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Table of content

Table of content I

List of Abbreviations III

Chapter I: General Introduction 1

1.1. Aim of this work 2

1.2. Neural degeneration 2

1.3. Neural plasticity 4

1.4. Interventions 5
1.4.1. Transcutaneous Electrical Nerve Stimulation (TENS) 5
1.4.2. Transcranial Direct Current Stimulation (tDCS) 7

1.5. Electrical current applications in patients and healthy subjects 9

1.6. Evaluation of regional brain functionality and neural plasticity 9

1.7. Evaluation sensorimotor functionality 10

1.8. Overview of the studies 10

1.9. References 14

Chapter II: Experimental work and results 23

Objectives 24

STUDY 1: The effect of long-term TENS on persistent neuroplastic changes in the human cerebral cortex 25

STUDY 2: Long-term TENS treatment improves tactile sensitivity in MS patients 49

STUDY 3: Long-term TENS treatment decreases cortical motor representation in multiple sclerosis 65

STUDY 4: Anodal TDCS increases corticospinal output and projection strength in multiple sclerosis 81

STUDY 5: A single session of 1mA anodal TDCS-supported motor training does not improve motor performance in patients with multiple sclerosis 97

STUDY 6: Is motor learning mediated by TDCS intensity? 115

Chapter III: General discussion and conclusions 127

3.1. Long-term TENS stimulation induces long-lasting functional and neuroplastic changes in MS 128

3.2. Evaluation of tDCS as an adjuvant therapy for MS rehabilitation 132

3.3. Limitations and future perspectives 134
3.3.1. Limitations of the current studies 134
3.3.2. Future perspectives 135

3.4. Conclusions 136

Chapter IV: Nederlandstalige samenvatting 143

Curriculum Vitae 147

Bibliography 148

Dankwoord 153

List of Abbreviations

ADM:	Abductor Digiti Minimi
ANOVA:	Analysis of Variance
APB:	Abductor Pollicis Brevis
atDCS:	Anodal Transcranial Direct Current Stimulation
AURC:	Area Under Recruitment Curve
BDNF:	Brain Derived Neurotrophic Factor
CoG:	Center of gravity
CS:	Corticospinal
ECR:	Extensor Carpi Radialis
EDSS:	Expanded Disability Status Scale
EMG:	Electromyography
FCR:	Flexor Carpi Radialis
FDI:	First Dorsal Interosseous
GABA:	Gamma-Aminobutyric Acid
ITI:	Inter Tap Interval
LTD:	Long-Term Depression
LTP:	Long-Term Potentiation
M1:	Primary Motor Cortex
MoCa:	Montreal Cognitive Assessment
MS:	Multiple Sclerosis
rMT:	Resting Motor Threshold
stDCS:	Sham Transcranial Direct Current Stimulation
tDCS:	Transcranial Direct Current Stimulation
TENS:	Transcutaneous Electrical Nerve Stimulation
TMS:	Transcranial Magnetic Stimulation
VAS:	Visual Analogue Scale

Chapter I:

General Introduction

1.1. Aim of this work

Neurorehabilitation is a complex process aiming at functional recovery from neural degeneration or injury. Besides the behavioral changes resulting from recovery, underlying processes of neural plasticity occur at the level of the central and in the peripheral nervous system. This work aims to unveil underlying neuroplastic mechanisms, necessary to develop and evaluate novel rehabilitation therapies focusing on sensorimotor recovery in patients with neurodegeneration. Therefore, functional effects as well as underlying mechanisms of peripheral [transcutaneous electrical nerve stimulation (TENS)] and central [transcranial direct current stimulation (tDCS)] electrical current applications were evaluated. Although a number of studies already investigated some aspects of these interventions, little is known about the effect of manipulating intervention parameters (amplitude, frequency, duration, etc.). Some basic mechanisms underlying these interventions still remain unclear. Until now, the application of interventions such as TENS and tDCS in the rehabilitation of multiple sclerosis (MS) patients has been sparse even though the implementation of these promising therapeutic interventions as an adjuvant therapy might optimize sensorimotor recovery in this population. Since electrical signal transfer between central and peripheral regions is disturbed, these interventions, which have a direct impact on the central and peripheral nervous system, might restore electrical signal transfer.

1.2 Neural degeneration

Neural degeneration is characterized by the loss of structure and functional activity of neurons. Many diseases such as MS, Parkinson's disease, Alzheimer and even neural changes in healthy aging occur as a result of neurodegenerative processes.

Although MS is primarily a central inflammatory disease, neural degeneration in MS is characterized by random, multifocal demyelination limited to the central nervous system in which the myelin sheaths around the axons located in the brain and spinal cord are damaged. Consequently, signal transfer between the central and peripheral regions is disturbed (Schmierer et al., 2000) leading to a variety of symptoms including visual disturbances, cognitive impairment, reduced tactile sensitivity, muscle weakness, spasticity, fatigue, coordination loss and balance problems. As compared to other neurodegenerative diseases such as amyotrophic lateral sclerosis, Parkinson's disease and stroke, which show rather focal lesions, the lesions in MS are distributed (Dutta and Trapp, 2006), resulting in a complex pathophysiology. This might complicate the application of

rehabilitation protocols since it's not always possible to identify which cortical areas or network are impaired due to MS.

Evidence gathered with neuroimaging techniques (such as magnetic resonance imaging, MRI) and brain stimulation techniques (such as transcranial magnetic stimulation, TMS) has shown significant differences in brain structure and functionality in MS patients as compared to healthy subjects. While MRI provides information about the localization of brain activity, TMS is mainly used to study changes in excitability (facilitation vs. inhibition) and motor conduction time with a resolution of milliseconds. Nonetheless there are many other techniques, which can be used to study brain processes. Some main (f)MRI and TMS findings will be discussed next to illustrate the mechanisms of neural degeneration and neural plasticity in MS.

Findings from functional MRI (fMRI) studies reported increased cortical activation patterns in MS patients while performing a simple motor task. In patients with minimal signs of MS increased activity was primarily reported in the contralateral and ipsilateral motor areas with respect to motor tasks (Lee et al., 2000, Rocca et al., 2003). This increased brain activity could be explained by manifestation of adaptive or compensatory mechanisms that allow normal performance despite neural damage or loss (Pantano et al., 2006). Furthermore, patients with primary progressive MS even activated areas that do not belong to the classical motor network (Filippi et al., 2002). Importantly, the extent of increased motor activation correlated with damage of both the brain tissue (Lee et al., 2000, Reddy et al., 2000, Rocca et al., 2002) and the corticospinal tract (Pantano et al., 2002).

TMS studies have reported deficits in corticospinal (CS) conduction in MS, resulting in prolonged central motor conduction time and reduced motor evoked potential (MEP) amplitudes. These pathology-related declines correlated significantly with demyelination, axonal loss (Hess et al., 1986, Hess et al., 1987, Caramia et al., 1988, Jones et al., 1991, Ravnborg et al., 1992) and with the expanded disability scale score (EDSS) (Salle et al., 1992, Conte et al., 2009, Kale et al., 2010). Furthermore, MEP abnormalities are usually present in muscles that show clinical weakness (Di Lazzaro et al., 1999).

As described above MRI, TMS and other neuroimaging techniques have the ability to unveil processes of functional reorganization and neural plasticity. In the next paragraph, the concept of neural plasticity will be explained, followed by interventions capable of inducing neuroplastic changes.

1.3. Neural plasticity

Pioneering experiments performed by Hubel and Wiesel have illustrated the concept of neural plasticity (Hubel and Wiesel, 1962, 1964, 1970). In their work with kittens, one eye was visually deprived by closure of the eyelid and at the same time cortical brain maps were recorded. Interestingly, Hubel and Wiesel found that the cortical area corresponding to the closed eye processed visual information of the open eye, indicating that the brain had found a way to re-wire itself.

Currently, the concept of neural plasticity refers to changes in neural pathways and synapses, which are due to changes in behavior, environmental and neural processes, as well as changes resulting from brain injury (Pascual-Leone et al., 2011). Anatomical changes, neurochemical and metabolic changes, and removal/emergence of connections are the main mechanisms responsible for neuroplastic changes. Anatomical changes occur when the structure of a neuron changes, for example, when an axon forms new connections with other pathways in the nervous system. These changes can then lead to strengthening of existing connections and/or lead to recovery of damaged neural pathways. Neurochemical and metabolic changes involve, for example, changes in the production of neurotransmitters and uptake of nutrients by the brain. Removal of connections can occur when neural pathways have not been used across shorter and larger time frames, for example after immobilization or amputation of a limb. Neurophysiological processes such as long-term potentiation (LTP) and long-term depression (LTD) often play a role in regulating synaptic plasticity and more specifically in manipulating synaptic strength. Whereas LTP results in activity-dependent increase in the efficacy of neuronal synapses, LTD reduces it. At the cellular level, these processes are likely to be driven by N-Methyl-D-aspartate (NMDA) receptor dependent calcium influx, activating kinases such as calcium/calmodulin-dependent protein kinase II (CaMKII) and protein kinase A (PKA). LTP is then established either by trafficking new α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors to activated synapses or by acting on the biophysical properties of existing AMPA receptors. Conversely, a smaller calcium influx through NMDA receptors can lead to LTD via the opposite mechanisms (Crair and Shah, 2009).

Throughout the 20th century the brain was considered a static organ, which was immutable after the critical period during infancy. Currently, a wide body of research indicates that the structure of the central nervous system is constantly changing as a consequence of different kinds of experiences (Sanes and Donoghue, 2000, Pascual-Leone et al., 2005, Dayan and Cohen, 2011, Caroni et al., 2012). In patients with neurodegenerative disease, rehabilitation influences recovery of neural function and promotes functional reorganization in the brain. Besides the intensity and the amount of training embedded in the intervention program, an early start of the

program following brain injury are crucial factors for successful rehabilitation (Teasell et al., 2005). Alongside the traditional rehabilitation program (medication intake, physiotherapy, etc.), electrotherapeutic interventions can be used to optimize the rehabilitation process in different neurodegenerative diseases (Hummel et al., 2005, Boggio et al., 2006, Fregni et al., 2006, Celnik et al., 2007, Conforto et al., 2010, Lefebvre et al., 2012, Cho et al., 2013). Until now, the effects of these interventions on the rehabilitation process remain mainly unclear. More specifically, the combination of different intervention parameters (current strength, duration, frequency, waveform, etc.) might play an important role. Whereas non-invasive stimulation techniques are commonly used in neurodegenerative diseases such as stroke and Parkinson's disease, the application of these techniques is almost not explored in MS. As mentioned earlier, the focality of the lesions in MS is less pronounced (Dutta and Trapp, 2006) as compared to other neurodegenerative diseases. Consequently, it is possible that central or peripheral stimulation is not or less effective due to widespread damage of other (non-stimulated) regions, which might also be involved in determining the rehabilitation outcome. Next, the interventions that have been used to promote neural plasticity and sensorimotor rehabilitation in MS will be described in detail.

1.4. Interventions

1.4.1.Transcutaneous Electrical Nerve Stimulation (TENS)

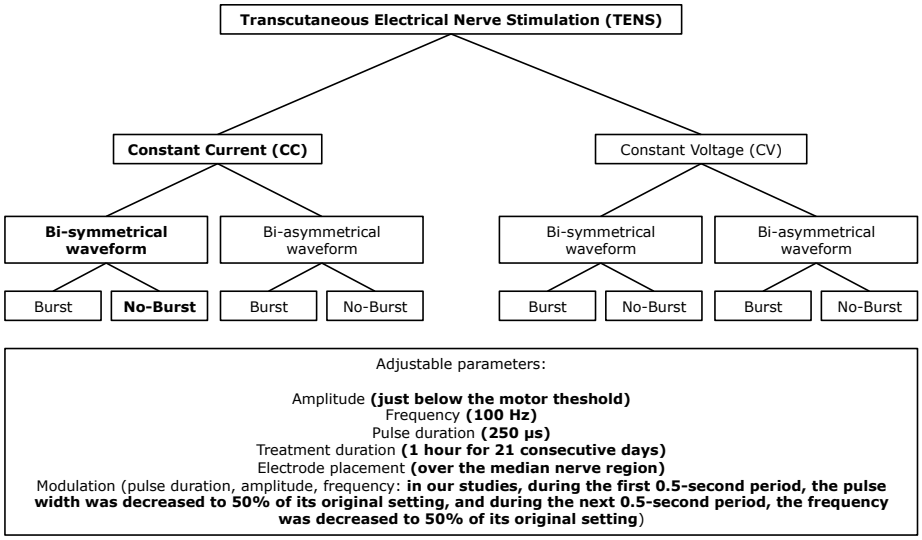
TENS is a non-invasive, simple-to-use, save and cheap therapeutic intervention. The stimulator consists of a hand held device that is used to transmit low electrical currents to the skin trough a pair (or more) of electrodes. TENS has been mainly used as a treatment for acute and chronic pain (Nnoaham and Kumbang, 2008, Walsh et al., 2009, Dubinsky and Miyasaki, 2010, Hurlow et al., 2012). Currently, TENS is also being used for other purposes such as bladder and bowel dysfunction (Monga et al., 2012, Rawashdeh et al., 2012), spasticity (Armutlu et al., 2003), motor recovery in stroke (Ng and Hui-Chan, 2007, Laufer and Elboim-Gabyzon, 2011), writer's cramp (Tinazzi et al., 2006), fibromyalgia (Gur, 2006), and as an adjuvant therapy for other pathologies.

The combination of parameters used for TENS stimulation are quite extensive as shown in the scheme below. These combinations resulted in several commonly used modalities. The two most commonly used modalities in clinical practice are high frequency, low intensity (conventional) TENS and low frequency, high intensity (acupuncture-like) TENS (Jones and Johnson, 2009).

Until now, the exact mechanisms underlying the effects of TENS are mainly unclear. The most known TENS theory is the gate control theory of pain (Melzack and Wall, 1965). According to this theory, signal transfer by nociceptive C and Aδ fibers (represented in the dorsal horn of the spinal cord) to the cortex, can be modulated by stimulating large diameter Aβ afferents with TENS, leading to pain inhibition. More specifically, a pain stimulus in a peripheral nociceptive neuron is transferred to the dorsal horn via the primary afferent neuron. The dorsal horn consists of grey matter (lamina I-VI), represented in the spinal cord. Lamina II is called the substantia gelatinosa (SG). Aδ fibers innervate lamina I-III of the SG, while C- fibers innervate lamina I and II. The SG functions as a gatekeeper and consists of short inhibitory interneurons projecting to lamina I and V, which then project to the thalamus. The SG regulates signal transfer at the first synapse (between the primary afferent neurons and the spinothalamic tract) of the nocioceptive pathway. SG hyperpolarizes the spinothalamic tract neurons. Primary afferent neurons (C and Aδ) inhibit the SG, while large afferent neurons (Aα and Aβ) can activate the SG.

Animal research provides evidence that TENS can lead to reorganization of substantia gelatinosa (SG) interneurons (Nakatsuka et al., 1999). Moreover these changes mainly occurred in the superficial dorsal horn neurons (lamina II) (Kohama et al., 2000). Furthermore, Nakatsuka et al. (1999) found that SG neurons in inflamed animals received more direct Aβ-afferent inputs compared to those in healthy animals. Another animal study revealed that high frequency TENS increased the release of the neurotransmitter gamma-aminobutyric acid (GABA) in the deep dorsal horn of the spinal cord (Maeda et al., 2007).

The therapeutic effects of TENS applications depend on the combination of different parameters such as stimulation location, waveform, current frequency, stimulation frequency, intensity and pulse width. In general, a single session of conventional TENS seems to induce (cortical) inhibition of the stimulated muscle (Tinazzi et al., 2005, Tinazzi et al., 2006), while for acupuncture-like TENS the opposite effects (facilitation) are reported (Hamdy et al., 1998, Ridding et al., 2000, Fraser et al., 2002). Additionally, stimulation intensity also seems to play a crucial role in modulating excitability of the corticomotor pathway of the stimulated muscles (Chipchase et al., 2011). There is a trend towards increased excitability when intensity is above motor threshold, whereas opposite findings are reported for stimulation intensities below the motor threshold, but sufficient to induce sensory perception (Chipchase et al., 2011).



Note: Parameters marked in bold represent the parameters included in our TENS protocol, that was similar throughout the experiments described in this work.

