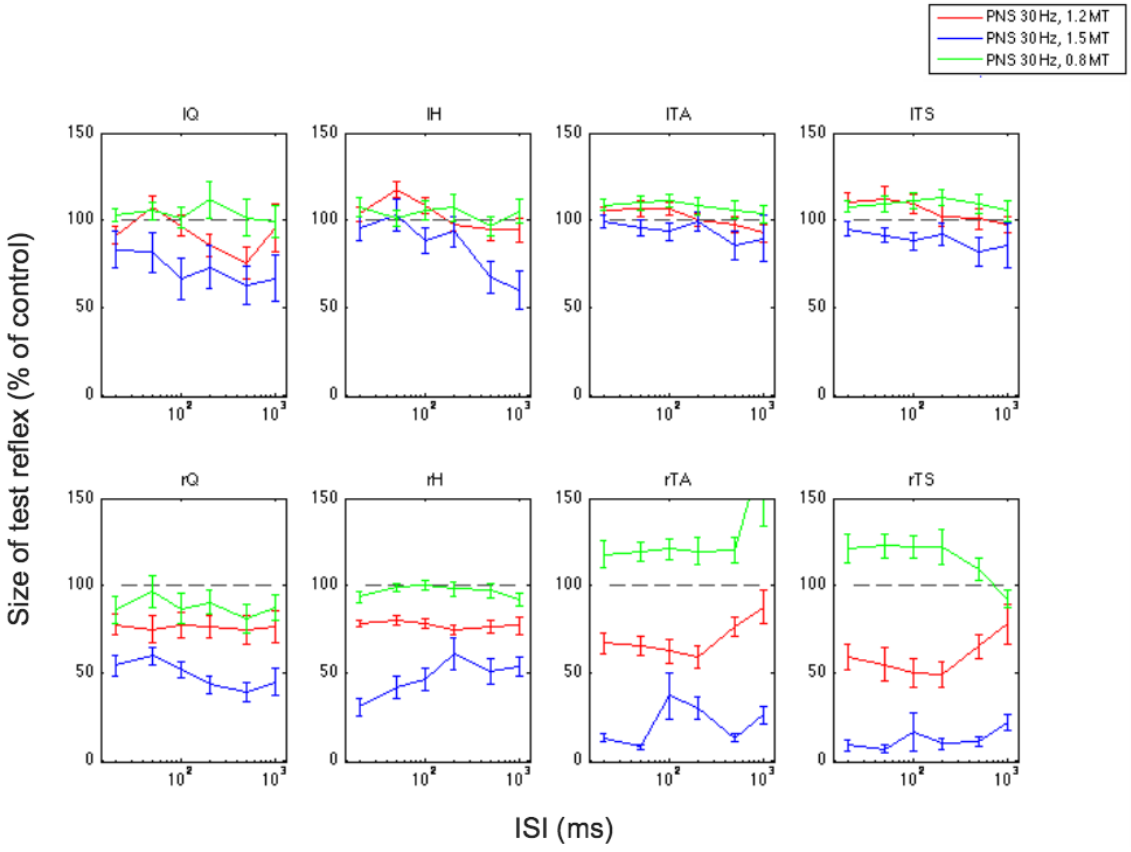

Figure 18: PNS 30 Hz at stimulation intensities 1.2 MT (red), 1.5 MT (blue) and 0.8 MT (green). X-axis is ISI in ms, y-axis is the size of the test reflex in percentage of the size of the control response. The first row displays the contralateral muscles (lQ, lH, lTA, lTS; 'l' for 'left'), the second row displays the ipsilateral muscles (rQ, rH, rTA, rTS; 'r' for 'right'). There is an ipsilateral suppression of the reflex responses for above-threshold stimulation (1.2 MT and 1.5 MT) and a contralateral suppression at stimulation intensity 1.5 MT.

4.1.2 PNS 1.2 MT

At a stimulation intensity of 1.2 MT, ipsilateral muscle responses are suppressed at all frequencies, with the effect being greater in TA and TS.

At 15 Hz and 120% MT the responses in the contralateral muscle groups, with the exception of the quadriceps, are increased.

At 50 Hz and 120% MT the contralateral quadriceps response is suppressed, while those of the remaining contralateral muscles slightly deviate from the control values.

At 30 Hz and 120% MT no conclusive statement can be made about the contralateral responses (Figure 19).

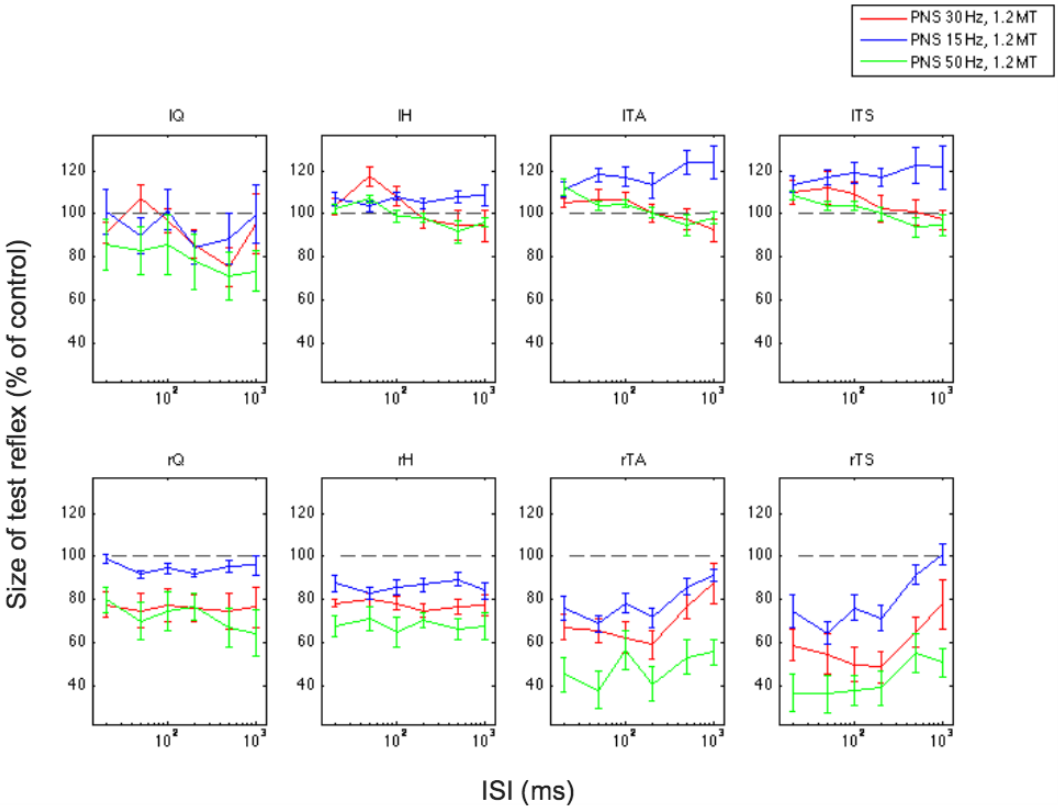

Figure 19: PNS 120% MT at stimulation frequencies 30 Hz (red), 15Hz (blue) and 50 Hz (green). X-axis is ISI in ms, y-axis is the size of the test reflex in percentage of the size of the control response. The first row displays the contralateral muscles (lQ, lH, lTA, lTS; 'l' for 'left'), the second row displays the ipsilateral muscles (rQ, rH, rTA, rTS; 'r' for 'right'). There is an ipsilateral suppression of the reflex responses for all stimulation frequencies (15 Hz, 30 Hz and 50 Hz) and an increased response in contralateral H, TA and TS (but not Q) at stimulation frequency 15 Hz.

4.1.3 Summary of results

To summarise the obtained results and conclusions:

- The main conditioning effect is the suppression of the ipsilateral PRM reflex for up to 1 second.
- The higher the stimulation intensity, the greater the observed effect.
- The higher the frequency, the greater the effect.
- For subthreshold stimulation no conclusion can be drawn due to the small sample size.
- No statement can be made regarding the contralateral effects, as they are inconclusive.

4.2 Control measurements

Figure 20 to Figure 23 illustrate the results of the control measurements.

Figure 20 and Figure 21 illustrate the control responses before and after the conditioned tests, with colour coding for experiments and subjects, respectively. The instability of the controls can be seen in their arbitrary fluctuation. No trend can be demonstrated, as the control values seemingly increase or decrease at random after 15 minutes of stimulation (approximate length of a series of conditioned stimulation).