1.4.2.Transcranial direct current stimulation (tDCS)

tDCS is a non-invasive electrical stimulation technique applying very weak direct currents to the human brain. A battery-driven constant current stimulator delivers electrical current, which is applied to the scalp using (saline soaked sponge) electrodes. tDCS is able to modulate cortical excitability in a polarity dependent manner (Nitsche and Paulus, 2000). More specifically, anodal tDCS is able to increase excitability, whereas cathodal tDCS can diminish excitability. The after-effects of tDCS depend mainly on the stimulation duration. A single session of tDCS can elicit changes in excitability up to 90 minutes (Nitsche and Paulus, 2001). Although many mechanisms underlying tDCS remain still unclear, tDCS is presumed to strengthen synaptic connections through a mechanism similar to long-term potentiation (LTP), a cellular mechanism that underlies learning (Cheeran et al., 2008, Stagg and Nitsche, 2011). Recently, tDCS has been used to improve learning and performance on a variety of cognitive and motor tasks in healthy humans subjects (Nitsche and Paulus, 2000, 2001, Nitsche et al., 2005, Stagg and Nitsche, 2011) and to enhance motor recovery in patients suffering from various neurological diseases such as stroke (Hummel et al., 2005, Hummel et al., 2006, Tanaka et al., 2011) and Parkinson's disease (Fregni et al., 2006).

In this work only atDCS over the primary motor cortex (M1) was applied. This choice was based on the beneficial of atDCS reported in previous work. Moreover, as stated earlier, it was shown that a single session of anodal tDCS (atDCS) over the primary motor cortex (M1) was sufficient to significantly improve motor performance, reaction time (Fregni

et al., 2006, Hummel et al., 2006), pinch force (Hummel et al., 2006, Tanaka et al., 2011), motor control (Hummel et al., 2005, Madhavan et al., 2011), and motor learning (Galea and Celnik, 2009, Fritsch et al., 2010, Tecchio et al., 2010). Nonetheless, the mechanisms underlying tDCS are mainly unclear, recent work is slowly gaining insights in these mechanisms. Previous findings revealed that the effects of tDCS are mainly intracortical (Nitsche and Paulus, 2000, 2001, Nitsche et al., 2003b) and relatively local (Miranda et al., 2006). As in MS signal transfer is disturbed due to demyelination of efferent and afferent pathways, it might be possible that intracortical tDCS modulation will not be sufficient/efficient in modulating motor performance. Moreover, potential motor improvement as a result of atDCS might be cancelled out by sensory deficits. TMS studies revealed abnormalities with respect to central motor conduction time in MS (Mills and Murray, 1985, Kandler et al., 1991). Therefore, it might be hypothesised that the efficacy of tDCS might depend on the severity of these conduction abnormalities. It is also noteworthy that these abnormalities correlated significantly with disability (Schmierer et al., 2000, Thickbroom et al., 2005).

Nonetheless evidence (Nitsche and Paulus, 2000, 2001) showed that atDCS is capable of increasing corticospinal excitability by polarizing neurons beneath the electrodes, very little is known about exactly which neurons are modulated.

According to an early study of Purpura and McMurtry (1965), different cortical neurons are targeted depending on the current intensity (density). Moreover, the authors suggested that nonpyramidal neurons are targeted by low suprathreshold intensities (current density: 4-8 mA/cm^2), while pyramidal neurons are modulated when stronger intensities (current density:10-20 mA/cm^2) are applied (Purpura and McMurtry, 1965). Recent evidence revealed that subthreshold electric field therapies (current density: 0.029 -0.8 mA /m^2), such as tDCS, preferentially polarize layer V (pyramidal cells) cell somas (Radman et al., 2009).

Previously it is reported that the (after)effects of tDCS mainly depend on membrane depolarization (Nitsche et al., 2003a). For atDCS, in particularly, modulation of GABAergic and glutamatergic synapses play a role in the modulation of neuroplasticity (Nitsche et al., 2005). Furthermore, TMS measurements applying short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF) protocols, indicate that these modulations are localized in the intracortical interneurons within the cortex. In addition, these neuromodulatory effects are modulated by the catecholamines acetylcholine (Kuo et al., 2007) and serotonin (Nitsche et al., 2009).

1.5. Electrical current applications in patients and healthy subjects

As we are aware of the fact that many patients with MS suffer from symptoms such as fatigue, reduced mobility, pain, depression etc, we have tried to optimize our experiments by minimizing the effort to participate in the experiments for this vulnerable population. Therefore, experimental procedures were mostly applied in healthy subjects first. There are two reasons for this rationale: Firstly, from a therapeutic perspective it is not (always) appropriate to evaluate experimental therapies in patient populations without knowing the effects and effort required to carry out the therapy. Secondly, to unravel the underlying mechanisms of disease, it might be appropriate to evaluate the therapy in both healthy subjects and patient populations. It is possible that a therapy that has proven its effectiveness in patients is not effective in healthy subjects or vice versa because of ceiling effects (in sensory and/or motor function) or because of differences in neurological 'hardware' resulting from disease.

1.6. Evaluation of regional brain functionality and neural plasticity

In this work, transcranial magnetic stimulation (TMS) was applied to evaluate regional brain functionality and neural plasticity. During TMS a brief high-current pulse is produced inside an insulated coil of wire held over the scalp. The electrical pulse then induces a rapidly changing magnetic field with lines of flux running perpendicular to the coil. When TMS is applied to the primary motor cortex (M1) and the pulse intensity is above the motor threshold, a muscle response called 'motor evoked potential (MEP)' can be recorded by electromyographical (EMG) recordings. The size of the MEP is assumed to reflect the number of recruited motor neurons and can be used to evaluate corticospinal (CS) excitability changes in healthy subjects and patients with neurodegenerative disease. Throughout this work we used two TMS methods for evaluating regional brain functionality and neural plasticity: recruitment curves (measuring CS excitability) and TMS mapping (measuring representation of the CS projection). Recruitment curves measure MEP amplitudes at a range of intensities (Devanne et al., 1997, Ridding and Rothwell, 1997, Carroll et al., 2001). For hand muscles this measurement results in a sigmoidal curve with a steeply rising slope and a plateau (Devanne et al., 1997). The slope is related to the number of CS neurons activated at different intensities (Ridding and Rothwell, 1997) and depends on the strength of the CS projection relative to the target muscle (Abbruzzese et al., 1999). During TMS mapping, MEPs at various scalp sites are measured resulting in a 'motor map' (Thickbroom et al., 1999). The motor output can be quantified by map area (number of excitable scalp positions) and map volume (MEP amplitudes at all excitable points).

Additionally, the hotspot (point of maximum response) and the centre of gravity (amplitude-weighted centre of the map) provide an estimation of the centre or most excitable region of the map (Thickbroom et al., 1999).

1.7. Evaluation of sensorimotor functionality

Sensory (Semmes-Weinstein monofilaments) and motor tests (9-hole pegtest, unimanual sequence task) were performed to evaluate the initial status and/or the effect of the intervention over time.

1.8. Overview of the studies

The main goal of this work is to investigate and evaluate non-invasive electrotherapeutic interventions, the underlying mechanisms and their functional outcome in neurodegeneration. Below, the different studies that were conducted are introduced in more detail.

1.8.1. Study 1: The effect of long-term TENS on persistent neuroplastic changes in the human cerebral cortex

This study was based on results of unpublished data of our group. Moreover, the effects of 3 long-term therapies (active training, tendon vibration and TENS) and a control condition on reorganization of the motor cortex (using TMS mapping) were evaluated in healthy subjects. These specific therapies were chosen because they are commonly used by physical therapists for nerve and/or muscle rehabilitation. Our pilot findings revealed that TENS was able to induce enlargements in cortical motor maps and that these enlargements were not restricted to the stimulated muscle but also extended to other neighboring muscles. The effects of tendon vibration on cortical reorganization were restricted to the stimulated muscle. In both, the active training and the control condition, no significant changes in cortical reorganization were reported. These observations were of primary interest as they point to the potential of TENS in (re)shaping 'global' somatosensory networks. Based on this pilot data, a new study evaluating the effects of TENS was carried-out. Moreover, the long-term application of TENS (3 weeks, one hour a day) on the reorganization of corticomotor representations was evaluated in healthy adults. Confronto et al. (2010) already revealed that multiple sessions of peripheral nerve stimulation can facilitate training effects on motor function after subacute stroke, however the mechanisms underlying these effects were unclear (Conforto et al.,

2010). Changes in motor cortex representations were assessed by means of TMS mapping. Besides mapping the motor representations of the stimulated muscle, other hand and forearm muscles were also mapped in order to evaluate the focality of the intervention. The results of study 1 revealed that long-term TENS leads to increased and long-lasting corticomotor representations of both the stimulated and adjacent muscles in healthy subjects.

Note: The data-collection of this study was already completed before the start of my PhD. However, I was involved in the data-analysis and writing/reviewing of the manuscript.

1.8.2. Study 2: Long-term TENS treatment improves tactile sensitivity in MS patients

Because long-term TENS has shown to reorganize cortical representations in healthy subjects (study 1), we hypothesized that this intervention might lead to changes in tactile sensitivity in MS patients suffering from sensory deficits. Therefore, the effect of the same long-term TENS intervention on tactile sensitivity was evaluated immediately after the intervention and 3 weeks later at follow-up. Because the previously reported TENS-induced changes in corticomotor representation in healthy subjects were not restricted to the stimulation site, sensitivity measurements were not only assessed at the stimulated region, but also at adjacent regions. Our results showed that long-term TENS was able to ameliorate tactile sensitivity in MS patients suffering from sensory deficits. These improvements were not restricted to the stimulated region but were also found in adjacent regions. In addition, long-term beneficial after-effects (i.e. increased sensitivity for at least 3 weeks after the end of the intervention) were reported.

1.8.3. Study 3: Long-term TENS treatment decreases cortical motor representation in multiple sclerosis

In this study the mechanisms underlying long-term TENS application in MS-patients were evaluated using a TMS mapping protocol. The hypothesis was that TENS would lead to enlarged corticomotor representation as was also reported in healthy subjects (study 1). In contrast, our results showed a decrease in corticomotor representation of the stimulated muscle. This finding was rather unexpected, but might be explained by several factors underlying the disease. However the most reasonable explanation might be that the observed reduction of corticomotor representation, following the long-term somatosensory intervention, may have been due to the formation of new inhibitory connections that were

previously impaired by the MS pathology. Similar studies combined with administration of pharmacological substances are recommended to get further insight and possibly confirmation of these findings.

1.8.4. Study 4: Anodal tDCS increases corticospinal output and projection strength in multiple sclerosis

Recently, tDCS has been shown to be capable of eliciting corticospinal (CS) excitability changes, leading to enhanced motor recovery in patients suffering from various neurological diseases such as stroke and Parkinson's disease. Since the effect of tDCS on CS excitability has not yet been evaluated in patients suffering from multiple sclerosis, the present study aimed to evaluate the effect of a single session (20 min) of tDCS in MS patients. Our results revealed that a single session of anodal tDCS was able to enhance CS output and projection strength in MS patients.

1.8.5. Study 5: A single session of 1mA anodal tDCS-supported motor learning does not improve motor performance in patients with multiple sclerosis.

Whereas study 4 showed that a single session of anodal tDCS was able to enhance CS output and projection strength in MS patients, it is not yet known if these changes are paralleled by training-induced changes in motor performance. In this experiment, patients were asked to practice a unimanual motor (sequence) learning task. In a double blind crossover design, motor learning was either combined with 1mA anodal or sham tDCS over the motor cortex for 20 minutes. Based on beneficial results reported in healthy subjects, stroke patients and other neurodegererative populations, we hypothezised that anodal tDCS-supported motor training was superior to sham tDCS-supported motor training. However, our results revealed no significant differences between both training conditions. It might be possible that mechanisms underlying the disease prevent the expression of tDCS-induced motor performance or that a single session or the implemented current intensity is not sufficient to induce neuroplastic changes required for improving motor performance.

1.8.6. Study 6: Is motor learning mediated by tDCS intensity?

Although tDCS has been shown to improve motor learning, previous studies reported rather small effects. Since physiological effects of tDCS seem to depend on intensity, the present study evaluated this parameter in order to enhance the effect of tDCS on skill acquisition. The effect of

different stimulation intensities of anodal tDCS (atDCS) was investigated in a double blind, sham controlled crossover design. In each condition, thirteen healthy subjects were instructed to perform a unimanual motor (sequence) learning task. Our results showed (1) a significant increase in the slope of the learning curve and (2) a significant improvement in motor performance at retention for 1.5mA atDCS as compared to sham tDCS. No significant differences were reported between 1mA atDCS and sham tDCS; and between 1.5mA atDCS and 1mA atDCS. Although statistical power in this study was low, our findings indirectly indicate that tDCS intensity plays a crucial role in obtaining the desired result.

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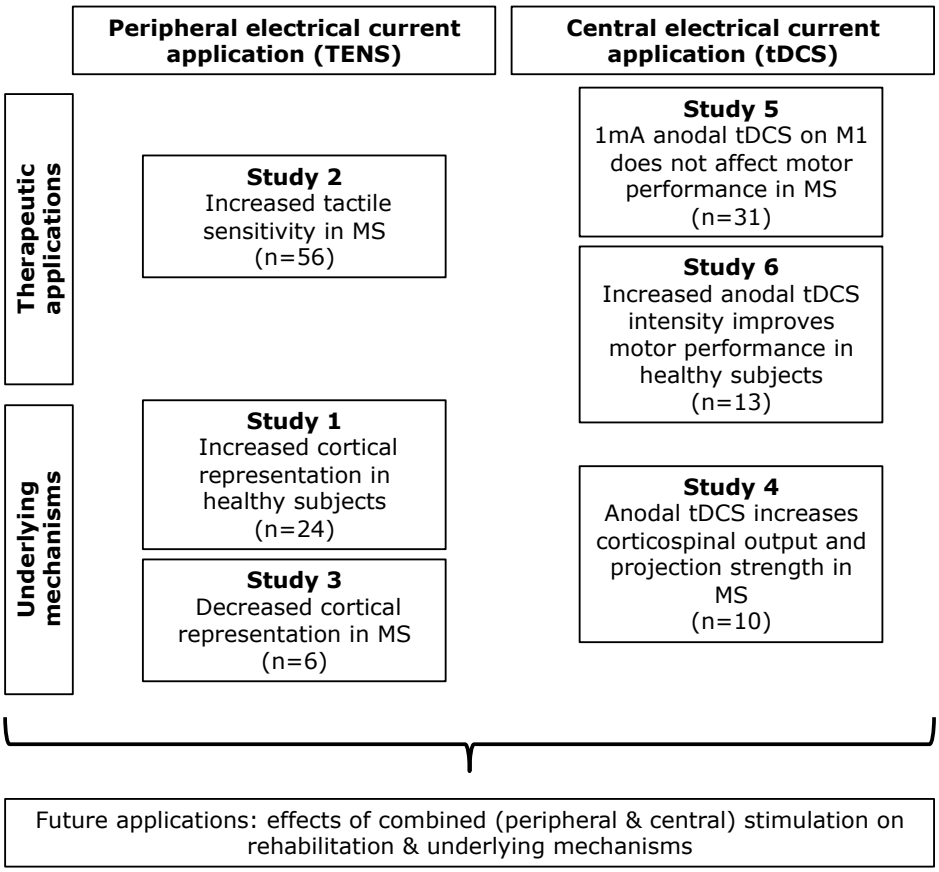
Chapter II:

Experimental work and results

Objectives

The studies performed in this work aimed to evaluate the effects of peripheral or central electrical current applications on neural plasticity and sensorimotor functions in healthy subjects as well as in patients with MS and to unravel the underlying mechanisms (neural plasticity) after application of these therapies. The studies carried out are presented in the form of six original research papers that have been published, submitted or are in preparation for publication to peer-reviewed journals with a focus on fundamental neuroscience or clinical and/or neurological rehabilitation.



Schematic presentation of the objectives of the performed studies

STUDY 1

The effect of long-term TENS on persistent neuroplastic changes in the human cerebral cortex

Adapted from: R.L.J. Meesen, K. Cuypers, J.C. Rothwell, S.P. Swinnen, & O. Levin

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2.1.1. Abstract

The long-term effect of daily somatosensory stimulation with transcutaneous electrical nerve stimulation (TENS) on reorganization of the motor cortex was investigated in a group of neurologically intact humans.

The scalp representation of the corticospinal projection to the finger (APB, ADM) and forearm (FCR, ECR) muscles was mapped by means of transcranial magnetic stimulation (TMS) before and after a 3 week intervention period, using map area and volume, and topographical overlaps between the cortical motor representations of these muscles as primary dependent measures.

Findings revealed a significant increase in cortical motor representation of all four muscles for the TENS group from pre- to post-test (all, $p \leq 0.026$). No significant changes in cortical motor representations were observed in the control group.

The present observations highlight the potential benefit of sensory training by means of TENS as a useful complementary therapy in neurorehabilitation.

2.1.2. Introduction

Somatosensory stimulation activates large parts of the motor and sensory networks, both in the contralateral and ipsilateral hemispheres (Deletis et al. 1992;Nudo et al. 1996;Nelles et al. 1999;Naito et al. 1999;Matteis et al. 2003;Radovanovic et al. 2002;Duclos et al. 2007). For example, medical imaging techniques have shown activation in contralateral sensorimotor cortex, inferior parietal cortex (bilateral), ipsilateral cerebellum and (medial) premotor areas (Carel et al. 2000;Naito et al. 1999;Nelles et al. 1999). This activity reflects the anatomical connectivity of the stimulated M1/S1, known to possess direct monosynaptic connections with all of the aforementioned areas (Classen et al. 1998;Nelles et al. 2001).

The effect of somatosensory stimulation on corticomotor excitability has been explored in humans by using transcranial magnetic stimulation (TMS) during and/or after the application of e.g., electrical nerve stimulation (Fraser et al. 2002;Hamdy et al. 1998;Ridding et al. 2001), muscle-tendon vibration (Rosenkranz and Rothwell 2003;Steyvers et al. 2003a;Steyvers et al. 2003b), cyclical passive movement (Lewis and Byblow 2004;Mace et al. 2008) or temporary deafferentation (Ziemann et al. 1996). These observations have recently been linked with the emergence of lasting facilitation or depression of excitability of neuronal populations in the primary motor cortex (Forner-Cordero et al. 2008;Mace et al. 2008;Rosenkranz and Rothwell 2006;Rosenkranz et al. 2008;Steyvers et al. 2003a). Yet, the long-lasting changes in corticomotor excitability induced by direct activation of Ia afferent pathways via passive movement or muscle vibration were somewhat more feeble and/or focal (Lewis and Byblow 2004;Mace et al. 2008) as compared to those scenarios in which facilitation (or depression) in excitability of the corticospinal projections to targeted musculature were monitored following training with electrical nerve stimulation (Fraser et al. 2002;Hamdy et al. 1998;Ridding et al. 2001;Tinazzi et al. 2005).

Electrical nerve stimulation in general, and transcutaneous electrical nerve stimulation (TENS) in particular have been applied successfully in neurorehabilitation, such as in the treatment of stroke (Levin and Hui-Chan 1992;Ng and Hui-Chan 2007;Sonde et al. 1998), urinary symptoms (Skeil and Thorpe 2001), spinal cord injury (Fung and Barbeau 1994;Goulet et al. 1996), multiple sclerosis (Armutlu et al. 2003;Miller et al. 2007), writer's cramp (Tinazzi et al. 2006) and/or to reduce movement disorders caused by tremor, myoclonia or dystonia (Bending and Cleeves 1990;Foley-Nolan et al. 1990;Toglia and Izzo 1985). This body of literature suggests that daily training with TENS may effectively activate larger parts of the underlying distributed sensorimotor networks of the brain. Studies that have evaluated the long-term effects of TENS on the reorganization of corticomotor representations are sparse. Nonetheless, a recent study revealed that

multiple sessions of peripheral nerve stimulation can facilitate training effects on motor function after subacute stroke (Conforto et al, 2010).

The present study aimed to reveal the effects of a daily somatosensory stimulation with TENS over the right abductor pollicis brevis (APB) muscle on cortical representations of hand and forearm muscles in healthy volunteers (Fig. 1). Based on the known connectivity between primary sensory and motor cortical areas (Quartarone et al. 2003;Zarzecki et al. 1978), we hypothesized positive effects of the sensory intervention on the motor map representation(s) after 3 weeks of daily training. More specifically, as a result of the existence of (1) wide-spread connectivity between e.g., the median and ulnar nerves (Kimura et al. 1983) and/or (2) simultaneous activation of primary and secondary afferent networks (Quartarone et al. 2006), we hypothesized that TENS applied to the APB would induce a global effect on hand (APB, ADM) and forearm (FCR, ECR) representations. Changes in motor cortex representations were assessed by means of a TMS mapping protocol, examining differences in loci and size of the cortical motor maps of the hand and forearm musculature (Thickbroom et al. 1999;Wassermann et al. 1992;Wilson et al. 1996). This technique has been used previously to assess changes in corticomotor representations of upper/lower limb musculature following limb amputation (Cohen et al. 1991;Fuhr et al. 1992) or specific skill learning (Pascual-Leone et al. 1993;Pascual-Leone et al. 1994).

2.1.3. Methods

2.1.3.1. Subjects

Participants were twenty-four healthy and neurologically intact right-handed volunteers (10 males, 14 females, mean age 27.4, SD 14.8, range 18-54 years). They were naive about the purpose of the experiment, were screened for potential risk of adverse events during TMS (Wassermann 1998), and provided written informed consent prior to participation. Handedness was determined by the Edinburgh Handedness Inventory (Oldfield 1971). The experimental procedures were approved by the local Ethics Committee for Biomedical Research at Katholieke Universiteit Leuven, according to the Declaration of Helsinki.

2.1.3.2. Experimental Procedure

Participants were randomly assigned to two groups (n=12 each): transcutaneous electrical nerve stimulation (TENS), and control (CONTROL). All subjects underwent two TMS mapping sessions, i.e., prior to the start of (at day 1) and following the intervention (at day 22). The intervention consisted of daily sessions over a period of 21 days. Participants in the control group preserved their normal daily activities without any intervention.

TENS (biphasic symmetrical rectangular pulse-wave at 100 Hz, 250μs pulse width) was delivered for a total duration of 60 minutes per day to the right APB muscle, using a constant current stimulator (Intelect Digitens, Chattanooga Group, Hixson TN USA). Self-adhesive electrodes (dura Stick II, 1.5 × 4 cm) were placed on the belly of the right abductor pollicis brevis (APB) and their locations were marked over the skin with a surgical pen to preserve locations across the daily sessions. Stimulus intensity was set at a sensory threshold just below motor threshold, producing a continuous tingling sensation in the stimulated area without any visible muscle twitch or pain.

2.1.3.3. Transcranial Magnetic Stimulation

TMS mapping of the right abductor pollicis brevis (APB), abductor digiti minimi (ADM), flexor carpi radialis (FCR) and extensor carpi radialis longus (ECR) was done by means of a magnetic stimulator (Dantec MagLite r-25, Medtronic, Skovlunde, Denmark). Single-pulse magnetic stimuli were delivered with a figure-of-eight coil (MC-B70 magnetic coil transducer; outer radius diameter: 50 mm; maximal output: 1.5 Tesla). For each subject a

customised cap was made out of thermoplastic material (Aquaplast-T Solid, 20cm x 15cm x 0.24cm, Sammons Preston Polyon, Cedarburg, USA) with references to anatomical landmarks (left and right external auditory meatus and occiput) to ensure reproducibility of measurements across the experiment. An orthogonal coordinate system referenced to the vertex was marked on each cap. The coil was positioned tangentially to the scalp over the subjects' left hemisphere with the coil handle pointing backward and rotated 45º away from the midsagital line (Fig. 2).

The optimal location (hotspot) for eliciting motor evoked potentials (MEPs) from the right APB, i.e., the target muscle, was identified and marked with a soft-tip pen to ensure reproducibility of coil positioning in each participant. In all cases, a response of the ADM, FCR and ECR was also evoked in this position. Next, the rest motor threshold (rMT) was determined at the optimal scalp positions of the APB, ADM, FCR and ECR. For all muscles, the rMT was defined as the lowest intensity of magnetic stimulation required to evoke MEPs larger than 50 μV amplitude in at least five out of ten trials in the relaxed muscle. The stimulation intensity for mapping was then set at 120 % of the APB rMT. This intensity was preserved during both the pre and post mapping sessions. A grid of 225 (15 x 15) positions, spaced 1 cm along both the medio-lateral and antero-posterior axes, was marked on each cap. In producing maps, single TMS pulses were applied in 1 cm-steps in a clockwise spiral course, beginning at the hotspot of the APB muscle (Fig. 1). Each position was stimulated 8 times (interstimulus interval: 5-8 s, at random) before moving to the adjacent grid point, until the border of the motor maps of all four muscles was defined.

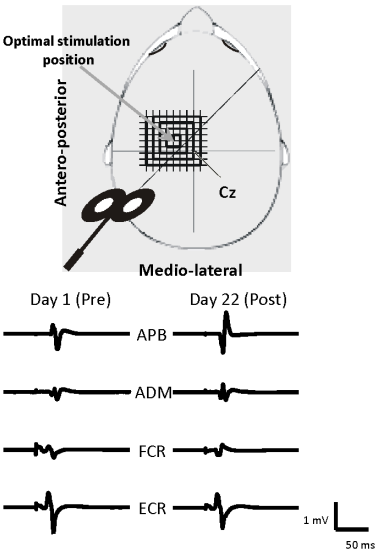

Figure 1. Illustration of the TMS mapping procedure.

2.1.3.4. EMG recordings

Electromyographic (EMG) signals from the APB, ADM, FCR and ECR of the right upper limb were collected by means of disposable, self-adhesive disc electrodes (Nutrode, Ag-AgCl sensor with Hydrogel, 35 mm diameter, GE Medical Systems Accessories Europe, Nanterre Cedex, France). For each subject, specific electrode placement for each of the four muscles was photographed and saved in a database. Skin surface was carefully shaved and degreased prior to electrode placement to achieve optimal conduction. The electrodes were additionally fixed to the skin with tape

to ensure maximal contact (Leukopor, skin-friendly, non-woven tape, 1.25 cm, BSN Medical GmbH and Co, Hamburg, Germany). After amplification (gain = 1000) and bandpass filtering (4-1500 Hz) (MEGA MESPEC 8000, Finland), the recorded EMG signals were digitized at 5000 Hz (CED Signal Version 3.03, Cambridge Electronic Design, Cambridge, UK) and were stored on a laboratory computer for offline analysis. Data collection was initiated 50 ms prior to the delivery of TMS and lasted 150 ms. EMG activity from all four muscles was continuously monitored online during the mapping session. Background EMG activity was minimized by continuous online EMG monitoring during the experiment, as well as by offline analysis of the trials.

2.1.3.5. Data Analysis

The size of the APB, ADM, FCR and ECR MEPs was measured offline by calculating the peak-to-peak amplitude of each waveform in a time window from 10 to 40 ms after the TMS stimulation pulse onset. The number of active positions in each map was determined as points whose stimulation evoked a mean MEP in the target muscles with a peak-to-peak amplitude of at least 100 μV. 3D representations of mean motor outputs for the four muscles were then composed by linear interpolation of the mean MEP amplitudes between adjacent stimulation positions (Matlab 6.5, MathWorks, Inc.).

For both pre and post mapping sessions, the cortical motor representation of the APB, ADM, FCR and ECR was defined as the number of stimulus positions whose stimulation evoked a mean MEP in the target muscle with a peak-to-peak amplitude of at least 100 μV (= 'active' stimulation positions). The peak-to-peak amplitudes obtained at the same stimulation site were averaged and normalized by their respective mean MEP amplitudes at the hotspot. The motor representation area of each muscle was defined as the number of stimulus positions whose stimulation evoked a mean MEP in the target muscle with a magnitude of at least 10 % of its respective normalized peak. Map area referred to the contour, whereas Map volume referred to the sum of the mean amplitudes at all active stimulation positions. The center of gravity (CoG) was computed separately for each muscle as a measure of the amplitude-weighted centre of the motor representational map (Wassermann et al. 1992). It is expressed as a bivariate measurement with a mediolateral (x) and anteroposterior coordinate (y), using the following formula: $CoG = [\Sigma a_i x_i/\Sigma a_i, \Sigma a_i y_i/\Sigma a_i]$, for stimulation position coordinates x_i, y_i and amplitudes a_i. The magnitude of the CoG displacement vector, i.e. the Euclidean distance between the CoG locations at day 1 (pre intervention mapping session) and day 22 (post intervention mapping session) was calculated.

2.1.3.6. Statistics

Advanced linear models applications (STATISTICA 6.0, StatSoft Inc.) were used for statistical analysis. Effects of interventions on corticospinal excitability and corticomotor representation were studied using the following dependent measures: mean MEP amplitude at optimal stimulation position (hotspot), location of hotspot, mean map area and mean map volume, map overlaps and the displacement of the map center of gravity (CoG). A 2 × 2 × 4 (GROUP × TIME × MUSCLE) repeated measures analysis of variance (ANOVA) was used with GROUP (TENS, CONTROL) as between-subjects factor, and TIME (PRE POST mapping session) and MUSCLE (APB, ADM, FCR, ECR) as within-subjects factors. Differences in topographical overlaps between cortical representations of the target (APB) and non-target (ADM, FCR and ECR) muscles in the pre and post mapping sessions were tested using a 2 × 2 ×6 ANOVA with the factors GROUP (two levels), TIME (two levels) and MAP OVERLAP (six levels: APB-ADM, APB-FCR, APB-ECR, ADM-FCR, ADM-ECR, FCR-ECR). T-tests were used to estimate whether potential changes in map area, volume or map overlaps differed significantly from zero. The magnitude of displacement of the estimated CoG positions along the anteroposterior and mediolateral axes were statistically compared using a 2 × 4 (GROUP × MUSCLE) ANOVA, to determine CoG stability between pre and post mapping sessions. The level of significance was set at $p < 0.05$.

2.1.4. Results

2.1.4.1. MEP amplitude at hotspot

Three weeks of TENS did not affect the size of MEPs at the hotspots, suggesting that levels of corticospinal excitability at hotspot remained unchanged (Table 1). Specifically, the 2 × 2 × 4 (GROUP × TIME × MUSCLE) ANOVA revealed a significant main effect for MUSCLE ($p < 0.01$) but the remaining main effects and the two and three factor interactions were not significant (all: $p > 0.2$).

Table I: Mean (± SD) magnitudes of the MEPs (mV) in the pre and post intervention mapping sessions for the TENS and CONTROL groups.

	TENS		CONTROL	
	Pre	Post	Pre	Post
APB	1.81 ± 0.39	1.83 ± 0.40	2.10 ± 0.39	2.14 ± 0.40
ADM	1.13 ± 0.24	1.51 ± 0.30	0.96 ± 0.24	0.71 ± 0.30
FCR	0.73± 0.10	0.69 ± 0.12	0.39± 0.10	0.40 ± 0.12
ECR	1.26 ± 0.25	1.15 ± 0.23	0.74 ± 0.25	0.60 ± 0.23

2.1.4.2. Location of the hotspot

The location of the hotspots did not change significantly between pre and post mapping sessions (all muscles: $p > 0.08$).

2.1.4.3. Map Area and Map Volume

Examples of individual maps are illustrated in Fig. 2a (TENS) and 2b (CONTROL). Changes in mean map area and volume of the representations of the target (APB) and non-target muscles (i.e., ADM, FCR and ECR) from pre- to post-test are demonstrated in Fig. 3.

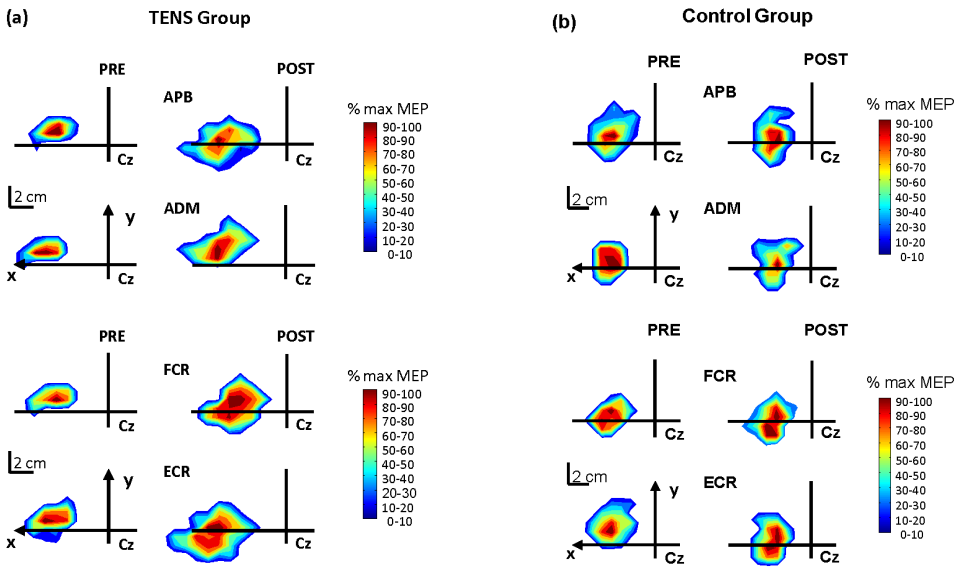

Figure 2. Representative map areas of the ABP, ADM, FCR and ECR muscles before (Pre – left hand column) and after 3 weeks (Post – right hand column) of sensory intervention with TENS **(a)** as compared to control **(b)**.