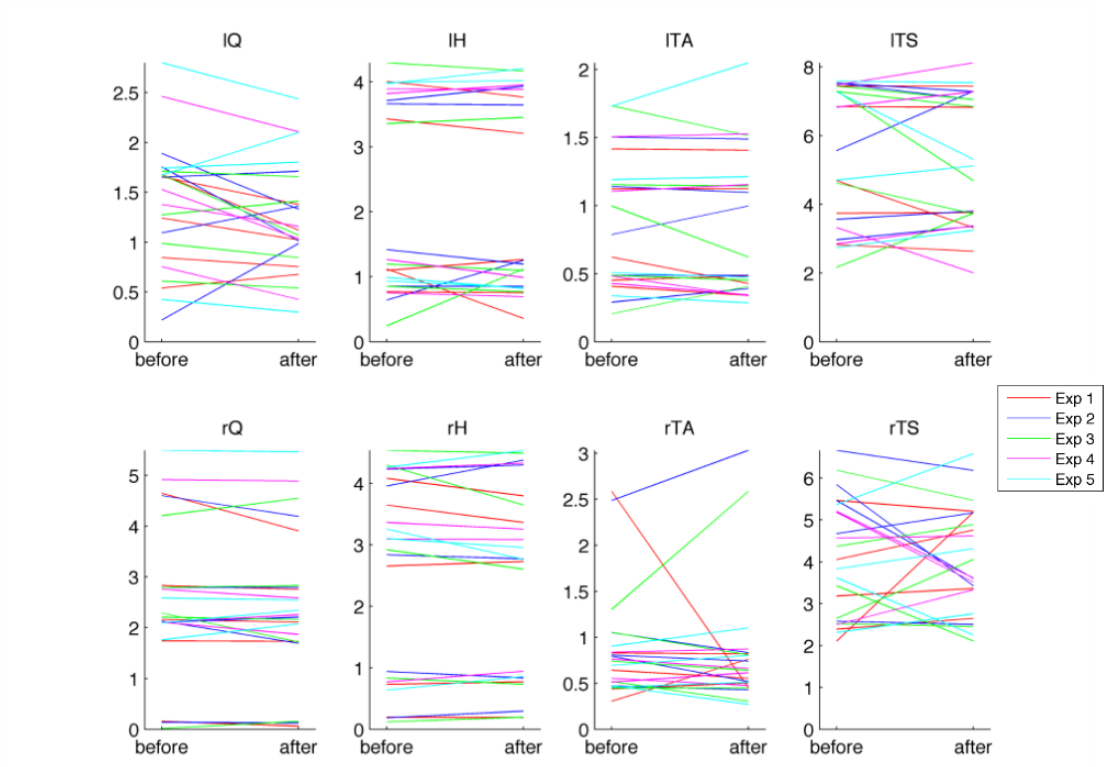

Figure 20: Control responses before and after the conditioned tests, colour-coded by experiments. Exp 1–5 are 30 Hz, 0.8 MT; 30 Hz, 1.2 MT; 30 Hz, 1.5 MT; 15 Hz, 1.2 MT; and 50 Hz, 1.2 MT, respectively. The first row displays the contralateral muscles (lQ, lH, lTA, lTS; 'l' for 'left'), the second row displays the ipsilateral muscles (rQ, rH, rTA, rTS; 'r' for 'right'). The control values seemingly increase or decrease at random.

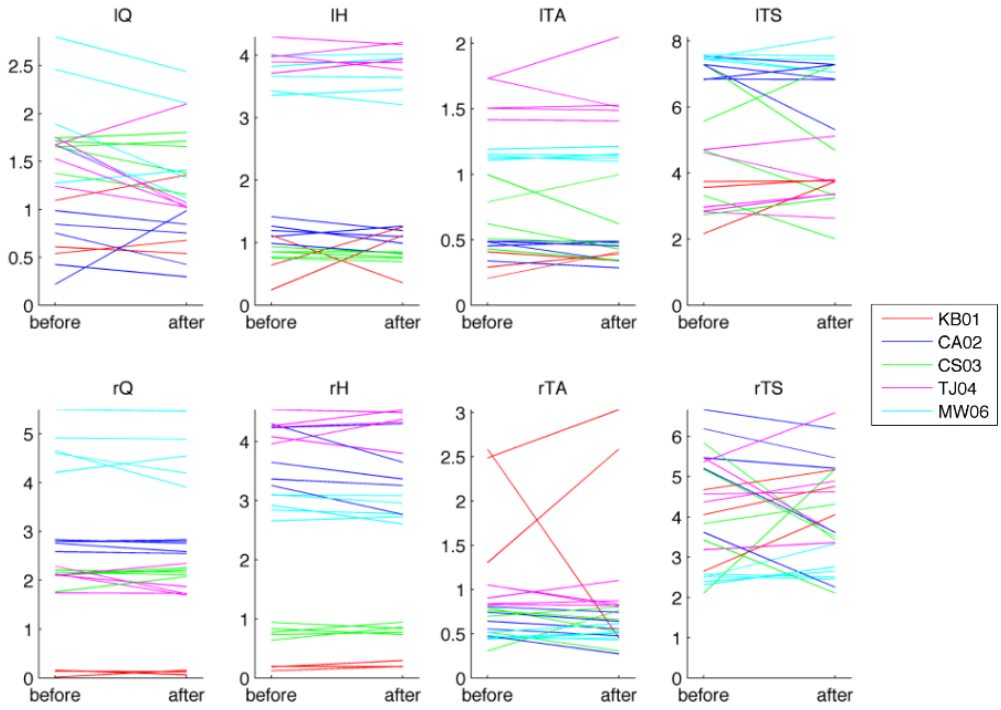

Figure 21: Control responses before and after the conditioned tests, colour-coded by subjects. The first row displays the contralateral muscles (lQ, lH, lTA, lTS; 'l' for 'left'), the second row displays the ipsilateral muscles (rQ, rH, rTA, rTS; 'r' for 'right'). The control values seemingly increase or decrease at random.

For each muscle, the minimum, maximum, mean value and standard deviation of the absolute value of the difference of the responses before and after the conditioned tests were found, as shown in Table 2. The minimum over all muscles is 0.00028 mV, the maximum is 3.074530 mV, the overall mean is 0.36454 mV and the standard deviation is 0.514826.

Table 2: Minima, maxima, mean values and standard deviations (STD) of the absolute values of the differences of the responses of each muscle before and after the conditioned tests.

	lQ	lH	lTA	lTS	rQ	rH	rTA	rTS
Min	0.05539	0.00028	0.00075	0.00542	0.00105	0.00465	0.000540	0.04772
Max	0.76838	0.87479	0.37559	2.60885	0.73998	0.64891	2.13408	3.07453
Mean	0.30714	0.20721	0.09758	0.71576	0.18647	0.18024	0.28788	0.93406
STD	0.22335	0.23310	0.10651	0.70660	0.19932	0.16539	0.48539	0.80030

In order to show the general deviation of the controls from their mean, their coefficients of variation were found by dividing each control of a test series (each control stimulation was performed up to 10 times) by the mean of all controls of that series, before and after the conditioned test. Thus the mean value is 1. Then the mean of the coefficiants of variation was plotted for all muscles, subjects and experiments in Figure 22 and Figure 23. With the exception of a few outliers, the controls are close to the mean.

Table 3 lists the minimum, maximum, mean value and standard deviation of the coefficients of variation for each muscle. The minimum over all muscles is 0.006731, the maximum is 1.036319, the overall mean is 0.135451 and the standard deviation is 0.112120.

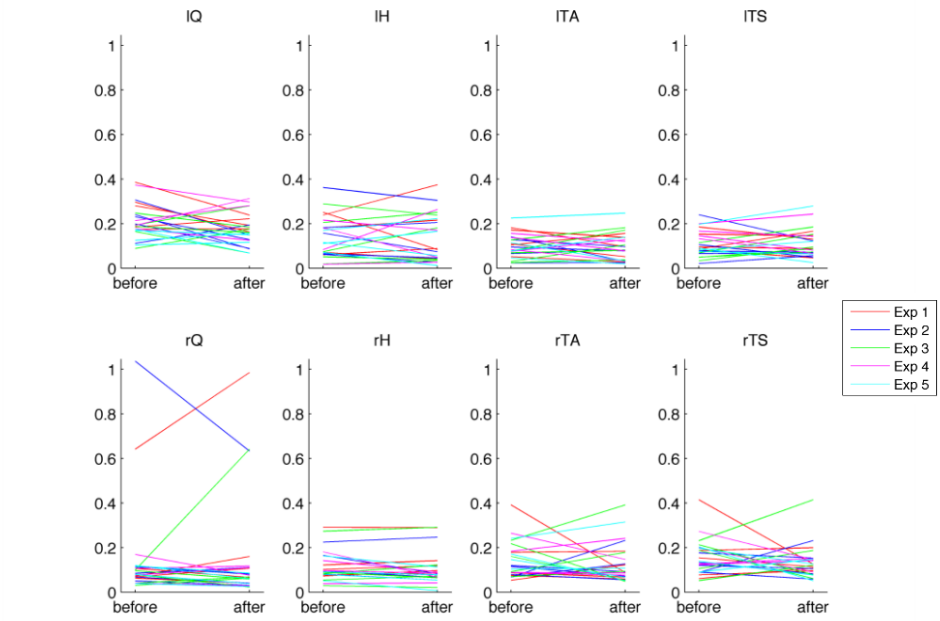

Figure 22: Coefficient of variation of control responses before and after the conditioned tests, colour-coded by experiment. Exp 1–5 are 30 Hz, 0.8 MT; 30 Hz, 1.2 MT; 30 Hz, 1.5 MT; 15 Hz, 1.2 MT; and 50 Hz, 1.2 MT, respectively. The first row displays the contralateral muscles, the second row the ipsilateral muscles. With the exception of a few outliers, the controls are close to the mean.