Overall, changes in the group mean areas (Fig. 3a) and volumes (Fig. 3b) between pre and post mapping sessions were observed for all four muscles in the TENS group. The control group did not reveal any changes. The aforementioned observations were largely confirmed by the 2 × 2 × 4 (GROUP × TIME × MUSCLE) ANOVA, as discussed next.

With respect to map area, the main effects for TIME ($p < 0.01$) and GROUP ($p < 0.05$) and the GROUP × TIME interaction ($p < 0.01$) were significant. The main effect for MUSCLE ($p = 0.079$) and the remaining two and three factor interactions (all: $p > 0.5$) were not significant, suggesting that all four muscles present a similar increase in the representation of their map areas after the intervention. This finding enabled us to explore the significant GROUP × TIME interaction in detail. Specifically, pair-wise post-hoc tests (Tukey HSD) revealed a significant increase of map area size in POST ($M = 25.0$ cm^2) relative to PRE ($M = 14.9$ cm^2) mapping session in the TENS group ($p = 0.0003$) while map area sizes in the CONTROL group were statistically identical: $M = 14.5$ cm^2 (PRE) vs. $M = 15.2$ cm^2 (POST) ($p > 0.9$).

Across group effects were also significant: TENS-POST vs. CONTROL PRE/POST (both, $p < 0.005$). Finally, t-tests for single means (TENS group: POST-PRE comparison to zero) showed that the map area of the target (APB) and the three non-target muscles (ADM, FCR and ECR) was significantly increased: APB (69 ± 15 % increase POST vs. PRE, $p = 0.001$), FCR (73 ± 21 % increase, $p = 0.007$), ECR (89 ± 27 % increase, $p = 0.008$) and ADM

(54 ± 21 %, p = 0.026). As expected, no consistent change in map areas between pre and post mapping sessions were observed in the CONTROL group (all muscles, p > 0.3).

With respect to map volume, effects were largely similar but the main effect for GROUP (p > 0.1) was not significant. Again, t-tests for single means showed that the map volume in the TENS group was significantly increased in all four muscles: APB (55 ± 12 %, p = 0.001), ADM (46 ± 18 %, p = 0.026), FCR (91 ± 29 %, p = 0.010) and ECR (97 ± 26 %, p = 0.004).

In summary, participants in the TENS group showed a markedly increased map area and volume in all four muscles.

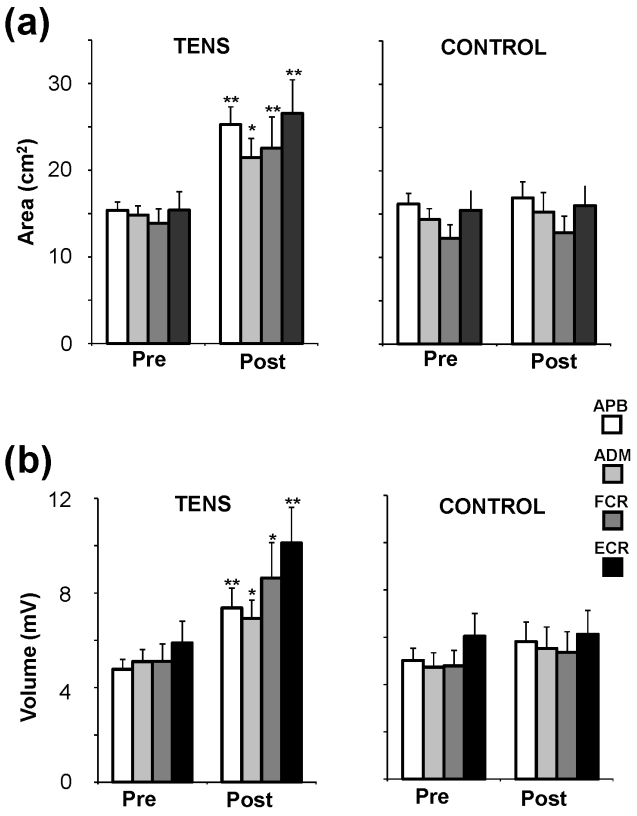

Figure 3. Group data showing motor representation area and volume of the four muscles at Pre and Post mapping sessions; *p < 0.05 and ** p < 0.01 compared to the Pre –values.

2.1.4.4. Overlaps between muscle cortical representations

Representative examples of topographical overlaps between cortical representations of the target (APB) and non-target (ADM, FCR and ECR) muscles in the pre and post mapping sessions are illustrated for two individuals (TENS and CONTROL) in Fig. 4.

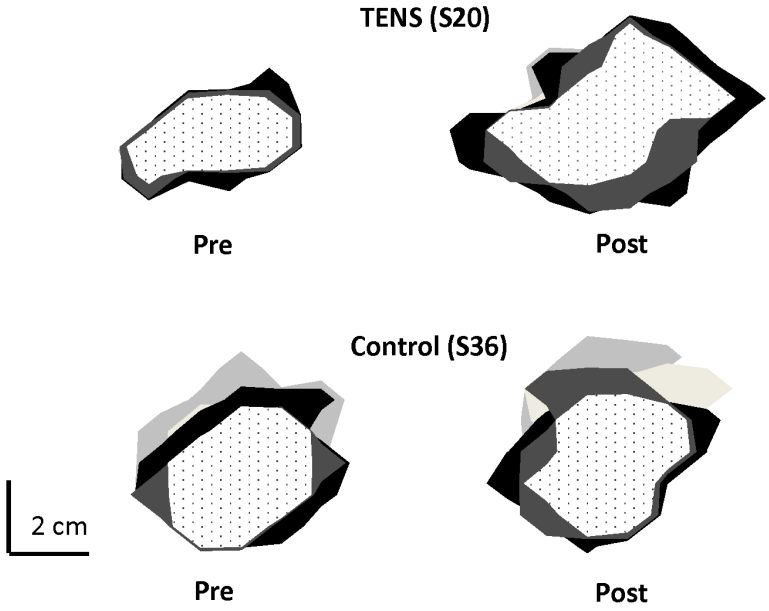

Figure 4. Representative map areas of the ABP (light-gray), ADM (intermediate gray), FCR (dark gray) and ECR (black) and their overlaps (dotted area) before (Pre – left hand column) and after 3 weeks (Post – right hand column) of sensory intervention with TENS versus control.

The 2 × 2 × 6 ANOVA revealed significant main effects for TIME ($p < 0.01$) and GROUP ($p < 0.05$) and a significant GROUP × TIME interaction ($p < 0.01$. The main effect for MAP OVERLAP ($p > 0.1$) and the remaining two and three-factor interactions were not significant (all: $p > 0.3$). Pair-wise post-hoc test (Tukey HSD) on the significant GROUP × TIME interaction revealed a significant increase of map overlap size in the POST ($M = 19.5$ cm^2) relative to PRE ($M = 12.6$ cm^2) mapping session in the TENS group ($p = 0.0008$) while no enlargements in map overlaps were noticed for the CONTROL group: $M = 11.3$ cm^2 (PRE) vs. $M = 11.7$ cm^2 (POST) ($p > 0.9$). Across group effects were also significant: TENS-POST vs. CONTROL PRE/POST (both, $p < 0.01$). Yet, further inspection of our data showed that

changes in topographical overlaps of the hand (i.e., APB, and ADM) and forearm (i.e., FCR and ECR) musculature after three weeks intervention with TENS were more pronounced for some muscles than others. Specifically, significant enlargements in map overlaps were noticed for the representations of the APB, ADM and ECR (TENS Group, contrast analyses for TIME (Tukey HSD); all, $p \leq 0.034$) but not for the aforementioned three muscles with FCR (all, $p > 0.06$). Group means (± SD) are shown in Table 2.

Table II. The effect of training with TENS on overlaps between cortical representations of the target muscle (APB) and the three non-target muscles (ADM, FCR, ECR). Data are expressed as group mean ± standard deviation (SD) for pre (baseline) and post intervention mapping sessions. * $p < 0.05$ and ** $p < 0.01$, Post- compared to Pre –values NS = not significant

Cortical representations	*Innervation*	*Pre* (cm^2)	*Post* (cm^2)
APB ∩ ADM	median/deep branch of ulnar (APB) & ulnar (ADM)	12.0 (1.9)	18.3 (4.8)*
APB ∩ FCR	median (APB & FCR) & deep branch of the ulnar (APB)	11.7 (4.2)	17.5 (8.7)NS
APB ∩ ECR	median/deep branch of ulnar (APB) & radial (ECR)	13.0 (3.8)	22.0 (8.0)**
ADM ∩ FCR	ulnar (ADM) & median (FCR)	11.8 (4.1)	17.1 (8.6)NS
ADM ∩ ECR	ulnar (ADM) & radial (FCR)	13.1 (3.7)	22.6 (11.2)**
FCR ∩ ECR	median (FCR) & radial (ECR)	13.9 (3.7)	19.3 (11.2)NS
APB ∩ ADM ∩ FCR ∩ ECR	median (APB & FCR), ulnar (APB & ADM) and radial (ECR)	9.8 (3.6)	12.8 (7.8)NS

Further, t-tests for single means (TENS group: POST-PRE comparison to 0) indicated that TENS intervention significantly increased the overlaps among cortical maps in the post as compared to the pre mapping session between the representations of the APB and ADM (PRE vs. POST, 52 % increase; $p = 0.024$), APB and ECR (PRE vs. POST, 70 % increase; $p=0.002$) and ADM and ECR (PRE vs. POST, 73 % increase; $p = 0.015$). Changes in size of overlaps between the FCR and the two finger muscles or FCR and ECR were either marginally significant or did not reach significance (all, $p > 0.05$). An increase in the size of the topographical overlap of the four cortical maps was also visible and reached marginal significance ($p = 0.052$).

In summary, in addition to a noticeably increased map area in all four muscles, the TENS intervention was also shown to increase the topographical overlaps between cortical representations of the finger and forearm muscles. However, this effect was more prominent for the APB, ADM and ECR than FCR.

2.1.4.5. Displacement of the center of gravity (COG)

The CoGs of the motor maps did not change between pre and post mapping sessions (all muscles: $p > 0.1$).

2.1.5. Discussion

In the present study we investigated the effect of a somatosensory stimulation intervention with TENS across a 3 week period on the cortical motor representations in the intact human brain. Our observations demonstrate for the first time that the size of motor map representations of APB, ADM, FCR and ECR muscles increased after TENS applied to the median nerve territory over the APB muscle.

The absolute size and spatial location of the map representation depend on factors such as coil orientation (Wilson et al. 1993;Wilson et al. 1996), current spread (Roth et al. 1991), coil distance (Thickbroom et al. 1998), and the excitability of the underlying corticospinal projection (Ridding and Rothwell 1997). All these parameters were held constant in the present study. Even so, all muscles underwent a considerable increase (> 50 %) in the area of their cortical motor representation in the absence of changes in excitability of the most excitable zone ("hotspot") or the CoG of the motor maps. This suggests that TENS led to a change in the distribution of excitability in the motor output zones to each muscle, with the largest effects in the least excitable peripheral areas. This led to marked changes in the topographical overlaps between cortical representations of the target (APB) muscle and two of the three remaining muscles (i.e., ADM and ECR).

2.1.5.1. Modulation of cortical motor representation

Our data, showing enlargement of the borders of cortical maps of the hand and forearm muscles, are in agreement with the findings of previous studies regarding the immediate post-stimulation effects with electrical nerve stimulation (Hamdy et al. 1998;Ridding and Rothwell 1997;Ridding et al. 2001) or muscle vibration (Forner-Cordero et al. 2008), as well as with the long-term effects induced by specific skill learning (Pascual-Leone et al. 1995) or constraint-induced movement therapy in stroke patients (Liepert et al. 2000). Such changes are usually considered to reflect an expansion of the cortical motor representation of the muscles being investigated (Pascual-Leone et al. 1993;Pascual-Leone et al. 1995;Ridding et al. 2001;Thickbroom et al. 1999); for review see (Siebner and Rothwell 2003). However, increased motor map expansion does not necessarily signify a true expansion of the underlying cortical projection zone (Ridding et al. 2001). For example, it has been demonstrated that an increase in the excitability of corticospinal tracts at the original projection would equally lead to an increase in motor map area (e.g., Ridding and Rothwell 1997). Nevertheless, further inspection of our data qualifies this possibility. First, the primary increase of the cortical motor map representations of the APB, ADM, FCR and ECR in the post-intervention mapping session was not accompanied by underlying changes in the excitability of corticospinal projections to those

muscles. Specifically, we found neither significant enlargements nor reductions in the amplitudes of MEP at the hotspot between the pre and post mapping sessions. This finding contrasts with the increases in excitability of cortico-cortical and corticospinal circuitry, such as seen after electrical nerve stimulation (Fraser et al. 2002;Hamdy et al. 1998;Ridding and Rothwell 1997). However, these rarely last more than 2h after the end of the intervention and often show a slight depression 24h post intervention (Ridding et al. 2001). Second, we observed no enlargement of the hotspots, neither in the target (APB) muscle nor in the remaining muscles tested. Specifically, we found no significant changes in the motor representation areas of all muscles at those active sites where the mean amplitude of MEPs, recorded at the pre/post mapping sessions, was larger than 40% of the mean MEP recorded at the hotspots. In other words, enlargement of cortical motor representations following three weeks of TENS occurred around the borders of the cortical motor maps, as established at the pre mapping session.

The mechanism responsible for the expansion of the maps cannot be determined from the present data. It could be caused by true reorganization of the connectivity patterns in the cortex; alternatively it could simply result from an increase in the excitability of connections that were already present but not detected by the TMS method. However, the fact that the maps expanded in muscles distant from the site of stimulation is interesting. In this respect, it is plausible that the observed expansion of the output zones to all muscles may have been partially due to high overlaps in topographic and/or neuronal representations between the APB and the remaining muscles.

Interestingly, the observed increase in TENS-induced representational reorganization of cortical maps is neither restricted to the motor areas nor to common innervation pathways (table 2). For example, prolonged (three weeks) TENS treatment over the median nerve in MS patients has revealed that increases in tactile sensitivity were not restricted to thumb and index finger areas but also expanded to the fifth finger area (Cuypers et al. 2010). The fact that TENS-induced enlargements in the hand motor and/or sensory representations occur at multiple sites, suggests that somatosensory stimulation of peripheral afferent pathways with TENS might spread to non-stimulated parts of the somatosensory network. Electrophysiological and/or neuroimaging mapping suggests that this phenomenon may occur both at the peripheral and supraspinal (cortical) levels (Kimura et al. 1983;Krause et al. 2001;Kurth et al. 2000;Sato et al. 2005).

The present findings provide indirect support for a strong connectivity between the somatosensory and motor areas. Electrophysiological animal research has revealed topographically and functionally specific corticocortical excitatory connections between somatosensory areas and primary motor cortex (M1) (Caria et al. 1997;

Ghosh and Porter 1988; Huerta and Pons 1992; Lucier et al. 1975; Murphy et al. 1974).

2.1.5.2. The mechanisms underlying motor reorganization

We can only speculate on the mechanisms involved in the changes we observed here. Long-lasting changes in cortical motor excitability produced by repetitive central or peripheral stimulation have been shown to depend on long-term potentiation (LTP) or long-term depression (LTD). For example, it has been demonstrated that layer II/III horizontal connections in rat primary motor cortex, which are capable of LTP and LTD, are strengthened during acquisition of a new motor skill (Rioult-Pedotti et al. 2007). Furthermore, animal studies suggest that LTP-like processes in motor cortex transiently increase synaptic strength, by insertion of glutamate receptors to existing synapses (Harms et al. 2008;Rioult-Pedotti et al. 2007). In humans, learning of new motor skills is also shown to be associated with LTP-like changes in activity of cortical synapses which are most likely mediated by down-regulation of $GABA_A$ergic inhibition (Rosenkranz et al, 2007). Our observations indicated that (1) the three weeks intervention with TENS expanded the overlaps between output zones of neighboring muscles without inducing discernible changes in the level of corticospinal excitability around the hotspots, and that (2) enlargement of cortical motor maps occurred around the borders of their pre-intervention regions. In line with the aforementioned observations, it is reasonable to assume that long stimulation periods may result in the formation of new connections. Studies using pharmacological interventions may help to further unravel these mechanisms.