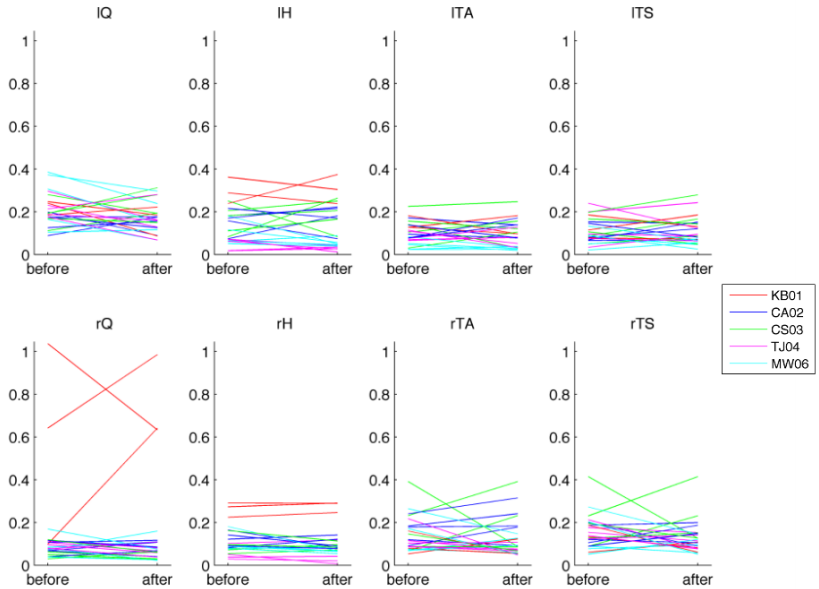

Figure 23: Coefficient of variation of control responses before and after the conditioned tests, colour-coded by subjects. The first row displays the contralateral muscles, the second row the ipsilateral muscles. With the exception of a few outliers, the controls are close to the mean.

Table 3: Minima, maxima, mean values and standard deviations of the coefficients of variation of each muscle.

	lQ	lH	lTA	lTS	rQ	rH	rTA	rTS
Min	0.06769	0.01199	0.01930	0.02093	0.02279	0.00673	0.04844	0.05183
Max	0.38574	0.37406	0.24722	0.27894	1.03632	0.29109	0.39134	0.41450
Mean	0.19195	0.13922	0.09723	0.11034	0.15323	0.10995	0.13590	0.14580
STD	0.07591	0.09775	0.05594	0.06020	0.23460	0.07240	0.08634	0.07878

CHAPTER 5

DISCUSSION

5.1 Central state of excitability

5.1.1 Excitability of motoneuronal network

The central state of excitability in healthy subjects differs from that in spastic SCI patients. In a study on the stretch reflexes in the lower limbs of patients with partial or complete lesions of the spinal cord, Dimitrijevic and Nathan showed that while the onset of the discharge of motor units (the reflex latency time) following a tendon tap, is the same in healthy as well as SCI individuals, in SCI patients the jerks have larger spikes, multiple rather than single spikes and an after-discharge of motor units. A tendon tap usually results in a short excitation of the agonist and inhibition of the antagonist; however, in spastic patients it can also cause activity in other muscles [Dimitrijevic and Nathan, 1967]. Neuroplasticity refers to the changes (adaptive and maladaptive) in the sensorimotor systems caused by spinal cord injury. The training of movement

through electrical stimulation can be used to facilitate neuroplasticity [Dietz & Fouad, 2014]. Different factors influence the effectiveness of this training. For locomotion, spastic muscle tone is required in order to induce a locomotor-like EMG-pattern in SCI individuals [Dietz et al., 1995]. Therefore, while spasticity may be undesirable in non-ambulatory SCI patients, as it can cause painful spasms, in ambulatory patients it is welcome since it is connected to an increased training effect. Heightened excitability of the motoneurons is one of the main features of spasticity [Dimitrijevic and Nathan, 1967]. Therefore, the excitability of the network in SCI is a good indicator of the effectiveness of training. The question remains, whether and how the spasticity, and thus excitability, of motoneurons could be influenced in SCI patients with low spasticity. Due to the altered state of excitability in SCI it cannot be assumed that the inhibitory suppression observed in our measurements on healthy subjects would also occur in SCI patients. Further studies are needed to determine the conditioning effects in SCI individuals.

In another study, Dimitrijevic and Nathan examined the effects of repetitive cutaneous stimulation of the plantar surface of the foot on the flexion-withdrawal reflex. In healthy subjects the reflex response decreases with repeated stimulation due to habituation. In SCI patients the response increases at first, then it also diminishes, which suggests that the central organisation of the response is different in SCI patients as opposed to healthy individuals. In addition to habituation, repetitive stimulation also causes the excitability of the whole spinal cord to decrease, so the whole process must be controlled by the spinal cord. [Dimitrijevic and Nathan, 1970]. These results also show that assumptions about the state of excitability in SCI patients based on results obtained from tests on healthy subjects need to be verified with additional tests on SCI patients.

An interesting effect was observed in our measurements: although the peroneal nerve, which controls only one muscle group, is stimulated, not only

agonist and antagonist are affected, but all leg muscles. This suggests that there exists a possibility for modification on a large scale as opposed to only causing localised change. The aim is to find a way to increase the excitability of the motoneuronal network. While in our case we achieved the opposite effect, i.e. a decrease of excitability, the fact that this occurs in all leg muscles is promising. Further tests must be conducted to find a way to increase excitability, perhaps by stimulating a different nerve.

5.1.2 Excitability of interneuronal network

A drawback of our measurements is their restriction to the study of the excitability of the motoneuronal network. However, it is not desirable for a motoneuron to be too excitable in a healthy subject. The inhibition of the Ia afferent – motoneuron pathway does not necessarily mean that the interneuronal network is less excitable. In order to study the interneuronal network one option could be to observe a reflex other than the H or PRM reflex – for instance the withdrawal reflex, which is polysynaptic and thus involves more neurons. In the following study the 'spinal reflex' (withdrawal reflex) evoked by tibial nerve stimulation was observed [Dietz et. al, 2009] (this reflex is commonly known as 'cutaneomuscular reflex'. Dietz and colleagues, hoewever, use the term 'spinal reflex', which they define as a below-nociceptive threshold to tibial nerve stimulation, since a noxious stimulus cannot be determined in complete spinal cord injury subjects). Dietz and colleagues examined whether there is a common mechanism for changes in cutaneomuscular reflex and locomotor activity in complete SCI. The cutaneomuscular reflex was elicited by a 40 ms train of 8 high-frequency (200 Hz) 2 ms bursts [Muller and Dietz, 2006], stimulation intensity was 2 MT. Leg muscle EMG activity was evoked by mechanically assisted locomotion. The results showed that the reflex changed with the SCI subject's locomotion ability. At around 8 weeks after the injury,

both a short latency cutaneomuscular reflex component appeared and leg-muscle activity was present during assisted locomotion. At around 6 months post-injury, an additional long latency reflex component appeared (which remained 15 years post-injury), while the short latency component decreased considerably 18 months post-injury. At the same time, EMG activity declined, and EMG amplitude decreased from the 2-minute point to the 10-minute point during assisted locomotion. These findings suggest that the change of the cutaneomuscular reflex is related to the locomotion ability of the SCI individual. Thus, perhaps a certain influence on the cutaneomuscular reflex, possibly peripheral nerve stimulation, can in turn heighten the central state and thus increase locomotion ability. Evidence for suppression of the central state has already been shown, further research in this area is needed to determine whether and how the central state can be increased.

5.2 Central effect of FES

Functional electrical stimulation (FES) is used to produce targeted patterned muscle contractions and movements for specific function in SCI individuals whose motor control is impaired by upper motoneuron dysfunction. As opposed to sustained stimulation, which is often used in other neuromodulation therapies, in FES, timed sequences of short bursts of electrical pulses are used [Holsheimer, 1998]. The muscles are activated either by electrical stimulation of the supplying lower motoneurons [Holsheimer, 1998] or by stimulation of the peripheral afferent and the subsequent spinal reflex [Kralj et al., 1983]. The main application of FES, which Liberson discovered in 1961 [Liberson et al., 1961], is the correction of the drop foot (also: foot drop). Drop foot is a symptom of an underlying neurological, muscular or anatomical disorder, which causes difficulty with dorsiflexion (raising the front part of the foot) during the swing phase of walking, due to a decreased capacity of the tibialis anterior. As a

result, the affected individual drags the foot or engages in steppage gait [NIH, 2009]. Drop foot can be easily corrected, since only one muscle needs to contract in order to provide dorsiflexion. According to Otto Bock, the company that produces the ActiGait® for drop foot correction, this is achieved by stimulating the peroneal nerve with a short train (30 to 50 Hz) [Otto Bock ActiGait®, 2014]. However, it is not clear if the 30 Hz stimulation is in fact necessary to achieve dorsiflexion, since even a 1 Hz simulation causes a muscle twitch. The flexion reflex elicited by stimulating the peroneal nerve causes hip, knee and ankle movements, and enhances the swing phase during walking [Liberson et al., 1961; Vodovnik et al., 1978; Weber et al., 2005]

While the results of our measurements (the suppression of the PRM reflex in all muscles of the ipsilateral leg following stimulation of the peroneal nerve) suggest that there is indeed a central effect of FES, the details of the central effect of this particular stimulation for the correction of drop foot remains to be determined. It is possible that in some patients the stimulation elicits a reflex, but that is not the case in a healthy individual.

5.3 Frequency dependence of SCS

In the motor complete SCI individual, epidural spinal cord stimulation of the L2 segment can elicit rhythmic locomotor-like EMG activity and/or movement in the lower limbs, with a stimulation frequency of 25–50 Hz [Dimitrijevic et al., 1998; Minassian et al., 2004]. At a frequency of 5–15 Hz, the stimulation can cause the extension of the lower limbs [Jilge et al., 2004] and a frequency of 15 Hz was sufficient to induce enough strength to enable full weight-bearing standing [Harkema et al., 2011]. Spasticity in SCI patients can be effectively controlled with epidural stimulation at 50–100 Hz [Pinter et al., 2000]. These studies all suggest that the frequency of epidural stimulation of the spinal cord of the motor complete SCI individual is not arbitrary but rather plays an important role. In

addition, for spasticity control in the incomplete SCI individual, transcutaneous SCS at 50 Hz may be as effective as epidural stimulation [Hofstoetter et al., 2014]. In our case, an increased frequency resulted in a larger suppression of the reflex response of healthy subjects. This could be due to the increased input, i.e. more pulses; there is no evidence of an altered configuration of the network as a result of the higher frequency. To gain more insight, more data should be collected from healthy subjects and the experiments should also be conducted on SCI patients.

5.4 Transition Ia interneurons – motoneurons

Our tests are limited to the investigation of the transition from Ia interneurons to motoneurons in individuals with intact spinal cords, where brain control is intact. Alternative tests are needed to gain information about the excitability of the network, perhaps using a different reflex that does not run from Ia interneurons to motoneurons, since this transition can be masked by presynaptic inhibition, which prevents us from learning about the network's excitability.

There probably exist more interneurons than we are aware of at present. In cats, there can be up to 60000 synaptic contacts onto a motoneuron [Ulfhake & Cullheim, 1988]. In the spinal segment D9 of the turtle there are eight interneurons for every motoneuron; in the lumbar enlargement in chickens there are six and in mice, fifteen interneurons for every motoneuron [Walløe et al., 2011]. This large number of interneurons suggests that there is recurrent connectivity and therefore higher processing capabilities [Petersen et al., 2014].

Ib inhibitory interneurons have numerous connections. They not only project to their own and synergist motoneurons [Eccles et al., 1957], but also receive inputs from cutaneous and proprioceptive afferents and project to antagonistic motoneurons. In addition, Ib inhibitory interneurons project to each other, which results in disinhibition [McCrea, 1986]. All these connections

suggest that Ib inhibitiory interneurons can influence not only agonists and antagonists, but possibly other segments as well. Repetitive stimulation may not be necessary to achieve this but may generate more pronounced effects.

Consequently, we can assume that the spinal cord plays a larger role than it is given credit for, and further tests should be conducted where not only the transition from Ia interneurons to motoneurons is examined, since, as was shown, there are probably many more relevant interneurons with numerous connections and functions.

5.5 Inhibitory pathways

We intended to show that the amount of reciprocal inhibition can be measured. However, reciprocal inhibition is relatively short (2–3 ms) and the suppression of the PRM reflex in our measurements lasted considerably longer (ISIs up to 1000 ms). Thus, evidence suggests that the suppression was not caused by reciprocal inhibition alone.

The long duration of the above-threshold suppression of the PRM reflex suggests that Renshaw cells play a substantial role in the inhibition. However, the very long duration of one second indicates that other inhibitory interneurons are involved as well (Renshaw cells inhibit for about 100 ms [Hultborn et al, 1971; Hultborn et al., 1979] or up to 200 ms with strong stimulation [Windhorst, 1990]).

Presynaptic inhibition is characterised by a long central latency of about 5ms [Eccles, 1964] and a very long duration of 300–400 ms [Rudomin & Schmidt, 1999]. Therefore it is possibly involved in the suppression of the PRM reflex as well, yet it is probably also not solely responsible, given the long duration of the inhibition.

It is noteworthy that in our measurements, reflex inhibition occurred not only in the agonist, but in all muscles of the respective leg. The peroneal nerve

inhibits the TS and repetition causes presynaptic inhibition of the TA response. However, presynaptic inhibition alone does not explain why the Q and H are inhibited as well. Perhaps the Ia interneurons make connections to Q and H and cause the suppression of their responses. Or maybe the process is polysynaptic and inhibitory. Even presynaptic inhibition itself is selective – an input that is regularly repeated is gated. As a rule, everything that is repetitive does not provide new information. Dimitrijevic and Nathan showed that repetitive application of a stimulus which causes a flexion reflex leads to habituation [Dimitrijevic and Nathan, 1970]. Once motor units have thus been made irresponsive, they are reactivated in numerous ways: by an increase in stimulus intensity; by an increase or decrease in stimulus rate; by other kinds of stimuli applied to the same site; by the same or other kinds of stimuli applied to different sites in the same limb, which can be as close as 2 cm to the original site; and by stimuli applied to the contralateral limb. A decrease in stimulus intensity does not reactivate the irresponsive motor units [Dimitrijevic and Nathan, 1971]. In short, repetition, or ‘old input’, causes a suppression of the reflex response. A small alteration of the stimulation – ‘new input‘ – results in an uninhibited response, which in turn can decrease again due to repetition.

More research is needed to determine what exactly is involved in the inhibitory pathways and the suppression of all muscle groups of the leg, as well as the topological principle and why the components responsible for inhibition seem to be so widespread.

5.6 Limitations due to instability of controls

The self-built voltage-controlled stimulator used for the measurements produced unstable controls, as is illustrated in Figure 20 to Figure 23.

Moreover, the stimulator was also easily affected by changes in the impedance of the electrodes. In addition, the sample size (number of subjects)

is low, as the stimulator broke after having completed the test series on merely a small number of subjects. Therefore, there is a lot of noise and only rough conclusions can be drawn.

Figure 20 and Figure 21 illustrate the control responses before and after the conditioned tests, with colour coding for experiments and subjects, respectively. The instability of the controls can be seen in their arbitrary fluctuation. No trend can be demonstrated, as the control values seemingly increase or decrease at random after 15 minutes of stimulation (approximate length of a series of conditioned stimulation). Due to these fluctuations of reflex responses, it was necessary to constantly adapt the stimulation intensity. This presents another limitation.

In order to show the general deviation of the controls from their mean, their coefficients of variation were found by dividing each control of a test series (each control stimulation was performed up to 10 times) by the mean of all controls of that series. Thus the mean value is 1. Then the mean of the standard deviations was plotted for all muscles, subjects and experiments in Figure 22 and Figure 23. With the exception of a few outliers, the controls are close to the mean, which allows for their use in this analysis.

Due to the limitations mentioned above, a negative statement, i.e. the lack of a conditioning effect in a certain case, cannot be made. The most obvious effect observed remains the consistent suppression of the posterior root-muscle reflex in all subjects.

CHAPTER 6

CONCLUSION

In SCI individuals, it is desirable to, on the one hand, increase presynaptic inhibition, as it is nearly non-existent due to its being controlled by the brain stem, and on the other hand, increase the central state of excitability. We have shown that in healthy individuals it is possible to change the excitability of the motoneuronal network with peripheral nerve stimulation. This change is multisegmental and unilateral – we observed a generalised ipsilateral suppression, i.e. an effect that is not limited to the stimulated spinal cord segments, but is rather more widespread. It is not entirely clear what causes this effect. However, it is known that peripheral stimulation has an antispastic effect, without decreasing the excitability of motoneurons, unlike some drugs used to treat spasticity. In locomotor training, it is desirable to increase the excitability of motoneurons and interneurons, but without triggering spasms. In this case peripheral stimulation, SCS or both, could be used. The advantages of peripheral stimulation are that it can be applied in a more targeted way and that it is possible to adjust it in case there is an area with insufficient activity. The

behaviour in SCI individuals remains to be studied. In order to discover more about the processes involved in the alteration of the central state of excitability, other test reflexes, both in healthy and SCI individuals, should also be used. A single-pulse stimulation, as opposed to repetitive stimulation, is not likely to cause a change that lasts longer than a few milliseconds. The long time constant and the spreading of the effect to muscles other than the ones innervated by the stimulated nerves are a promising sign that repetitive stimulation of peripheral nerves can cause prolonged changes of the central state of excitability.

.

BIBLIOGRAPHY

Baret, M., Katz, R., Lamy, J. C., Pénicaud, A., & Wargon, I. (2003). Evidence for recurrent inhibition of reciprocal inhibition from soleus to tibialis anterior in man. *Experimental Brain Research*, *152*(1), 133–6.

Bear, M. F., Connors, B. W., & Paradiso, M. A. (2007). *Neuroscience: Exploring the brain*. Philadelphia, PA: Lippincott WIlliams & Wilkins.

Brooke, J. D., Misiaszek, J. E., & Cheng, J. (1993). Locomotor-like rotation of either hip or knee inhibits soleus H reflexes in humans. *Somatosensory & Motor Research*, *10*(4), 357–64.

Capaday, C., & Stein, R. B. (1987). Difference in the amplitude of the human soleus H reflex during walking and running. *The Journal of Physiology*, *392*, 513–22.

Chiu, S. Y., Ritchie, J. M., Rogart, R. B., & Stagg, D. (1979). A quantitative description of membrane currents in rabbit myelinated nerve. *The Journal of Physiology*, *292*, 149–66.

Crone, C., & Nielsen, J. (1989). Spinal mechanisms in man contributing to reciprocal inhibition during voluntary dorsiflexion of the foot. *The Journal of Physiology*, *416*, 255–72.

Crone, C., Hultborn, H., & Jespersen, B. (1985). Reciprocal Ia inhibition from the peroneal nerve to soleus motoneurones with special reference to the size of the test reflex. *Experimental Brain Research*, *59*(2), 418–22.