It is also meaningful to address how the present data can be reconciled with previous reports on the effect of single sessions of TENS on cortical excitability. Tinazzi et al (2005) have shown that prolonged somatosensory stimulation with TENS induces long-lasting depression in MEPs from the target muscle while having the opposite effect (i.e., long-lasting facilitation) on the non-target muscles, however this was not confirmed by others (Fernandez-del-Olmo et al. 2008). Nevertheless the suggestion of simultaneous changes in excitatory and inhibitory pathways made by Tinazzi et al (2005) may be one way in which it is possible to account for the simultaneous enlargement of cortical motor representations in the stimulated (APB) and non-stimulated muscles in the absence of a change in global excitability (e.g., rest motor threshold) of the descending motor pathways to these muscles. In this respect, the contribution of the GABAergic inhibitory system to the emergence of TENS-induced plasticity may remain a viable but not exclusive means for inducing cortical map changes, as observed in the present study. In the absence of direct recordings of synaptic dynamics or pharmacological manipulation, any

inference about the physiological basis of the present findings must be made with caution.

Even though the present findings are encouraging, some limitations need to be recognized. Firstly, no follow-up measurements were conducted beyond the post-intervention test to examine the lasting effects of the intervention. Secondly, no sham TENS group beyond our current control group was included to examine placebo effects. However, since participants were healthy and naïve about the purpose of the study, it is unlikely that our observations were biased by placebo effects. Finally, changes in the motor representation of the hand may be caused by unspecific reasons such as attentional drift away from the zone of stimulation. However, observations from the present and other studies (e.g., Cuypers et al, 2010; Ridding et al, 2001) suggest that TENS-induced modulations in cortical motor representations (or tactile sensitivity) extended beyond the boundaries of the stimulated zone, indicating spread of activation from stimulated to non-stimulated parts of the somatosensory network.

2.1.5.3. Clinical applications

The present observations highlight the potential of somatosensory stimulation to serve as a useful complementary therapy in neurorehabilitation. We have shown that TENS-induced enlargements in cortical motor maps were not restricted to the stimulated muscle but also extended to other hand and forearm muscles. However, therapeutic choices for global versus local effects will have to be made according to the specific disorder under treatment. In any case, it is clear that interventions that are first and foremost sensory in nature, do impact upon motor representations that persist for more than 20 hours following the intervention. More research will be necessary to differentially 'tune' M1 reorganization by further manipulation of the type of somatosensory stimulation and its parameters.

Since only healthy volunteers were included in the present study, generalization of the results to patient populations should be made with caution. However, a recent study using exactly the same intervention protocol (i.e. TENS to the right APB muscle, 1 hour a day for 3 weeks) in patients with MS, showed long-lasting improvements in tactile sensitivity. MS patients reached the same level of sensitivity as healthy subjects immediately after the intervention, and significant long-term effects were reported up to 3 weeks following the last intervention (Cuypers et al, in press). Overall, this suggests that TENS may be particularly useful as a complementary therapeutic tool in neurorehabilitation.

2.1.6. References

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STUDY 2

Long-term TENS treatment improves tactile sensitivity in MS patients

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2.2.1. Abstract

Transcutaneous electrical nerve stimulation (TENS) is commonly used in neurorehabilitation for the treatment of pain and spasticity.

In this study the long-term effects of sensory stimulation by means of transcutaneous electrical nerve stimulation (TENS) on hand sensitivity were investigated in patients with Multiple Sclerosis (MS). TENS was applied for three weeks (one hour/day) on the median nerve region of the dominant hand. Sensitivity was assessed by the Semmes-Weinstein monofilaments before and twelve hours following the last intervention as well as three weeks later.

Long lasting increases in tactile sensitivity were achieved by repetitive stimulation of sensory afferents with TENS in MS-patients but not in healthy subjects. This increased sensitivity was not restricted to the median nerve area but also expanded to the ulnar nerve area. Remarkably, MS patients reached the same level of sensitivity as healthy subjects immediately after the intervention and long-term effects were reported three weeks later.

Our findings demonstrated lasting improvements in tactile sensitivity of the fingers as a result of a long-term TENS intervention in MS patients, who ultimately reached a level comparable to that of healthy subjects.

2.2.2. Introduction

TENS involves application of electrical currents to the skin at varying frequencies, pulse durations, and intensities. This results in recruitment of large diameter sensory nerve fibers and/or mechanoreceptors without creating significant muscle contraction (for review, see: (Sluka and Walsh, 2003). It is commonly used in neurorehabilitation for easing symptoms such as pain and spasticity. Nonetheless, only few studies have evaluated the clinical effect of TENS on multiple sclerosis (MS). For example, significant reduction in spasticity in the plantar flexor muscles of the ankle after four weeks of TENS treatment was reported in MS patients by Armutlu et al., whereas Miller et al. found that a majority of patients reported TENS to result in a reduction of pain, spasticity and joint stiffness. However, studies exploring the effect of peripheral sensory stimulation on recovery of sensory functions in MS are limited.

Approximately 25 percent of patients with MS may have a reduced tactile stimulation attributed to a pure sensory attack at disease onset (Armutlu et al., 2003) whereas in approximately 40 percent of onset cases, paresthesiae are present (Sanders and Arts, 1986). For example, Sanders et al. reported diminished sensation of at least one of the three sensation modalities (being touched, pain and vibration) during a clinical examination in 70 percent of MS patients.

There is evidence that increased afferent input following peripheral sensory stimulation can lead to changes in excitatory and inhibitory interactions within the adult mammalian cortex (Ridding et al., 2001; Ridding et al., 2005). Previous studies have demonstrated that sustained alterations in sensory input affect map representations in the somatosensory cortex (Simons and Land, 1987; Van der Loos and Woolsey, 1973), associated with recovery of sensorimotor deficits (Fraser et al., 2002; Ward et al., 2006). In contrast to MS, recovery of somatosensory modalities in stroke has been well documented (Carey, 1995). Nevertheless, functional neuroplastic reorganisation is not only reported in stroke (Ward et al., 2004) but even in MS (Comi et al., 2004; Filippi and Rocca, 2004). In this respect, brain plasticity may play a crucial role in limiting the clinical consequences of MS-related damage during the early stages of the disease in clinically stable patients (Rocca et al., 2007). Recent evidence has shown that peripheral stimulation can modulate sensitivity in MS. For example, Mima et al. found that a short-term intervention with TENS applied over hand muscles increased sensory thresholds during and immediately after intervention (Mima et al., 2004). However, the effects of repetitive peripheral stimulation have not yet been documented in MS, particularly in view of inducing long-term after-effects. This is the principal goal of the present study in which we will focus on the long-term effects of TENS on tactile sensitivity in MS patients. We hypothesize that long-term sensory stimulation by means of TENS will induce long-term sensory after-effects.

2.2.3. Methods

2.2.3.1. Subjects

Twenty-six patients with MS (7 males, 19 females) aged 25-67 years (mean 47.70 ± 9.29 years) and thirty healthy subjects (13 males, 17 females) aged 22-74 years (mean 47.63 ± 13.40 years) were included in this study. All subjects gave their written informed consent to the study. Patient characteristics are shown in Table 1. They all exhibited stable MS, showing no relapse for six months prior to the intervention. Patients with other pathologies associated with peripheral and/or central sensory dysfunction or under psychotropic or antiepileptic medication were excluded. Expanded disability status scale scores (EDSS) ranged between 3 and 6.5 (Mean 4.52 ± 0.96 SD). Initially the number of patients was 29. Three patients were excluded, two suffered from acute relapse and one did not show up for testing.

Healthy subjects were also screened for pathologies associated with peripheral and/or central sensory dysfunction and medication intake. There was no drop-out.

2.2.3.2. Procedure

Each group (both, MS patients and healthy subjects) was subdivided in an intervention and control group (Fig. 1). Groups were balanced for age, gender and handedness (all, $p > .05$). The intervention group received TENS on the dominant hand whereas the control group received no additional intervention. In both groups sensitivity of the dominant and non-dominant hand was examined at the onset of the study (baseline), as well as three (post-intervention) and six (follow-up) weeks later. To rule-out short-term effects in excitability, post-intervention measurements were taken 12 hours following the intervention. The dominant hand undergoing the intervention was called the target hand. Handedness was assessed according to the Edinburgh Handedness Inventory (Oldfield, 1971). A laterality quotient (L.Q.) of +100 represented extreme right hand preference, while a L.Q. of -100 represented extreme left hand preference. Twenty-four patients were right-handed (Mean L.Q. 79.91 ± 18.23 SD) and two patients were left-handed (Mean L.Q. -60.00 ± 14.14 SD), whereas twenty-eight healthy subjects were right-handed (Mean L.Q. 93.85 ± 10.71 SD) and two were left-handed (Mean L.Q. -78.66 ± 14.01 SD). Experimental procedures conformed to the declaration of Helsinki and were approved by the local ethics committee of the University of Hasselt.

Table 1. Patient characteristics. Patient ID, Group, Age, Sex, Year of first symptom, Year of diagnosis, MS type (RR: relapsing-remitting, PP: primary-progressive, SP: secondary-progressive), Functional system scores (Visual, Brainstem, Pyramidal, Cerebellar, Sensory, Bladder/bowel, Mental), EDSS score and Current medication intake.

					FUNCTIONAL SYSTEM SCORES								
AGE	SEX	FIRST SYMPTOM	DIAGNOSIS	MS TYPE	VISUAL	BRAINSTEM	PYRAMIDAL	CEREBELLAR	SENSORY	BLADDER/BOWEL	MENTAL	EDSS	MEDICATION
44	F	2005	2005	RR	3	1	3	1	3	0	1	4	avonex
56	F	1969	1982	PP	2	2	4	3	3	1	3	5,5	/
46	M	1984	1984	RR	1	2	3	2	2	1	2	4	/
59	F	1994	1999	PP	2	1	4	3	3	2	2	5,5	/
25	F	2001	2003	RR	2	2	4	2	3	4	2	5,5	/
41	F	1984	2002	RR	2	1	0	1	3	0	1	3,5	copaxone
47	M	1999	2001	SP	1	0	4	5	2	2	2	6,5	/
44	M	1999	1999	SP	1	0	3	2	1	0	1	4	rebif
42	M	1990	1990	RR	1	2	3	2	2	0	1	4	copaxone
63	F	2007	2007	SP	1	2	3	3	3	1	0	6	betaferon
54	F	?	1993	SP	5	2	2	1	0	1	1	3,5	rebif
40	F	1991	1991	PP	0	0	3	0	0	0	0	5,5	copaxone
60	M	1996	2001	PP	1	1	3	3	2	1	2	5	/
42	F	1996	1998	PP	0	0	3	3	2	2	2	5,5	betaferon
46	F	1979	1985	RR	0	1	3	2	2	1	1	4	/
48	F	1985	1985	RR	1	2	3	2	3	2	1	4	betaferon
48	F	1990	1992	SP	3	0	3	1	2	3	3	4	copaxone
50	M	1999	2000	PP	2	2	2	0	1	2	2	3	rebif
46	F	1979	1983	SP	0	2	3	4	3	2	2	4,5	/
49	F	1978	1996	RR	0	2	3	1	1	0	2	3,5	copaxone
57	F	1975	2000	SP	2	0	3	3	1	1	0	5,5	betaferon
50	F	1981	1981	PP	3	2	3	2	1	1	2	4	copaxone
67	F	1985	1985	PP	1	0	4	2	0	1	0	5,5	rebif
48	F	2000	2000	RR	1	0	3	2	2	3	1	4,5	/
49	M	1997	1997	SP	3	1	2	1	2	2	1	3	/
37	M	1991	2002	RR	2	2	2	2	2	0	2	4	copaxone

2.2.3.3. Intervention

TENS (Intelect Digitens, Chattanooga Group, Hixson TN USA) was applied on the median nerve region (more specifically, the thenar eminence) of the dominant hand, using self-adhesive electrodes (Dura-stick II, 1.5 x 4cm). A biphasic alternating current with a frequency of 100 Hz and pulse width of 250 μsec was automatically modulated to prevent habituation. More specifically, during the beginning of the 0.5 sec period, the width was decreased to 50% of its original setting, and during the next 0.5 sec period, the frequency was decreased to 50% of its original setting. This pattern was repeated every second across a duration of 1 hour. This was done to prevent nerve accommodation, such that no intensity changes were required for long and effective treatment. Stimulation intensity was below the motor threshold and produced a tingling sensation in the stimulated area without muscle twitch or pain. Intensity was first increased above motor threshold and was subsequently decreased until visual and tactile muscle contraction disappeared. For both intervention groups, the timing of stimulation was fixed, and occurred more specifically between 6 and 8 pm. This protocol was applied for three weeks with a duration of one hour per day.

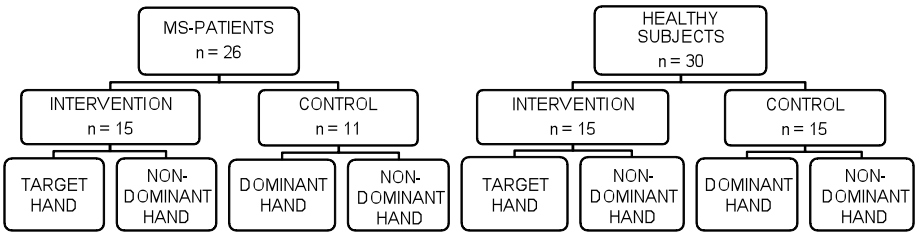

Figure 1. Overview of subject groups and subdivisions.

2.2.3.4. Sensitivity

The Semmes-Weinstein monofilaments (Smith & Nephew, Inc., Germantown, WI) were used to determine finger sensitivity. This test is known for its validity, reliability, reproducibility and responsiveness, and is widely used in research as well as in clinical settings (Bell-Krotoski and Tomancik, 1987; Jerosch-Herold, 2005). Participants were seated in front of the examiner with both hands relaxed in supination. The examiner was blind to the intervention. Five different monofilament diameters (2.83, 3.61, 4.31, 4.56, 6.65 expressed as the log of force in mg; the corresponding forces in grams are respectively: 0.07, 0.4, 2, 4 and 447.) were used, corresponding respectively with the following clinical classification: normal, diminished light

touch, diminished protective sensation, loss of protective sensation and untestable. The monofilaments were randomly presented in a descending or ascending order to the thumb, index and fifth finger. Each filament was pressed against the skin until it was buckled for approximately 1.5 sec. The participants were instructed to give a verbal response when they felt a touch. All filaments were tested 3 times with randomization in order and finger before switching to the following monofilament. The filament with the lowest pressure-score, which was felt 3/3 times on the fingertip, was recorded as the score for this fingertip.

2.2.3.5. Data Analysis

Advanced linear applications (SPSS 16.0) were used. Dominant and non-dominant hands were analysed separately to fulfil the assumption of independency. Differences in finger sensitivity between independent groups were analysed using the Mann-Whitney U test. Effects over time were analysed within groups using Friedman's repeated measures ANOVA. In addition, the Wilcoxon signed rank test was used for repeated measurements on a single sample. The level of significance was set at $p < .05$.

2.2.4. Results

Overall, as shown in Table 2, tactile sensitivity in MS patients was more impaired in comparison with healthy subjects for all fingers at baseline (all, $p < .05$). More specifically, most patients showed either diminished light touch (35.26%) or diminished protective sensation (40.38%), whereas healthy subjects showed mainly normal sensitivity (38.89%) or diminished light touch (53.33%). In addition, no significant differences in sensitivity between intervention and control subjects were observed within groups (both, healthy and MS) at baseline (all, $p > .05$).