Crone, C., Hultborn, H., Jespersen, B., & Nielsen, J. (1987). Reciprocal Ia inhibition between ankle flexors and extensors in man. *The Journal of Physiology*, *389*, 163–85.

Delwaide, P. J., Cordonnier, M., & Charlier, M. (1976). Functional relationships between myotatic reflex arcs of the lower limb in man: investigation by excitability curves. *Journal of Neurology, Neurosurgery, and Psychiatry*, *39*(6), 545–54.

Dietz, V., & Fouad, K. (2014). Restoration of sensorimotor functions after spinal cord injury. *Brain : A Journal of Neurology*, *137*(Pt 3), 654–67.

Dietz, V., Colombo, G., Jensen, L., & Baumgartner, L. (1995). Locomotor capacity of spinal cord in paraplegic patients. *Annals of Neurology*, *37*(5), 574–82.

Dietz, V., Grillner, S., Trepp, A., Hubli, M., & Bolliger, M. (2009). Changes in spinal reflex and locomotor activity after a complete spinal cord injury: a common mechanism? *Brain : A Journal of Neurology*, *132*(Pt 8), 2196–205.

Dimitrijevíc, M. R., & Nathan, P. W. (1967). Studies of spasticity in man. 2. Analysis of stretch reflexes in spasticity. *Brain : A Journal of Neurology*, *90*(2), 333–58.

Dimitrijević, M. R., & Nathan, P. W. (1970). Studies of spasticity in man. 4. Changes in flexion reflex with repetitive cutaneous stimulation in spinal man. *Brain : A Jounal of Neurology*, 93(4), 743–68.

Dimitrijević, M. R., & Nathan, P. W. (1971). Studies of spasticity in man. 5. Dishabituation of the flexion reflex in spinal man. *Brain : A Journal of Neurology*, *94*(1), 77–90.

Dimitrijevic, M. R., Gerasimenko, Y., & Pinter, M. M. (1998). Evidence for a spinal central pattern generator in humans. Annals of the New York Academy of Sciences, 860, 360–76.

Eccles, J. C., Eccles, R. M. & Lundberg, A. (1957). Synaptic actions on motoneurones caused by impulses in Golgi tendon organ afferents. *Journal of Physiology*. 138, 227–52.

Eccles, J. C., Fatt, P. & Koketsu, K. (1954). Cholinergic and inhibitory synapses in a pathway from motor-axon collaterals to motoneurones. *Journal of Physiology*. 126(3), 524-62.

Eccles, J.C. (1964). The Physiology of Synapses. Springer Verlag, Berlin.

Ethier, C., Imbeault, M.-A., Ung, V., & Capaday, C. (2003). On the soleus H-reflex modulation pattern during walking. *Experimental Brain Research*, *151*(3), 420–5.

Frank K., Fuortes M. G. F. (1957). Presynaptic and postsynaptic inhibition of monosynaptic reflexes. *Fed Proc*. 16:39–40.

Harkema, S., Gerasimenko, Y., Hodes, J., Burdick, J., Angeli, C., Chen, Y., Ferreira, C., Willhite, A., Rejc, E., Grossman, R. G., Edgerton, V. R. (2011). Effect of epidural stimulation of the lumbosacral spinal cord on voluntary movement, standing, and assisted stepping after motor complete paraplegia: a case study. *Lancet*, *377*(9781), 1938–47.

Hoffmann, P. (1952). Reflex inhibition and voluntary action of normal human muscles. *The Journal of Nervous and Mental Disease*, *116*(6), 579–84.

Hofstoetter, U. S., McKay, W. B., Tansey, K. E., Mayr, W., Kern, H., & Minassian, K. (2014). Modification of spasticity by transcutaneous spinal cord stimulation in individuals with incomplete spinal cord injury. *The Journal of Spinal Cord Medicine*, *37*(2), 202–11.

Holsheimer, J. (1998). Concepts and methods in neuromodulation and functional electrical stimulation: an introduction. *Neuromodulation : Journal of the International Neuromodulation Society*, *1*(2), 57–61.

Houk, J. C. (1979). Regulation of stiffness by skeletomotor reflexes. *Annual Review of Physiology*, *41*, 99–114.

Houk, J. C., & Rymer, W. Z. (1981). Neural Control of Muscle Length and Tension. In V. B. Brooks (Ed.), *Handbook of Physiology, Vol. 2, Part 1* (pp. 257–323). Baltimore: Williams and Wilkins.

Houk, J., & Henneman, E. (1967). Responses of Golgi tendon organs to active contractions of the soleus muscle of the cat. *Journal Of Neurophysiology*, *30*(3), 466–81.

Hultborn, H., Jankowska, E., & Lindström, S. (1971). Recurrent inhibition from motor axon collaterals of transmission in the Ia inhibitory pathway to motoneurones. *The Journal of Physiology*, *215*(3), 591–612.

Hultborn, H., Meunier, S., Morin, C., & Pierrot-Deseilligny, E. (1987). Assessing changes in presynaptic inhibition of Ia fibres: a study in man and the cat. *The Journal of Physiology*, *389*, 729–56.

Hultborn, H., Pierrot-Deseilligny, E., & Wigström, H. (1979). Recurrent inhibition and afterhyperpolarization following motoneuronal discharge in the cat. *The Journal of Physiology*, *297*, 253–66.

Iles, J. F., & Roberts, R. C. (1987). Inhibition of monosynaptic reflexes in the human lower limb. *The Journal of Physiology*, *385*, 69–87.

Jankowska, E. & Edgley, S.A. (2010). Functional subdivision of feline spinal interneurons in reflex pathways from group Ib and II muscle afferents; an update. *European Journal of Neuroscience 32*, 881–93.

Jankowska, E., McCrea, D. & Mackel, R. (1981). Pattern of “non reciprocal” inhibition of motoneurones by impulses in group Ia muscle spindle afferents in the cat. *The Journal of Physiology 316*, 393–409.

Jilge, B., Minassian, K., Rattay, F., Pinter, M. M., Gerstenbrand, F., Binder, H., & Dimitrijevic, M. R. (2004). Initiating extension of the lower limbs in subjects with complete spinal cord injury by epidural lumbar cord stimulation. *Experimental Brain Research*, *154*(3), 308–26.

Kandel, E., Schwartz, J., Jessell, T., Siegelbaum, S., & Hudspeth, A. J. (2012). *Principles of Neural Science, Fifth Edition* (p. 1760). McGraw-Hill.

Katz, R., Meunier, S., & Pierrot-Deseilligny, E. (1988). Changes in presynaptic inhibition of Ia fibres in man while standing. *Brain*, *111*(2), 417–37.

Katz, R., Penicaud, A., & Rossi, A. (1991). Reciprocal Ia inhibition between elbow flexors and extensors in the human. *The Journal of Physiology*, *437*, 269–86.

Kido, A., Tanaka, N., & Stein, R. B. (2004). Spinal reciprocal inhibition in human locomotion. *Journal of Applied Physiology, 96*(5), 1969–77.

Kitano, K., & Koceja, D. M. (2009). Spinal reflex in human lower leg muscles evoked by transcutaneous spinal cord stimulation. *Journal of Neuroscience Methods*, *180*(1), 111–5.

Knikou, M. (2006). Effects of changes in hip position on actions of spinal inhibitory interneurons in humans. *The International Journal of Neuroscience*, *116*(8), 945–61.

Knikou, M., & Rymer, Z. (2002). Effects of changes in hip joint angle on H-reflex excitability in humans. *Experimental Brain Research*, *143*(2), 149–59.

Kralj, A., Bajd, T., Turk, R., Krajnik, J., & Benko, H. (1983). Gait restoration in paraplegic patients: a feasibility demonstration using multichannel surface electrode FES. *Journal of Rehabilitation R&D / Veterans Administration, Department of Medicine and Surgery, Rehabilitation R&D Service*, *20*(1), 3–20.

Ladenbauer, J., Minassian, K., Hofstoetter, U. S., Dimitrijevic, M. R., & Rattay, F. (2010). Stimulation of the human lumbar spinal cord with implanted and surface electrodes: a computer simulation study. *IEEE Transactions on Neural Systems and Rehabilitation Engineering : A Publication of the IEEE Engineering in Medicine and Biology Society*, *18*(6), 637–45.

Lavoie, B. A., Devanne, H., & Capaday, C. (1997). Differential control of reciprocal inhibition during walking versus postural and voluntary motor tasks in humans. *Journal of Neurophysiology*, *78*(1), 429–38.

Liberson, W. T., Holmquest, H. J., Scot, D., & Dow, M. (1961). Functional electrotherapy: stimulation of the peroneal nerve synchronized with the swing phase of the gait of hemiplegic patients. *Archives of Physical Medicine and Rehabilitation*, *42*, 101–5.

Lloyd, D. P. C. (1943). Reflex action in relation to pattern and peripheral source of afferent stimulation. *Journal of Neurophysiology*, *6*(2), 111–19.

Mao, C. C., Ashby, P., Wang, M., & McCrea, D. (1984). Synaptic connections from large muscle afferents to the motoneurons of various leg muscles in man. *Experimental Brain Research*, *56*(2), 341–50.

Marchand-Pauvert, V., Nicolas, G., Burke, D., & Pierrot-Deseilligny, E. (2002). Suppression of the H reflex in humans by disynaptic autogenetic inhibitory pathways activated by the test volley. *The Journal of Physiology*, *542*(Pt 3), 963–76.

McCrea, D. A. (1986). Spinal cord circuitry and motor reflexes. *Exercise and Sport Sciences Reviews*, *14*, 105–41.