			FINGER	BASELINE					POST					FOLLOW-UP				
				N	DLT	DPS	LPT	U	N	DLT	DPS	LPT	U	N	DLT	DPS	LPT	U
INTERVENTION	MS	TARGET	I	0	7	7	0	1	5	9	1	0	0	2	8	4	1	0
			II	0	6	8	1	0	8	7	0	0	0	1	10	4	0	0
			V	0	5	10	0	0	3	10	2	0	0	1	5	9	0	0
		NON-DOM	I	0	6	7	1	1	0	7	7	1	0	0	6	7	1	1
			II	0	6	7	2	0	0	8	7	0	0	0	6	7	2	0
			V	0	5	9	1	0	1	6	8	0	0	0	5	9	1	0
	HEALTHY	TARGET	I	4	9	2	0	0	4	11	0	0	0	6	8	1	0	0
			II	5	10	0	0	0	6	9	0	0	0	7	8	0	0	0
			V	7	7	1	0	0	6	8	1	0	0	9	6	0	0	0
		NON-DOM	I	5	8	2	0	0	7	6	2	0	0	9	4	2	0	0
			II	6	9	0	0	0	7	7	1	0	0	9	6	0	0	0
			V	5	10	0	0	0	8	6	1	0	0	10	5	0	0	0
			FINGER	BASELINE					POST					FOLLOW-UP				
				N	DLT	DPS	LPT	U	N	DLT	DPS	LPT	U	N	DLT	DPS	LPT	U
CONTROL	MS	DOM	I	2	5	2	1	1	2	4	3	0	2	2	5	2	1	1
			II	2	4	3	1	1	1	5	3	0	2	3	2	4	1	1
			V	2	4	3	2	0	2	4	2	1	2	2	5	2	2	0
		NON-DOM	I	3	2	3	2	1	1	5	3	0	2	3	2	3	1	2
			II	3	2	3	1	2	1	5	2	1	2	2	3	3	1	2
			V	3	3	1	3	1	3	3	1	2	2	2	4	3	1	1
	HEALTHY	DOM	I	6	6	3	0	0	6	7	2	0	0	10	3	2	0	0
			II	7	7	1	0	0	10	4	1	0	0	8	7	0	0	0
			V	7	8	0	0	0	8	7	0	0	0	9	6	0	0	0
		NON-DOM	I	5	7	3	0	0	7	6	2	0	0	9	3	3	0	0
			II	6	7	2	0	0	8	6	1	0	0	7	8	0	0	0
			V	7	8	0	0	0	8	7	0	0	0	8	7	0	0	0

Table 2. Sensitivity measurements for all subjects at baseline, post-intervention and follow-up. The black area indicates the shift in sensitivity for the target hand of MS-patients. After the intervention sensitivity increased compared to baseline and follow-up measurements. The data indicates the number of subjects represented in each clinical category (35): normal (N), diminished light touch (DLT), diminished protective sensation (DPS), loss of protective sensation (LPS) and untestable (U) .

2.2.4.1. MS-patients

Intervention vs. Control group

The Mann-Whitney U test for post-intervention vs. baseline revealed a significant group effect for the thumb (p = .002), index (p = .004) and fifth finger (p = .027) of the dominant (target) hand indicating a significant increase in sensitivity immediately after the intervention. For the index finger of the target hand this effect remained significant (p = .047) when the follow-up session was compared with baseline, indicating a long-lasting increase in sensitivity. For the non-dominant hand no significant effects were found (all, p > .05).

Intervention Group

Friedman's repeated measures ANOVA revealed significant effects of TENS on sensitivity over time in the thumb (p < .001), index (p <.001) and the fifth finger (p = .002) of the target hand. Wilcoxon matched-pairs signed rank tests showed that sensitivity of the thumb (p < .001), index (p < .001) and fifth finger (p = .004) increased significantly when comparing post-intervention with baseline measures (Fig. 2). At follow-up, sensitivity was still significantly higher in comparison with the baseline for thumb (p = .031) and index (p = .016), but not in the fifth finger (p = .500). For the non-dominant hand (Fig 3.), no significant effects were reported (all, p > .05).

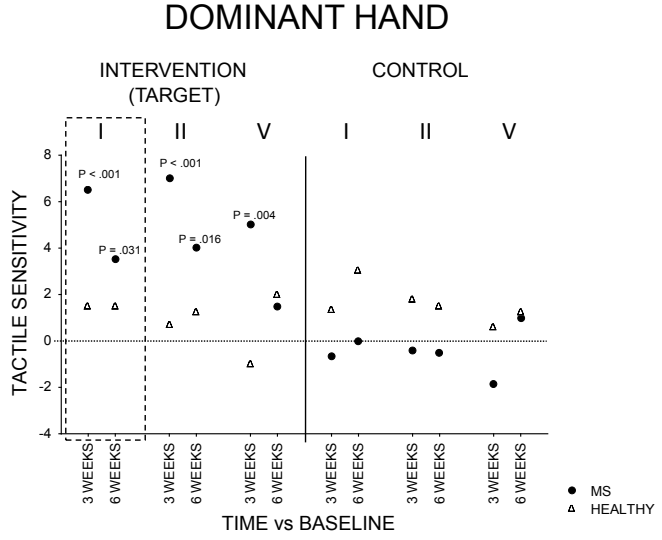

Figure 2. Semmes-Weinstein monofilaments for the dominant hand. Improvement scores (Wilcoxon matched pairs signed-rank test) for the thumb (I), index (II) and 5th finger (V) for the intervention (target) and control group are given as a mean rank. Mean ranks were higher when sensitivity increased. The dashed rectangle on the left represents the target finger.

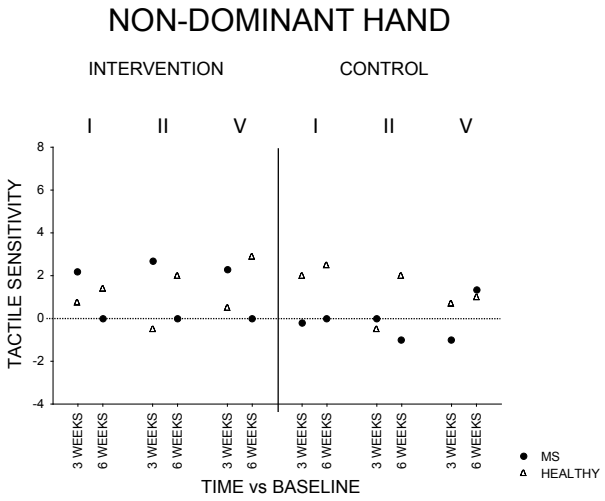

Figure 3. Semmes-Weinstein monofilaments for the non-dominant hand. Improvement scores (Wilcoxon matched pairs signed-rank test) for the thumb (I), index (II) and 5th finger (V) for the intervention and control group are given as a mean rank. Mean ranks were higher when sensitivity increased.

Control Group

Friedman's repeated measures ANOVA revealed no significant changes in sensitivity over time (all, $p > .05$). As shown in Figure 2 & 3, Wilcoxon matched-pairs signed rank tests revealed no significant changes in sensitivity at post-intervention or follow-up as compared to

baseline (all, $p > .05$).

2.2.4.2. Healthy subjects

Intervention vs. Control Group

The Mann-Whitney U tests showed no significant group effects for differences in finger sensitivity over time between the intervention and control group (all, $p > .05$), indicating that the level of sensitivity was similar in both groups.

Intervention Group

Friedman's repeated measures ANOVA revealed no significant effects of TENS on sensitivity over time. As shown in Figure 2 & 3, Wilcoxon matched-pairs signed rank tests revealed no significant changes in sensitivity at post-intervention or follow-up relative to baseline (all, $p > .05$).

Control Group

Friedman's repeated measures ANOVA revealed no significant sensitivity changes over time in any of the tested fingers (all, $p > .05$). As shown in figure 2 & 3, Wilcoxon matched-pairs signed rank tests revealed no significant changes in sensitivity at post-intervention or follow-up as compared to baseline (all, $p > .05$).

2.2.4.3. Impact of the intervention on sensitivity in MS patients

To evaluate the impact of the intervention on MS patients, additional analysis was carried out to compare baseline sensitivity measures of the target hand of MS patients with those of healthy subjects (intervention group). Mann-Whitney U tests showed that, at post-intervention, there were no significant differences in sensitivity of all tested fingers between MS patients and healthy subjects (all, $p > .05$). In contrast, when baseline and follow-up measures of MS patients were compared to baseline measures of healthy subjects, MS patients were less sensitive in all tested fingers (all, $p < .05$) except for the thumb at follow-up ($p = .219$). These results indicate that MS patients reached a level of sensitivity comparable to healthy subjects immediately after the intervention (Table 2). Nevertheless, 3 weeks after the end of the intervention, sensitivity in MS patients was similar to healthy subjects for the thumb only.

2.2.5. Discussion

The present study shows for the first time that a long lasting increase in tactile sensitivity can be achieved by repetitive stimulation of sensory afferents with TENS in MS-patients but not in healthy subjects. Moreover, increased sensitivity in MS-patients was not restricted to the median nerve area but also expanded to the ulnar nerve area. Remarkably, MS patients reached a level of sensitivity comparable to healthy subjects immediately after the intervention and long-term effects were reported three weeks later. Whereas Mima et al. (2004) reported that a short-term intervention with TENS increased sensory thresholds during and immediately after intervention, we demonstrated evidence for long-lasting changes induced by a repetitive sensory stimulation protocol. Additionally, sensitivity was still significantly increased in some fingers three weeks following the end of the intervention.

Potential mechanisms accounting for these long-lasting changes are long-term potentiation and depression (Garraghty and Muja, 1996; Glazewski et al., 1996), but these mechanisms have neither been proven to be necessary nor sufficient for cortical map reorganization. Other physiological mechanisms refer to short-term synaptic dynamics, which are altered by sensory experience (Finnerty et al., 1999) and alterations in inhibitory circuits (Foeller and Feldman, 2004).

The observed increase in sensitivity was not restricted to the stimulated area but extended beyond this. Cortical reorganization as a result of the present TENS paradigm could possibly account for this finding but convincing evidence to support this claim is currently lacking.That an increase in sensitivity was also found in the fifth finger in our study is rather surprising as the thumb and index share different neural pathways (median nerve) in comparison to the fifth finger (ulnar nerve). Although speculative, this phenomenon can perhaps be accounted for at the peripheral as well as cortical level. At the peripheral level (Kimura et al., 1983), evidence exists for ulnar to median nerve communication. At the cortical level, spatial and temporal overlap in the human somatosensory cortex has been established (Krause et al., 2001; Sato et al., 2005). More specifically, somatotopic overlapping representations of all five fingers of a single hand have been demonstrated in the primary somatosensory cortex by means of electrical stimulation (Kurth et al., 2000). Overall, this suggests that spread of activation from stimulated to non-stimulated parts of the somatosensory network might occur at different levels.

Interestingly, MS patients reached levels of sensitivity that were similar to healthy subjects immediately after the end of the intervention. Long-term effects were even reported three weeks later. This result shows that TENS can be used as a valuable neurorehabilitative tool for patients with stable MS and with limitations in daily activities according to the EDSS score. Remarkably, healthy subjects showed no significant increase in

sensitivity. Perhaps this result is not surprising because the healthy subjects behaved already close to their highest sensitivity level, limiting further improvements.

An interesting question for future MS-research is whether such TENS interventions also result in improvement of fine motor function, as was found in stroke patients (Celnik et al., 2007; Conforto et al., 2007). Evidence from animal research suggests that somatosensory input acts as a 'teacher' to help shape motor system plasticity (Asanuma and Pavlides, 1997). Moreover, stimulation of the somatosensory pathway in the thalamus or the somatosensory cortex induces long-term potentiation in the motor cortex, mediated by excitatory glutamatergic synapses (Iriki et al., 1989; Sakamoto et al., 1989). Additionally, synaptic density in the motor cortex can be modified by means of somatosensory stimulation (Keller et al., 1992). In humans, clinical studies also indicate that a prolonged period of electrical peripheral nerve stimulation induces short-term plasticity at multiple levels of the motor system (for review, see: (Kaelin-Lang, 2008).

To summarize, this paper demonstrates that long-lasting improvement in tactile sensitivity can be induced in MS patients by means of TENS and that these patients can reach comparable levels of sensitivity to those of healthy subjects after a long-term repetitive intervention protocol. An important future goal is to assess the role of the TENS-protocol parameters. In this respect, several studies (Fraser et al., 2002; Mima et al., 2004; Tinazzi et al., 2006) have reported varying outcomes using different stimulation parameters. Therefore a careful selection of the appropriate stimulation parameters (i.e. frequency, intensity and duration of the stimulus applied) is crucial to obtain the desired therapeutic result. The present observations highlight the potential advantage of TENS as a meaningful and valuable therapeutic tool in neurorehabilitation.

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STUDY 3

Long-term TENS treatment decreases cortical motor representation in multiple sclerosis

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2.3.1. Abstract

This study investigated the effect of a long-term transcutaneous electrical nerve stimulation (TENS) treatment on cortical motor representation in patients with multiple sclerosis (MS).

In this double blind crossover design, patients received either TENS or sham stimulation for 3 weeks (1 hour per day) on the median nerve region of the most impaired hand, followed by the other stimulation condition after a washout period of 6 months. Cortical motor representation was mapped using transcranial magnetic stimulation (TMS) at baseline and after the 3-week stimulation protocol.

Our results revealed that three weeks of daily stimulation with TENS significantly decreased the cortical motor representation of the stimulated muscle in MS patients.

Although the mechanisms underlying this decrease remain unclear, our findings indicate that TENS has the ability to induce long-term reorganization in the motor cortex of MS patients.

2.3.2 Introduction

Multiple sclerosis (MS) is often accompanied by sensory-motor dysfunctions that have a substantial impact on the quality of life of patients (Gallien and Robineau, 1999). Besides pain (O'Conner et al., 2008) and diminished sensation which has been reported during clinical examination in 70% of MS patients (Sanders and Arts, 1986), impaired motor control characterized by a loss of fine motor skills (Longstaff and Heath, 2006), increased intention tremor (Alusi et al., 2001) and spasticity (Barnes et al., 2003) have also been reported in MS patients. Evidence suggests that some of the aforementioned symptoms can be treated or reduced with electrotherapy. For example, Armutlu et al. (2003) reported a significant reduction in spasticity in the plantar flexor muscles of the ankle after a 4-week treatment with transcutaneous electrical nerve stimulation (TENS)(Armutlu et al., 2003). Sluka and Walsh (2003) found that a majority of patients reported TENS to result in a reduction of pain, spasticity, and joint stiffness (Sluka and Walsh, 2003). However, studies exploring the effect of peripheral sensory stimulation on recovery of motor functions in MS are sparse. Furthermore, no studies have explored the effect of long-term intervention with TENS on the reorganization of cortical motor representations of the hand musculature in MS. Electrical stimulation in general, and TENS in particular have been applied successfully in neurorehabilitation, such as in the treatment of stroke (Sonde et al., 1998, Ng and Hui-Chan, 2007), immobilization (Meesen et al., 2010) urinary symptoms (Skeil and Thorpe, 2001), spinal cord injury (Fung and Barbeau, 1994, Goulet et al., 1996), multiple sclerosis (Armutlu et al., 2003, Miller et al., 2007), writer's cramp (Tinazzi et al., 2006) and/or to reduce movement disorders caused by tremor, myoclonia, or dystonia (Toglia and Izzo, 1985, Bending and Cleeves, 1990). Recently our group showed that three weeks of daily stimulation with TENS resulted in a long-term increased tactile sensitivity in MS patients (Cuypers et al., 2010). In another study we showed that a three-week intervention with TENS resulted in significant enlargements of cortical motor maps in healthy individuals (Meesen et al., 2011). Based on these findings we predicted that a 3-week intervention with TENS, applied to the abductor pollicis brevis (APB) muscle, will induce a significant increase of the cortical motor representation of this muscle in MS patients.

2.3.3. Methods

2.3.3.1. Patients

Six female patients with MS (aged 34 to 60yrs, mean 49.67 ± 9.56) participated in this double blind crossover study (see Table I for patient characteristics). Expanded Disability Status Scale (EDSS) scores ranged between 2.5 and 4.5 (mean 3.30 ± 0.60). Handedness was assessed according to the Edinburgh Handedness Inventory (Oldfield, 1971). A laterality quotient (LQ) of +100 represented extreme right hand preference, whereas an LQ of -100 represented extreme left hand preference. All patients were right-handed (mean LQ = 89.92 ± 12.65). Patients were stable and showed no relapses for six months prior to the experiment. Patients with other pathologies associated with peripheral and/or central sensory dysfunction or under psychotropic or antiepileptic medication were excluded. All participants provided written informed consent prior to participation. Experimental procedures conformed to the Declaration of Helsinki and were approved by the local ethics committee of the University of Hasselt.