Meunier, S., & Pierrot-Deseilligny, E. (1998). Cortical control of presynaptic inhibition of Ia afferents in humans. *Experimental Brain Research*, *119*(4), 415–26.

Meunier, S., Pierrot-Deseilligny, E., & Simonetta, M. (1993). Pattern of monosynaptic heteronymous Ia connections in the human lower limb. *Experimental Brain Research*, *96*(3), 534–44.

Minassian, K., Hofstoetter, U. S., & Rattay, F. (2011). Transcutaneous Lumbar Posterior Root Stimulation for Motor Control Studies and Modification of Motor Activity after Spinal Cord Injury. In M. R. Dimitrijevic, B. A. Kakulas, W. B. McKay, & G. Vrbova (Eds.), Restorative Neurology of Spinal Cord Injury (pp. 226–255). Oxford University Press.

Minassian, K., Jilge, B., Rattay, F., Pinter, M. M., Binder, H., Gerstenbrand, F., & Dimitrijevic, M. R. (2004). Stepping-like movements in humans with complete spinal cord injury induced by epidural stimulation of the lumbar cord: electromyographic study of compound muscle action potentials. *Spinal Cord*, *42*(7), 401–16.

Minassian, K., Persy, I., Rattay, F., Dimitrijevic, M. R., Hofer, C., & Kern, H. (2007a). Posterior root-muscle reflexes elicited by transcutaneous stimulation of the human lumbosacral cord. *Muscle & Nerve*, *35*(3), 327–36.

Minassian, K., Persy, I., Rattay, F., Pinter, M. M., Kern, H., & Dimitrijevic, M. R. (2007b). Human lumbar cord circuitries can be activated by extrinsic tonic input to generate locomotor-like activity. *Human Movement Science*, *26*(2), 275–95.

Morin, C., Katz, R., Mazieres, L., & Pierrot-Deseilligny, E. (1982). Comparison of soleus H reflex facilitation at the onset of soleus contractions produced voluntarily and during the stance phase of human gait. *Neuroscience Letters*, *33*(1), 47–53.

Morita, H., Crone, C., Christenhuis, D., Petersen, N. T., & Nielsen, J. B. (2001). Modulation of presynaptic inhibition and disynaptic reciprocal Ia inhibition during voluntary movement in spasticity. *Brain : A Journal of Neurology*, *124*(Pt 4), 826–37.

Müller, R., & Dietz, V. (2006). Neuronal function in chronic spinal cord injury: divergence between locomotor and flexion- and H-reflex activity. *Clinical Neurophysiology : Official Journal of the International Federation of Clinical Neurophysiology*, *117*(7), 1499–507.

Nielsen, J., & Kagamihara, Y. (1993). The regulation of presynaptic inhibition during co-contraction of antagonistic muscles in man. *The Journal of Physiology*, *464*, 575–93.

NIH National Institute of Neurological Disorders and Strokes (2009, January 29). NINDS Foot Drop Information Page. Retrieved from

http://www.ninds.nih.gov/disorders/foot_drop/foot_drop.htm. Accessed 10.9.2014, 20:35.

Otto Bock ActiGait® (2014). Retrieved from http://www.ottobock.at/cps/rde/xchg/ob_com_en/hs.xsl/4762.html?id=9982#t9982. Accessed 10.9.2014, 20:47.

Pearson Education, Inc. (2011). Retrieved from http://889yoga.com/blog/wp-content/uploads/2013/07/reflex2.jpg. Accessed 4.9.2014, 11:01.

Petersen, P. C., Vestergaard, M., Jensen, K. H. R., & Berg, R. W. (2014). Premotor spinal network with balanced excitation and inhibition during motor patterns has high resilience to structural division. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *34*(8), 2774–84.

Pfützner, H. (2012). *Angewandte Biophysik*. Springer Vienna.

Pierrot-Deseilligny, E., & Burke, D. (2012). General Methodology. In *The Circuitry of the Human Spinal Cord: Spinal and Corticospinal Mechanisms of Movement* (pp. 1–64). Cambridge: Cambridge University Press.

Pierrot-Deseilligny, E., Bergego, C., Katz, R., & Morin, C. (1981). Cutaneous depression of Ib reflex pathways to motoneurones in man. *Experimental Brain Research*, *42*(3-4), 351–61.

Pinter, M. M., Gerstenbrand, F., & Dimitrijevic, M. R. (2000). Epidural electrical stimulation of posterior structures of the human lumbosacral cord: 3. Control of spasticity. *Spinal Cord*, *38*(9), 524–31.

Rattay, F. (1990). *Electrical Nerve Stimulation*. Springer Vienna.

Rattay, F., Minassian, K., & Dimitrijevic, M. R. (2000). Epidural electrical stimulation of posterior structures of the human lumbosacral cord: 2. quantitative analysis by computer modeling. *Spinal Cord*, *38*(8), 473–89.

Renshaw, B. (1941). Influence of discharge of motoneurones upon excitation of neighboring motoneurons. *Journal of Neurophysiology, 4*, 167–83.

Rothwell, J. C. (1987). *Control of Human Voluntary Movement*. Boston, MA: Springer US.

Rudomin, P., & Schmidt, R. F. (1999). Presynaptic inhibition in the vertebrate spinal cord revisited. *Experimental Brain Research*, *129*(1), 1–37.

Schieppati, M. (1987). The Hoffmann reflex: A means of assessing spinal reflex excitability and its descending control in man. *Progress in Neurobiology*, *28*(4), 345–76.

Schneider, C., Lavoie, B. A., & Capaday, C. (2000). On the origin of the soleus H-reflex modulation pattern during human walking and its task-dependent differences. *Journal of Neurophysiology*, *83*(5), 2881–90.

Sherrington CS. 1979. 1924 Linacre lecture. In: JC Eccles, WC Gibson (eds). Sherrington: His Life and Thought, p. 59. New York: Springer-Verlag.

Sherrington, C. S. (1906). *Integrative Actions of the Nervous System*. New Haven, CT: Yale University Press.

Stein, R. B., & Thompson, A. K. (2006). Muscle reflexes in motion: how, what, and why? *Exercise and Sport Sciences Reviews*, *34*(4), 145–53.

Sweeney, J., Mortimer, J., & Durand, D. (1987). Modeling of mammalian myelinated nerve for functional neuromuscular electrostimulation. *Proceedings of the 97th Annual Conference IEEE EMBS*, 1577–78.

UC San Diego (2014). https://health.ucsd.edu/specialties/neuro/specialty-programs/paralysis-center/PublishingImages/spinal%20cord%203.jpg Accessed 4.9.2014, 10:44.

Ulfhake, B., & Cullheim, S. (1988). Postnatal development of cat hind limb motoneurons. III: Changes in size of motoneurons supplying the triceps surae muscle. *The Journal of Comparative Neurology*, *278*(1), 103–20.

Vodovnik, L., Kralj, A., Stanic, U., Acimovic, R., & Gros, N. (n.d.). Recent applications of functional electrical stimulation to stroke patients in Ljubljana. *Clinical Orthopaedics and Related Research*, (131), 64–70.

Walløe, S., Nissen, U. V., Berg, R. W., Hounsgaard, J., & Pakkenberg, B. (2011). Stereological estimate of the total number of neurons in spinal segment D9 of the red-eared turtle. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *31*(7), 2431–5.

Weber, D. J., Stein, R. B., Chan, K. M., Loeb, G. E., Richmond, F. J. R., Rolf, R., James, K., Chong, S. L. (2004). BIONic WalkAide for correcting foot drop. *Conference Proceedings : Annual International Conference of the IEEE Engineering in Medicine and Biology Society. IEEE Engineering in Medicine and Biology Society. Annual Conference*, *6*, 4189–92.

Wikipedia (2014). http://en.wikipedia.org/wiki/File:Neuron_Hand-tuned.svg Accessed 4.9.2014, 9:32.

Windhorst, U. (1990). Activation of renshaw cells. *Progress in Neurobiology*, *35*(2), 135–79.