Table I. Patient characteristics

						FUNCTIONAL SYSTEM SCORES							
ID	AGE	LATERALITY QUOTIENT	FIRST SYMPTOM	DIAGNOSIS	MS TYPE	VISUAL	BRAINSTEM	PYRAMIDAL	CEREBELLAR	SENSORY	BLADDER/BOWEL	MENTAL	EDSS
1	44	100	jan-89	feb-89	RRMS	0	0	2	2	2	1	1	3
2	58	89.5	aug-07	mrt-09	RRMS	0	1	2	1	2	1	0	2.5
3	34	80	nov-09	nov-10	RRMS	0	0	1	2	2	1	0	2.5
4	60	70	jan-69	jan-83	SPMS	0	0	2	2	2	1	1	3
5	51	100	nov-89	nov-89	SPMS	0	2	2	3	3	1	1	4
6	51	100	aug-97	okt-02	SPMS	0	0	2	2	2	3	1	4.5

2.3.3.2. Intervention

In the TENS condition, TENS (Intelect Digitens, Chattanooga Group, Hixson, TN) was applied on the median nerve region (thenar eminence) of the most impaired hand (as determined by sensory testing using the Semmes-Weinstein monofilaments), using self-adhesive electrodes (Dura-stick II, 1.5 × 4cm). TENS consisted of a biphasic alternating current with a frequency of 100Hz and a pulse width of 250ms that was automatically

modulated to prevent habituation. More specifically, during the first 0.5-second period, the pulse width was decreased to 50% of its original setting, and during the next 0.5-second period, the frequency was decreased to 50% of its original setting. This pattern was repeated every second for 1 hour. Stimulation intensity was below the motor threshold and produced a tingling sensation in the stimulated area without muscle twitch or pain. Intensity was first increased above motor threshold and was subsequently decreased until visual and tactile muscle contraction disappeared.

In the sham condition, procedures were identical to those of the TENS condition. However although no current was applied, patients were told that they received subsensory stimulation. In both conditions, the timing of stimulation was fixed and occurred between 6 pm and 8 pm. To monitor if patients performed the stimulation protocol as requested, they were asked to fill in a diary. All patients completed all treatment sessions. Patients were instructed to apply stimulation on the relaxed muscle when seated in a comfortable chair. TENS or sham was applied across 3 weeks for 1 hour per day. Half of the patients started in the TENS condition and half in the sham condition. Both conditions were separated by a washout period of 6 months. The investigators performing the statistical analysis and the TMS measurements were blinded for the intervention. Only the principal investigator (RLJM) who did not perform any measurements or statistical analysis was aware of patient assignment.

2.3.3.3. Transcranial Magnetic Stimulation (TMS)

During the course of the experiment cortical motor representation of the abductor pollicis brevis (APB) was measured with TMS before (baseline) and after (post) the interventions with either TENS or sham. Cortical motor maps in the post-intervention session were taken at least 12h (but not more than 24h) after the last stimulation session. TMS was applied by means of a magnetic stimulator (Magstim BiStim^2, Whitland, South West Wales, UK). Single-pulse magnetic stimuli were delivered over the patients' hemisphere contralateral to the most impaired hand with a 70mm loop-diameter figure-of-eight coil. For each patient a customized cap was made out of thermoplastic material (Aquaplast-T Solid, 20cm x 15cm x 0.24cm, Sammons Preston Polyon, Cedarburg) with references to anatomical landmarks (left and right external auditory meatus and occiput) to ensure reproducibility of measurements across the experiment. An orthogonal coordinate system referenced to the vertex was marked on each cap. The magnetic coil was held tangentially to the scalp at an angle of 45° to the midline with the handle backwards. The optimal location (hotspot) for eliciting motor evoked potentials (MEPs) from the APB of the stimulated hand was identified and marked to ensure reproducibility of coil positioning

in each patient. Next, the rest motor threshold (rMT) was determined at the optimal scalp position. The rMT was defined as the lowest intensity of magnetic stimulation required to evoke MEPs larger than 50μV (peak-to-peak) in at least 5 of 10 trials in the relaxed muscle. The stimulation intensity for mapping was then set at 120% of the rMT of the baseline session and was the same at baseline and after the intervention. This was done for each condition. Both the location of the hotspot and the stimulation intensity was preserved within each stimulation condition. A grid of 225 (15 x 15) positions, spaced 1cm along both the medio-lateral and antero-posterior axes, was marked on each cap. In producing maps, single TMS pulses were applied in 1 cm-steps in a clockwise spiral course, beginning at the hotspot. Each position was stimulated 8 times (randomized interstimulus interval: 5–8s) before moving to the adjacent grid point, until the border of the motor map was defined (i.e. less than 4 MEPs with an amplitude above 100μV peak-to-peak).

2.3.3.4. Electromyographic (EMG) Recordings

EMG signals from the APB were collected. For each patient, the specific electrode placement was photographed and saved in a database. After amplification (gain = 1000), bandpass filtering (4–1500Hz) (Bagnoli-16, Delsys Inc, Boston, USA) and 50/60Hz noise elimination (Humbug, Quest Scientific, North Vancouver, Canada) the recorded EMG signals were digitized at 5000Hz (CED Sigal Version 3.03, Cambridge Electronic Design, Cambridge, UK) and were stored on a laboratory computer for offline analysis. Data collection was initiated 50ms prior to the delivery of TMS and lasted 150ms. Pre-trigger EMG activity was continuously monitored online during the mapping session and was below 5μV.

2.3.3.5. Data analysis

MEP size was measured offline by calculating the peak-to-peak amplitude of each waveform in a 10 to 40ms time window after the TMS pulse onset. Cortical motor representation was defined as the number of stimulus positions whose stimulation evoked a mean MEP with a peak-to-peak amplitude of at least 100μV (= 'active' stimulation positions). Map area referred to the contour, whereas map volume referred to the sum of the mean amplitudes at all active stimulation positions. The center of gravity (CoG) was computed as a measure of the amplitude-weighted centre of the motor representational map. It was expressed as a bivariate measurement with a mediolateral (x) and antero-posterior coordinate (y), using the following formula: CoG = $(\Sigma a_i x_i/\Sigma a_i, \Sigma a_i y_i/\Sigma a_i)$, for stimulation position

coordinates x_i, y_i and amplitudes a_i. The magnitude of the CoG displacement vector, i.e., the Euclidean distance between the CoG locations at baseline and post-intervention was calculated. Because the Shapiro-Wilk test indicated that our data was not normally distributed, non-parametric statistics (SPSS v20) were used for statistical analysis. Effects of the intervention on corticospinal excitability and cortical motor representation were studied using the following dependent measures: mean MEP amplitude at the hotspot, center of gravity, mean map area and mean map volume. A Wilcoxon signed rank test was used to analyze the effect of the intervention between conditions (TENS vs. sham) and within conditions (TENS or sham) over time (baseline vs. post-intervention). The level of significance was set at $p < 0.05$.

2.3.4. Results

2.3.4.1. Rest motor threshold (rMT) and MEP amplitude at the hotspot

TENS or sham did not affect the rMT or size of MEPs at the hotspots over time (all, $p > 0.05$), suggesting that levels of corticospinal excitability at the hotspots remained unchanged (Table II). Furthermore, no differences in rMT or size of MEPs at the hotspot were reported between conditions (all, $p > 0.05$).

Table II. Mean (±SD) resting motor threshold (rMT, in % of maximum stimulator output) and mean (±SD) MEPs amplitudes (mV) for baseline and post-intervention are shown for the TENS and the SHAM condition.

	TENS		SHAM	
	Baseline	Post	Baseline	Post
rMT	46.00 ± 7.82	45.67 ± 6.68	46.40 ± 5.68	44.50 ± 7.19
MEP at hotspot	0.66 ± 0.35	0.51 ± 0.30	0.84 ± 0.51	0.73 ± 0.84

2.3.4.2. Map area and Map volume

Individual changes in map area and volume are shown in Table III. The Wilcoxon signed rank test revealed a significant effect of the intervention on map area over time ($Z = 2.201$; $p = 0.031$) between conditions (see Fig. 1). Furthermore, within the TENS condition the Wilcoxon signed rank test showed a significant decrease in map area over time ($Z = 2.201$; $p = 0.031$). Within the sham condition no significant changes in map area were reported. Map volume did not change significantly, neither between nor within conditions over time (all, $p > 0.05$). An example of the

cortical motor maps for a typical patient is shown in Figure 2.

Table III. Individual changes in map area and volume for the TENS and the SHAM condition.

	MAP AREA % change (BASELINE - POST)		MAP VOLUME % change (BASELINE - POST)	
SUBJECT	TENS	SHAM	TENS	SHAM
1	-52.54	2.37	-67.73	0.51
2	-15.47	4.91	25.67	-17.72
3	-14.06	1.32	-17.56	-3.46
4	-9.54	-4.13	-9.09	18.42
5	-42.60	1.86	-63.06	4.95
6	-2.87	30.60	-17.38	42.27

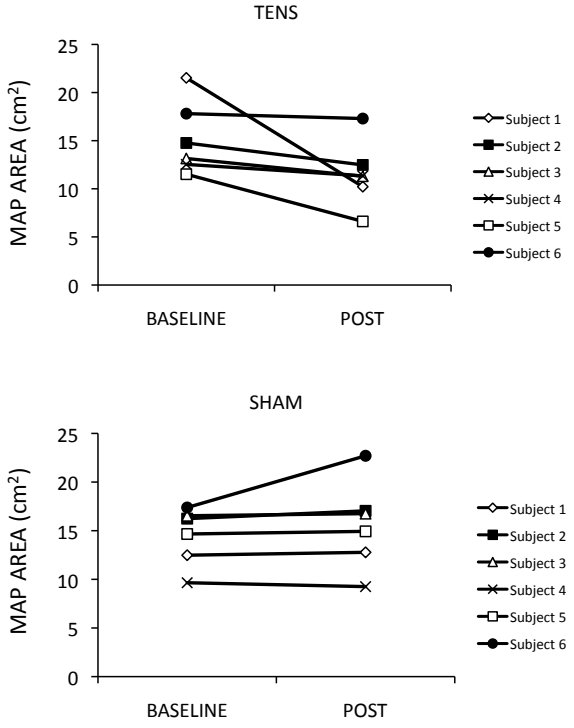

Fig. 1 Individual map areas at baseline and post-intervention.

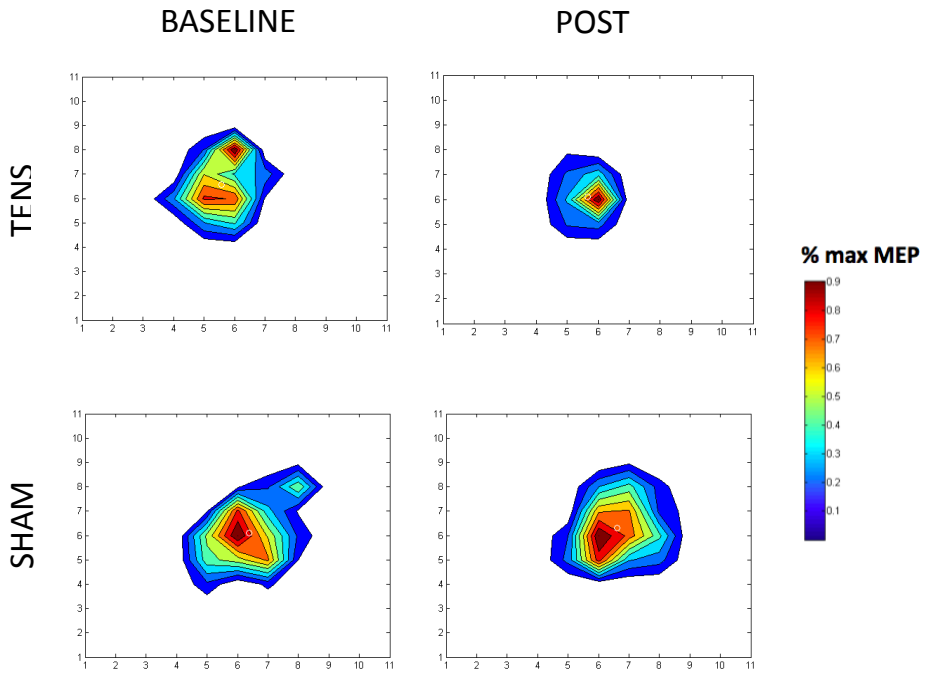

Fig. 2 Cortical motor maps for a typical patient at baseline and post-intervention. A decrease in map/volume area is shown only in the TENS condition.

2.3.4.3. Displacement of the center of gravity

No significant changes for the CoGs of the motor maps were reported over time (all, $p > 0.05$).

2.3.5. Discussion

The present study evaluated the effect of a long-term somatosensory stimulation with TENS on cortical motor representation in patients with MS. Our results revealed that three weeks of daily stimulation with TENS modulates cortical motor representation of the stimulated muscle in MS patients. Specifically, we found that long-term stimulation with TENS resulted in a significant reduction of the cortical representation of the stimulated muscle. This observation is in contrast with our previous findings in healthy subjects (Meesen et al., 2011) showing that three weeks of daily stimulation with TENS resulted in a significant enlargement of the cortical representations of the hand and forearm musculature on the stimulated side. It is noteworthy that the cortical motor maps in the post-intervention session were assessed at least 12 hours (but not more than 24h) after the last TENS stimulation to ensure that changes in cortical motor maps were not affected by underlying changes in the excitability of corticospinal projections to those muscles. No significant increases or reductions were found in the amplitudes of MEPs and the levels of rMT at the hotspot of the APB over the mapping sessions. This finding was consistent with the findings of Meesen et al, 2011, showing that three weeks of daily stimulation with TENS did not change amplitudes of MEPs at the hotspot in healthy subjects (Meesen et al., 2011). These observations indicate that the primary decrease of the cortical motor map representation in the post-intervention mapping session was not accompanied by underlying changes in corticospinal excitability at the hotspot, but might be caused by true reorganization of the connectivity patterns in the cortex. In line with our findings, previous studies (Ziemann et al., 1996b, a) revealed that changes in peripheral motor excitability are caused by gamma-aminobutyric acid (GABA)-controlled interneuronal circuits in M1, while changes in motor threshold are dependent on ion channel conductivity and may reflect membrane excitability. Furthermore, these studies showed that reinforcement of GABA action reduced intracortical excitability but had no effect on rMT. Therefore, we suggest that the decline in map area observed in the MS patients following the three weeks of TENS represents long-term changes in the cortical motor representation of the APB muscle rather than a temporary decline in corticospinal excitability as reported by Tinazzi et al. (2006) after a single session of TENS (Tinazzi et al., 2006).

The observed decrease in the area of the cortical motor maps in MS patients was rather unexpected as our early findings in healthy individuals (Meesen et al., 2011) clearly showed the opposite effect (i.e., a significant enlargement of the cortical motor maps). However, it is known that mechanisms regulating cortical plasticity in the neurodegenerative and healthy brain seem to differ. For example, in normal aging, more elaborate activation of cortical networks is required to perform simple motor tasks (Heuninckx et al., 2008). Evidence from other functional magnetic resonance

imaging (fMRI) studies showed comparable increased cortical activation patterns in normal aging and MS. In patients with minimal signs of MS (i.e., median EDSS = 1.25), increased activity was primarily reported in the contralateral and ipsilateral motor areas with respect to motor tasks (Lee et al., 2000, Rocca et al., 2003). This increased brain activity could be explained by manifestation of adaptive or compensatory mechanisms that allow normal performance despite neural damage or loss (Pantano et al., 2006). Moreover, patients with primary progressive MS also activated areas that do not belong to the classical motor network (Filippi et al., 2002). Importantly, the extent of increased motor activation correlated with damage of both the brain tissue (Lee et al., 2000, Reddy et al., 2000, Rocca et al., 2002) and the corticospinal tract (Pantano et al., 2002).

Interestingly, Levy et al. (2002) reported that inhibition of sensory input to the human sensorimotor cortex reduces GABA as detected by magnetic resonance spectroscopy (Levy et al., 2002). This indicates that GABAergic activity depends on the manipulation of sensory input. Consistent with these findings, Jacobs and Donoghue (1991) reported an expansion of the forelimb area in the motor cortex of the rat following stimulation of the adjacent vibrissae motor cortex while blocking the inhibitory action of GABA receptors with bicuculline (Jacobs and Donoghue, 1991). Therefore, it is plausible that the observed reduction of MAP area after a long-term somatosensory intervention may have been due to the formation of new GABAergic inhibitory connections that were previously impaired by the MS pathology (Dutta et al., 2006). Moreover, Clements et al. (2008) recently suggested that GABAergic interneurons at the level of the motor cortex are selectively affected by MS and therefore cause an imbalance between cortical excitability and inhibition (Clements et al., 2008).