SOURCE CODE

1. Processing and analysis of raw data

```
clear all
close all

pathDATA = '/Users/'; %insert path
pathRESULTS = [pathDATA 'Results/'];

[~,~,~]=mkdir(pathRESULTS);

FName = ListDirectory_v1(pathDATA,'asc');       % ListDirectory_v1 -
selfwritten function

muscle=[' lQ';' lH';'lTA';'lTS';' rQ';' rH';'rTA';'rTS'];
NumOfChannels = 8;

NumOfFiles = length(FName);

SamplingRate = 10000;
SamplingTime = 1/SamplingRate;
PreTrigger_sec = 0.05;
NumOfSamples = 2500;

NumOfTrials = 11;   %Controls

time_sec = (0:NumOfSamples-1)/SamplingRate-PreTrigger_sec;
time_ms = time_sec*1000;

HeaderSizeFile = 12;

PTPofControl = [];
PTPofPNSStim = [];
prePTPofPNSStim = [];

%sorting of FName
FileIndex = [];
for c = 1:NumOfFiles
    if ~isempty(strfind(FName(c).name,'Control'))
        FileIndex = [ c FileIndex ];
```

```
    elseif ~isempty(strfind(FName(c).name,'PNStim'))
        FileIndex = [ FileIndex c ];
    end
end

for fIT = FileIndex

    Data = nan(NumOfSamples,NumOfChannels,NumOfTrials);

    txtFName = FName(fIT).name;
    if (isempty(strfind(txtFName,'TrigRec')))
       continue
    end

    disp(['-------> Start: ' txtFName '
************************************']);

    %% CHECK HEADER SIZE OF DATA-FILE
    fid = fopen([pathDATA txtFName]);  %Datei ?ffnen
    for i = 1:15
        tline = fgetl(fid);

        if i == HeaderSizeFile;
            if isempty(strfind(tline,'ime'))
                disp(['ERROR - wrong header size: (' tline ')'] );
                pause
            end
        end
    end
    fclose(fid);

    %% Read Data

    rawData = dlmread([pathDATA txtFName],';',HeaderSizeFile,0);
    rawData(:,11:end) = [];     % delete unused channels

    ind = strfind(FName(fIT).name,'ISI');
    inde = strfind(FName(fIT).name(ind+3:end),'m');
    ISI = str2double(FName(fIT).name(ind+3:ind+1+inde));    %delay of
tSCStim after PNStim (ms)
    if isnan(ISI)
        ISI = 0;
    end
    if ISI == 0, ISI = 20; end

    ind = strfind(FName(fIT).name,'PNS-');
    inde = strfind(FName(fIT).name(ind+4:end),'_');
    Subject = FName(fIT).name(ind+4:ind+2+inde);
    SubNum = str2num(Subject(3:4));

    %% Convert stream data to matrix form
    for i = 1:NumOfChannels
        % get data set for each channel
        D = rawData(:,i+1);   %first colum is time

        currentNumOfTrials = fix(length(D)/NumOfSamples);

        D = D(1:NumOfSamples*currentNumOfTrials);
```

```
Data(:,i,1:currentNumOfTrials)=reshape(D,NumOfSamples,currentNumOfTria
ls);

    end

    PTP = nan(NumOfTrials,NumOfChannels);

    tStart_sec = 0.010;
    tEnd_sec = 0.060;
    IntervalPTP =
(round((tStart_sec+PreTrigger_sec)*SamplingRate)):(round((tEnd_sec+Pre
Trigger_sec)*SamplingRate));

    for chIT = 1:NumOfChannels
        PTP(:,chIT) = max(Data(IntervalPTP,chIT,:))-
min(Data(IntervalPTP,chIT,:));
    end

    if ~isempty(strfind(txtFName,'Control'))
        meanPTP = nanmean(PTP);
        PTPofControl = [PTPofControl; SubNum ISI meanPTP];
    end


    if ~isempty(strfind(txtFName,'PNStim'))
        meanControl = PTPofControl(PTPofControl(:,1)==SubNum,2:end);
        getMeanControl =
@(ch,isi)interp1(meanControl(:,1),meanControl(:,ch+1),isi);

        for chIT = 1:8
            PTP(1:currentNumOfTrials,chIT) =
PTP(1:currentNumOfTrials,chIT)/getMeanControl(chIT,ISI);
        end

        tones = ones(currentNumOfTrials,1);
        prePTPofPNSStim = [prePTPofPNSStim;SubNum*tones ISI*tones
PTP(1:currentNumOfTrials,:)];
    end

end


ISI = unique(prePTPofPNSStim(:,2));
for isi = ISI'

 ind = (prePTPofPNSStim(:,2)==isi);

 meanPTP = nanmean(prePTPofPNSStim(ind,3:10));
 stdPTP = nanstd(prePTPofPNSStim(ind,3:10))/sqrt(sum(ind)); %standard
error
 tmp =[meanPTP,stdPTP];
 PTPofPNSStim = [PTPofPNSStim; isi tmp];
end

for plotit = 0:1
if plotit, figure(9); end
```

```
    Xtick = unique(PTPofPNSStim(:,1));
    Xrange = [min(Xtick)*.7,max(Xtick)*1.3];
    Yvec = PTPofPNSStim(:,2:9)+PTPofPNSStim(:,10:17);
    Yvec = sort(reshape(Yvec,size(Yvec,1)*size(Yvec,2),1));
    Ymax = Yvec(end);
    Yvec = PTPofPNSStim(:,2:9)-PTPofPNSStim(:,10:17);
    Yvec = sort(reshape(Yvec,size(Yvec,1)*size(Yvec,2),1));
    Ymin = Yvec(1);

    Yrange = [min(1,Ymin)*100-5 max(1,Ymax)*100+5];

    for chIT = 1:8
        if ~plotit, figure(chIT); end
        if plotit, subplot(2,4,chIT); end
        errorbar(PTPofPNSStim(:,1),PTPofPNSStim(:,chIT+1)*100,
PTPofPNSStim(:,chIT+9)*100,'.-r','Linewidth',1);

        set(gca,'XScale','log');

        hold on
        plot(Xtick([1 end]),[100 100],'-.b')
        hold off
        if ~plotit, set(gca,'Xtick',Xtick); else
set(gca,'Xtick',[100,1000]);end %displays only used ISIs
        set(gca,'Xlim',Xrange);
        set(gca,'Ylim',Yrange);
        title(muscle(chIT,:))
        fileName = muscle(chIT,:);
        fileName = fileName(fileName~=' ');
        if ~plotit, print('-r600','-dpng',[pathRESULTS,
fileName,'.png']);end
    end
    if plotit,print('-r600','-dpng',[pathRESULTS, 'PTP.png']);end
end
```

2. Function ‘ListDirectory_v1’

```
function [ listDIR ] = ListDirectory_v1( path, extention  )

    list = dir(path);

    if isempty(list)
        disp('ERROR @ ListDirectory_v1 -> wrong directory path')
        listDIR = [];
        return
    end


    del = [];
    for d = 1:length(list)
      if(strcmp(list(d).name,'.')),  del = [del d]; end
      if(strcmp(list(d).name,'..')), del = [del d]; end
    end
    list(del) = [];
```

```
    if exist('extention','var')
        if ischar(extention)
            del = [];
            for d = 1:length(list)
              if(~strcmpi(list(d).name(end-2:end),extention)),  del =
[del d]; end
            end
            list(del) = [];
        else
            disp('WARNING @ ListDirectory_v1 -> Extention must be a
string')
        end
    end

    listDIR = list;

end


%% EXAMPLE
%(1) ----------------------------------------
% ListDirectory_v1( 'D:\path' )
%
% ... list all files and directories in D:\path\
%
%(2) ----------------------------------------
% ListDirectory_v1( 'd:\path','exe')
%
% .... list all files *.exe in D:\path\
```

3. Analysis of control measurements

Controls before and after tests:

```
clear all
close all

pathEXP = {'EXP01 30Hz 0.8MT','EXP01 30Hz 1.2MT','EXP01 30Hz
1.5MT','EXP02 15Hz 1.2MT','EXP03 50Hz 1.2MT'};
prepathDATA = '/Users/'; %insert path

pathRESULTS = [prepathDATA 'Results_controls_exp/'];
%pathRESULTS = [prepathDATA 'Results_controls_sub/'];

[~,~,~]=mkdir(pathRESULTS);


muscle = [' lQ';' lH';'lTA';'lTS';' rQ';' rH';'rTA';'rTS'];
colour = ['rbgmck'];
subjects= {'KB01','CA02','CS03','TJ04','MW06'};
NumOfChannels = 8;
```

```
SamplingRate = 10000;
SamplingTime = 1/SamplingRate;
PreTrigger_sec = 0.05;
NumOfSamples = 2500;

Ymax = 0;
BetragDiff =[];

NumOfTrials = 11;   %Controls

time_sec = (0:NumOfSamples-1)/SamplingRate-PreTrigger_sec;
time_ms = time_sec*1000;

HeaderSizeFile = 12;


for expIT = 1:size(pathEXP,2)
    pathDATA = [prepathDATA pathEXP{expIT} '/']
FName = ListDirectory_v1(pathDATA,'asc');
NumOfFiles = length(FName);

count = 0;
MeanOfControls = [];

for fIT = 1:NumOfFiles

    Data = nan(NumOfSamples,NumOfChannels,NumOfTrials);

    txtFName = FName(fIT).name;
    if (isempty(strfind(txtFName,'TrigRec')))
       continue
    end

    if (isempty(strfind(txtFName,'Control')))
       continue
    end

    count = count+1;

    disp(['-------> Start: ' txtFName '
**************************************']);

    %% CHECK HEADER SIZE OF DATA-FILE
    fid = fopen([pathDATA txtFName]);
    for i = 1:15
        tline = fgetl(fid);

        if i == HeaderSizeFile;
            if isempty(strfind(tline,'ime'))
                disp(['ERROR - wrong header size: (' tline ')'] );
                pause
            end
        end
    end
    fclose(fid);
```

```
    %% Read Data

    rawData = dlmread([pathDATA txtFName],';',HeaderSizeFile,0);
    rawData(:,11:end) = [];

    ind = strfind(FName(fIT).name,'ISI');
    inde = strfind(FName(fIT).name(ind+3:end),'m');
    ISI = str2double(FName(fIT).name(ind+3:ind+1+inde));
    if isnan(ISI)
        ISI = 0;
    end

    %% Convert stream data to matrix form
    for i = 1:NumOfChannels
        D = rawData(:,i+1);
        currentNumOfTrials = fix(length(D)/NumOfSamples);
        D = D(1:NumOfSamples*currentNumOfTrials);

Data(:,i,1:currentNumOfTrials)=reshape(D,NumOfSamples,currentNumOfTria
ls);
    end

    Mean = nan(NumOfTrials,NumOfChannels);

    tStart_sec = 0.010;
    tEnd_sec = 0.060;
    IntervalPTP =
(round((tStart_sec+PreTrigger_sec)*SamplingRate)):(round((tEnd_sec+Pre
Trigger_sec)*SamplingRate));

    for chIT = 1:NumOfChannels
         Mean(:,chIT) = max(Data(IntervalPTP,chIT,:))-
min(Data(IntervalPTP,chIT,:));
    end

    MeanOfControls(ceil(count/2),(1:8)+(1-mod(count,2))*8) =
nanmean(Mean);

    for subIT = 1:size(subjects,2)
        if (~isempty(strfind(txtFName,subjects{subIT})))
        colourIT(ceil(count/2)) = subIT;
           break;
        end
    end

end

for plotit = 0:1
if plotit, figure(9); end

    for chIT = 1:8
        if ~plotit, figure(chIT); end
        if plotit, subplot(2,4,chIT); end

        hold on

        % Subjects colour coded
%         for plotIT = 1:size(MeanOfControls,1)
```

```
%             plot([10 90]', [MeanOfControls(plotIT,chIT)
MeanOfControls(plotIT,chIT+8)]', colour(colourIT(plotIT)));
%         end

        % Experiments colour coded
        plot(repmat([10
90],size(MeanOfControls,1),1)',[MeanOfControls(:,chIT)
MeanOfControls(:,chIT+8)]',colour(expIT))

        hold off

        set(gca,'XTickLabel',{'before','after'},'Xtick',[10,90])
        ylim ([0 inf]);
        title(muscle(chIT,:))

        fileName = muscle(chIT,:);
        fileName = fileName(fileName~=' ');
        if ~plotit, print('-r600','-dpng',[pathRESULTS,
fileName,'.png']);end
    end

    if plotit,print('-r600','-dpng',[pathRESULTS, 'ALL.png']);end

end
BetragDiff = [BetragDiff;abs(MeanOfControls(:,1:8)-
MeanOfControls(:,9:16))];
end

fID = fopen([pathRESULTS, 'statistik.txt'],'w');
fprintf(fID,'Min:  %f %f %f %f %f %f %f %f\n', min(BetragDiff,[],1));
fprintf(fID,'Max:  %f %f %f %f %f %f %f %f\n', max(BetragDiff,[],1));
fprintf(fID,'Mean: %f %f %f %f %f %f %f %f\n', nanmean(BetragDiff));
fprintf(fID,'Std:  %f %f %f %f %f %f %f %f\n', nanstd(BetragDiff));
fprintf(fID,'#Ueber alles\n');

[Bn, Bm] = size(BetragDiff);
fprintf(fID,'Min:  %f\n', min(min(BetragDiff)));
fprintf(fID,'Max:  %f\n', max(max(BetragDiff)));
fprintf(fID,'Mean: %f\n', nanmean(reshape(BetragDiff,Bn*Bm,1)));
fprintf(fID,'Std:  %f\n', nanstd(reshape(BetragDiff,Bn*Bm,1)));

fclose(fID);
```

Coefficient of Variation

```
clear all
close all

pathEXP = {'EXP01 30Hz 0.8MT','EXP01 30Hz 1.2MT','EXP01 30Hz
1.5MT','EXP02 15Hz 1.2MT','EXP03 50Hz 1.2MT'};
prepathDATA = '/Users/'; %insert path

pathRESULTS = [prepathDATA 'Results_normalised_std_exp/'];
%pathRESULTS = [prepathDATA 'Results_normalised_std_sub/'];
```

```
[~,~,~]=mkdir(pathRESULTS);

Ymax = 0;
BetragDiff = [];

muscle=[' lQ';' lH';'lTA';'lTS';' rQ';' rH';'rTA';'rTS'];
colour=['rbgmck'];
subjects={'KB01','CA02','CS03','TJ04','MW06'};
NumOfChannels = 8;


SamplingRate = 10000;
SamplingTime = 1/SamplingRate;
PreTrigger_sec = 0.05;
NumOfSamples = 2500;

NumOfTrials = 11;

time_sec = (0:NumOfSamples-1)/SamplingRate-PreTrigger_sec;
time_ms = time_sec*1000;

HeaderSizeFile = 12;


for expIT = 1:size(pathEXP,2)
    pathDATA = [prepathDATA pathEXP{expIT} '/']
FName = ListDirectory_v1(pathDATA,'asc');


count = 0;
MeanOfControls = [];

for fIT = 1:NumOfFiles

    Data = nan(NumOfSamples,NumOfChannels,NumOfTrials);

    txtFName = FName(fIT).name;
    if (isempty(strfind(txtFName,'TrigRec')))
       continue
    end

    if (isempty(strfind(txtFName,'Control')))
       continue
    end

    count = count+1;

    disp(['-------> Start: ' txtFName '
**************************************']);

    %% CHECK HEADER SIZE OF DATA-FILE
    fid = fopen([pathDATA txtFName]);  %Datei ?ffnen
    for i = 1:15
        tline = fgetl(fid);
%         if i == 3,
%             tmpHour = tline(34); Hour = (str2num(tmpHour)+12)*100;
%             tmpMin = tline(36:37); Min = str2num(tmpMin);
```

```
%             Time = Hour+Min; txtTime = int2str(Time);
%         end

        if i == HeaderSizeFile;
            if isempty(strfind(tline,'ime'))
                disp(['ERROR - wrong header size: (' tline ')'] );
                pause
            end
        end
    end
    fclose(fid);

    %% Read Data

    rawData = dlmread([pathDATA txtFName],';',HeaderSizeFile,0);
    rawData(:,11:end) = [];

    ind = strfind(FName(fIT).name,'ISI');
    inde = strfind(FName(fIT).name(ind+3:end),'m');
    ISI = str2double(FName(fIT).name(ind+3:ind+1+inde));
    if isnan(ISI)
        ISI = 0;
    end

    %% Convert stream data to matrix form
    for i = 1:NumOfChannels
        D = rawData(:,i+1);

        currentNumOfTrials = fix(length(D)/NumOfSamples);
        D = D(1:NumOfSamples*currentNumOfTrials); %eine Spalte aus dem
Datensatz

Data(:,i,1:currentNumOfTrials)=reshape(D,NumOfSamples,currentNumOfTria
ls);

    end

    Mean = nan(NumOfTrials,NumOfChannels);

    tStart_sec = 0.010;
    tEnd_sec = 0.060;
    IntervalPTP =
(round((tStart_sec+PreTrigger_sec)*SamplingRate)):(round((tEnd_sec+Pre
Trigger_sec)*SamplingRate));

    for chIT = 1:NumOfChannels
         Mean(:,chIT) = max(Data(IntervalPTP,chIT,:))-
min(Data(IntervalPTP,chIT,:));
    end

    MeanOfControls(ceil(count/2),(1:8)+(1-mod(count,2))*8) =
nanstd(Mean./repmat(nanmean(Mean),size(Mean,1),1));

    for subIT = 1:size(subjects,2)
        if (~isempty(strfind(txtFName,subjects{subIT})))
        colourIT(ceil(count/2)) = subIT;
           break;
        end
```

```
    end

end

Ymax = max(max(max(MeanOfControls)),Ymax);

for plotit = 0:1
if plotit, figure(9); end

    for chIT = 1:8
        if ~plotit, figure(chIT); end
        if plotit, subplot(2,4,chIT); end
        hold on

        % Subjects colour coded
%         for plotIT = 1:size(MeanOfControls,1)
%             plot([10 90]', [MeanOfControls(plotIT,chIT)
MeanOfControls(plotIT,chIT+8)]', colour(colourIT(plotIT)));
%         end

        % Experiments colour coded
        plot(repmat([10
90],size(MeanOfControls,1),1)',[MeanOfControls(:,chIT)
MeanOfControls(:,chIT+8)]',colour(expIT))

        hold off
        set(gca,'XTickLabel',{'before','after'},'Xtick',[10,90])
        set(gca,'Ylim',[0 Ymax*1.01]);
        title(muscle(chIT,:))
        fileName = muscle(chIT,:);
        fileName = fileName(fileName~=' ');
        if ~plotit, print('-r600','-dpng',[pathRESULTS,
fileName,'.png']);end
    end
    if plotit,print('-r600','-dpng',[pathRESULTS, 'ALL.png']);end

end

BetragDiff =
[BetragDiff;MeanOfControls(:,1:8);MeanOfControls(:,9:16)];
end

fID = fopen([pathRESULTS, 'statistik.txt'],'w');
fprintf(fID,'Min:  %f %f %f %f %f %f %f %f\n', min(BetragDiff,[],1));
fprintf(fID,'Max:  %f %f %f %f %f %f %f %f\n', max(BetragDiff,[],1));
fprintf(fID,'Mean: %f %f %f %f %f %f %f %f\n', nanmean(BetragDiff));
fprintf(fID,'Std:  %f %f %f %f %f %f %f %f\n', nanstd(BetragDiff));
fprintf(fID,'#All\n');

[Bn, Bm] = size(BetragDiff);
fprintf(fID,'Min:  %f\n', min(min(BetragDiff)));
fprintf(fID,'Max:  %f\n', max(max(BetragDiff)));
fprintf(fID,'Mean: %f\n', nanmean(reshape(BetragDiff,Bn*Bm,1)));
fprintf(fID,'Std:  %f\n', nanstd(reshape(BetragDiff,Bn*Bm,1)));

fclose(fID);
```