Although the present study indicates persistent neuroplastic changes in the motor cortex of MS patients after a long-term intervention with TENS, findings of short-term TENS interventions can also be reconciled with our results. Firstly, an fMRI study showed that the activated volume in the primary motor cortex (M1) and supplementary motor area (SMA) during cyclical thumb movements decreased after a 15min somatosensory stimulation with TENS provided at the thenar area of the right hand in healthy subjects (Toma, 2003). Based on this observation Toma et al. (2003) proposed that a short-term somatosensory stimulation with TENS might account for recruitment of a smaller brain network to achieve the same motor output (Toma, 2003). Secondly, Schabrun et al. (2009) demonstrated that a single session of afferent stimulation in patients with focal hand dystonia induced short-term cortical reorganization (i.e. a decrease in map area and volume) and alleviated the symptoms (Schabrun et al., 2009). It is noteworthy that findings from the same group (Schabrun and Ridding, 2007), using the same paradigm as reported in Schabrun et al. (2009) showed no significant results changes in map area and volume in healthy subjects. The authors speculated that short-term reorganization in

dystonic subjects might be driven by excessive sensitivity in the sensorimotor cortex to afferent inputs (Schabrun et al., 2009). Therefore, it might be plausible that similar mechanisms are reflected in the MS pathology. Thirdly, previous studies in healthy subjects (Mima et al., 2004, Chipchase et al., 2011, Schabrun et al., 2012) reported decreased corticospinal excitability immediately after 30 min of TENS using comparable stimulation parameters as compared to the present study. Interestingly, Schabrun et al. (2012) provided evidence for co-modulation of the primary sensory cortex (S1) and M1 in response to afferent input. More specific, the authors reported a positive correlation between decreased somatosensory evoked potentials and corticomotor excitability (Schabrun et al., 2012).

In contrast to the short-term aftereffects reported after a single stimulation session, our results showed persistent aftereffects (≥ 12h after the end of the last stimulation) suggesting that long-term potentiation (LTP) or depression (LTD) may play a role in the reorganization of the motor cortex when afferent stimulation is repeated over several days.

Even though the present findings are encouraging, some limitations need to be recognized. First, we have to be careful generalizing our results given the relatively small sample size. However, because decline in map area was consistent for all patients in the TENS but not the sham condition (Table III), it seems reasonable to conclude that a three weeks intervention with TENS is sufficient to induce reorganization of cortical motor representation of the APB muscles in patients with MS. Second, no measures of sensory and/or motor function were performed in this study. Accordingly, it was not possible to relate changes in cortical motor representation with function.

In summary we can conclude that, although the mechanisms underlying our findings are still unclear, long-term TENS treatment is able to reorganize motor maps in patients with MS. Moreover, a robust reduction in map area was reported.

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STUDY 4

Anodal tDCS increases corticospinal output and projection strength in multiple sclerosis

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2.4.1. Abstract

The application of anodal transcranial direct current stimulation (atDCS) to the human brain has been shown to elicit corticospinal (CS) excitability changes. This study evaluated the effect of a single session of anodal transcranial direct current stimulation (atDCS) on corticospinal (CS) excitability in patients with multiple sclerosis (MS).

atDCS and sham tDCS (stDCS) were applied on the primary motor cortex (M1) for 20 minutes in a double-blinded crossover design. Changes in CS excitability were assessed using transcranial magnetic stimulation (TMS).

The area under the recruitment curves increased significantly after application of atDCS (+ 56.58%, $p = 0.023$) but not after stDCS. The sigmoidal curve-analysis revealed a higher plateau of the curve after atDCS (+22.2%, $p < 0.001$).

Our results show that atDCS has the ability to increase CS output and projection strenght in MS-patients and suggest that it can be used during neural rehabilitation to facilitate motor recovery in MS.

2.4.2. Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system in which the myelin sheaths around the axons of the brain and spinal cord are damaged. As a consequence, signal transfer between central and peripheral regions is disturbed (Schmierer et al., 2000) leading to a variety of symptoms including visual disturbances, cognitive impairment, reduced tactile sensitivity, muscle weakness, coordination and balance problems. Previous transcranial magnetic stimulation (TMS) studies have reported deficits in corticospinal (CS) conduction in MS, resulting in prolonged central motor conduction latencies and reduced motor evoked potential (MEP) amplitudes. These pathology-related declines correlated significantly with demyelination, axonal loss (Hess et al., 1986, Hess et al., 1987, Caramia et al., 1988, Jones et al., 1991, Ravnborg et al., 1992) and with the expanded disability scale score (EDSS) (Salle et al., 1992, Conte et al., 2009, Kale et al., 2010). Based on the aforementioned findings, it is reasonable to suggest that a therapy enabling the modulation of MEP parameters might significantly contribute to the rehabilitation process in MS-patients.

The transcranial application of weak direct currents to the human brain has been shown to be able to elicit CS excitability changes (for a review, see Stagg and Nitsche 2011) (Stagg and Nitsche, 2011). Depending on the stimulation location and intensity, transcranial direct current stimulation (tDCS) has been shown to improve learning and performance on a variety of cognitive and motor tasks in healthy humans subjects (Nitsche and Paulus, 2000, 2001, Nitsche et al., 2005, Stagg and Nitsche, 2011). Recently, tDCS has been used to enhance motor recovery in patients suffering from various neurological diseases such as stroke (Hummel et al., 2005, Hummel et al., 2006, Tanaka et al., 2011) and Parkinson's disease (Fregni et al., 2006). Even though the underlying mechanisms of tDCS remain largely unclear, electrophysiological data suggests that direct current stimulation elicits polarity-dependent and long lasting cortical excitability changes (i.e. MEP changes) outlasting the stimulation period by up to 90 min (Nitsche and Paulus, 2000, 2001). Anodal tDCS (atDCS) over the primary motor cortex (M1) increases excitability, whereas cathodal tDCS diminishes it (Nitsche and Paulus, 2000). Furthermore, tDCS is presumed to strengthen synaptic connections through a mechanism similar to long-term potentiation (LTP), a cellular process that underlies learning (Stagg and Nitsche, 2011).
To the best of our knowledge, so far no studies have evaluated the effect of atDCS on CS excitability in patients with multiple sclerosis (MS). Because atDCS has been shown to increase CS excitability (Nitsche and Paulus, 2000) we hypothesized that M1 excitability will increase in MS-patients, following a single session of atDCS as compared to sham tDCS (stDCS) treatment.

2.4.3. Methods

2.4.3.1. Subjects

Ten MS-patients, 4 men and 6 women, aged 27 to 65 years (mean 44.90 ± 13.79) participated in this double-blinded crossover study (see Table I, for detailed patient characteristics). Patients were recruited at the REVAL Research Institute in Diepenbeek and at the Multiple Sclerosis and Rehabilitation Hospital in Overpelt. Expanded Disability Status Scale (EDSS) scores ranged between 1.5 and 4 (mean 2.50 ± 0.71). Patients showed no cognitive deficits [Montreal Cognitive Assessment (MoCa) score ≥ 26], exhibited stable MS (no relapse 3 months prior to inclusion), and they were screened for other pathologies associated with peripheral and/or central sensory dysfunction and for central nervous system-acting, psychotropic or antiepileptic medication intake. Finally, patients were screened for TMS contra-indications (Wassermann, 1998). Each participant provided written informed consent and experimental procedures were approved by the local Ethics Committee of the University of Hasselt according to the Declaration of Helsinki.

Table I. Patient characteristics

										FUNCTIONAL SYSTEM SCORES						
ID	AGE	SEX	MoCa	OLDFIELD (LQ)	STIMULATION SITE	FIRST SYMPTOM	DIAGNOSIS	MS TYPE	VISUAL	BRAINSTEM	PYRAMIDAL	CEREBELLAR	SENSORY	BLADDER/BOWEL	MENTAL	EDSS
1	32	M	26	-100	L	jan-04	may-04	RRMS	0	2	2	2	2	0	0	3
2	60	M	27	100	R	jan-96	jan-96	RRMS	1	0	3	3	2	1	2	4
3	27	F	30	100	R	jun-09	jul-09	RRMS	0	0	1	1	2	0	1	2
4	44	F	27	100	R	jan-89	feb-89	RRMS	0	0	2	2	2	1	1	3
5	58	F	29	89.5	R	aug-07	mrt-09	RRMS	0	1	2	1	2	1	0	2.5
6	38	F	29	100	R	jan-09	oct-10	RRMS	0	1	2	1	1	1	1	2
7	65	M	27	100	R	jan-01	jan-02	PPMS	0	1	0	1	1	2	2	2.5
8	43	F	27	40	L	jan-05	may-09	RRMS	0	1	2	1	0	1	1	2
9	54	F	26	90	R	dec-04	dec-04	RRMS	0	1	2	1	2	1	1	2.5
10	28	M	30	100	R	jan-08	may-08	RRMS	0	0	1	1	0	0	0	1.5

2.4.3.2. Experimental design

First, the Nine-hole Peg Test was administered to determine the most impaired hand. Subsequently, patients participated in a double-blind crossover procedure. In two pseudo-randomized, counterbalanced sessions separated by at least one week, patients received either atDCS or stDCS. The effect of the intervention on CS excitability was measured using TMS, before and immediately after the intervention.

2.4.3.3. Transcranial direct current stimulation (tDCS)

Patients received either atDCS (HDCstim, Newronika, Italy) with an intensity of 1 mA for 20 min or stDCS (1mA for 12sec, to mimic the initial sensation associated with atDCS) in two separate sessions. The site for stimulation was determined by TMS. Specifically, the anode (surface 25cm^2) was centered on the hotspot (i.e. the optimal scalp position) of the First Dorsal Interosseous (FDI) contralateral to the most impaired hand and the cathode (surface 50cm^2) was fixed on the contralateral supraorbital region. The cathode size was increased to make this electrode functionally inert (Nitsche et al., 2007).

2.4.3.4. Transcranial magnetic stimulation (TMS)

Magnetic stimuli (Magstim BiStim^2, Whitland, South West Wales, UK) were delivered by a 70-mm loop-diameter figure-of-eight coil. To ensure reproducibility of measurements across sessions, a customized cap was made out of thermoplastic material (Aquaplast-T Solid, 20 cm x 15 cm x 0.24 cm, Sammons Preston Polyon, Cedarburg) with references to anatomical landmarks (left and right external auditory meatus, occiput and vertex) for each subject. An orthogonal coordinate system was marked on each cap. Then, the hotspot of the FDI muscle was determined. The coil was positioned on the hemisphere contralateral to the most impaired hand with the coil handle pointing backward and rotated 45° away from the midsagital line. Next, the rMT was defined as the lowest stimulation intensity evoking MEPs with an amplitude larger than 50 μV peak-to-peak in at least five of ten consecutive trials. Finally, the recruitment curve was determined using TMS intensities of 70, 90, 110, 130, 150, 170 and 190% relative to the rMT. The interval between TMS stimuli was randomized (5-8sec). Stimulation intensities were provided 5 times in a pseudo-randomized order.

2.4.3.5. Electromyographic recordings (EMG)

Electromyographic signals from the FDI muscle were measured using EMG. After amplification (gain = 1000), bandpass filtering (4–1500 Hz) (Bagnoli-16, Delsys Inc, Boston, USA) and 50/60 Hz noise elimination (Humbug, Quest Scientific, North Vancouver, Canada) the recorded EMG signals were digitized at 5000 Hz (CED Signal Version 3.03, Cambridge Electronic Design, Cambridge, UK) and were stored on a laboratory computer for offline analysis.

2.4.3.6. Data analysis

Before analysis, individual MEPs were screened and excluded (<3%) if the root mean square EMG exceeded 5 μV during the 50-ms period immediately preceding the onset of the TMS pulse. To correct for inter-individual differences in absolute MEP amplitudes, MEPs were normalized to the maximum MEP value (i.e. mean of 5 pulses) measured at baseline for each intervention session. The Kolmogorov-Smirnov test was used to test the assumption of normality of the data.

Advanced linear applications (SAS 9.2, SAS institute Inc., Cary, NC) were used for statistical analysis of the area under the recruitment curve (AURC). AURC is a robust marker of overall CS output and projection strength (Carson et al., 2013; Pitcher et al., 2009; Talelli et al., 2008) and was calculated with the following algorithm: $yi(xi + 1 - xi) + (1/2)(yi + 1 - yi)(xi + 1 - xi)$, where y is the stimulus intensity and x is the MEP amplitude at a given intensity. Paired sample t-tests were applied to test for differences in AURC between conditions (atDCS post – atDCS baseline vs. stDCS post – stDCS baseline) and within each condition (atDCS post vs. atDCS baseline and stDCS post vs. stDCS baseline).

Advanced non-linear models applications (SAS 9.2, SAS institute Inc., Cary, NC) were used for statistical analysis of the 5-parameter sigmoidal curve as described by the following equation (see also, Pitcher et al. 2003)(Pitcher et al., 2003):

$$f = f_0 + a/(1 + \exp(- (int - int_0)/b))^c,$$

Where 'f' is defined as the normalized MEP value at each TMS intensity, calculated as the ratio between the MEP value at that intensity and the maximum MEP value at baseline within the corresponding session. With respect to the five parameters model the following definitions were used: 'f_0'represents the normalized MEP value at the lowest TMS intensity. Since for all subjects this value was equal to zero, the parameter was not included in the model. 'a' was defined as the largest value for 'f', which is reached at

the higher intensities. 'b' represents the difference between the TMS intensity at 25% and 75% of the maximum 'f' value. 'int_0' was defined as the TMS intensity required to obtain 50% of the maximum 'f' value. 'c' was defined as the slope constant. Finally, it was explored to what extent the above mentioned parameters were related to tDCS condition (atDCS vs. stDCS) and time (baseline vs. post) and/or their interaction. The significance level was set at $p < 0.05$.

2.4.4. Results

2.4.4.1. Rest motor threshold

Mean rMTs for both tDCS conditions were similar (rMT stDCS ± StDev. = 36.3 ± 5.17; rMT atDCS ± StDev. = 36.6 ± 5.23; t = 0.19, p > 0.05).

2.4.4.2. Recruitment curve characteristics

Data illustrating CS excitability changes after atDCS and stDCS are shown in Figure 1.

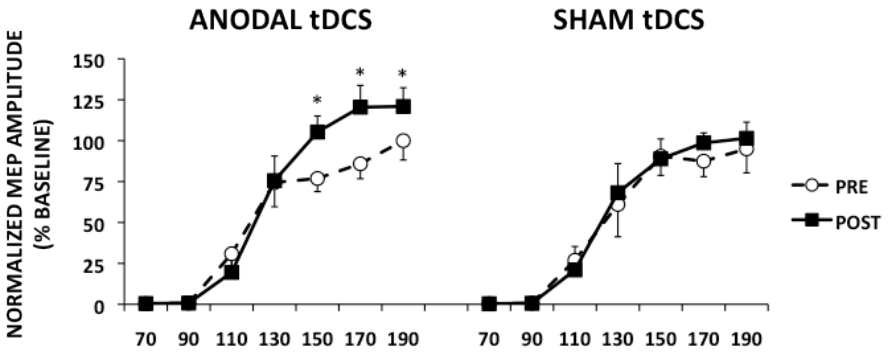

Figure. 1 Recruitment curves for anodal tDCS and sham tDCS are shown. The normalized MEP amplitude (expressed as % of the maximum MEP at baseline for each subject) for each TMS intensity is represented before (white dots) and after (black squares) the intervention.

Area under the recruitment curve (AURC)

A paired sample t-test revealed a significant difference between the increase of AURC for atDCS as compared to stDCS (net difference: +56.58%, t = 2.74, p = 0.023). Within the atDCS condition AURC was significantly higher at post atDCS as compared to baseline atDCS (+66.5%, t = 4.51, p = 0.001). For the stDCS condition no significant change in AURC was found (+9.92%, t = 0.59, p > 0.05).

Sigmoidal curve-analysis

Statistical analysis revealed a significant interaction effect between treatment and time on parameter 'a' ($p < 0.001$). In other words, the maximum MEP value ('f') was significantly higher at post atDCS. More specifically, the final plateau was estimated to be 22.2% higher for post atDCS (parameter estimate: 119.77 ± 3.19) as compared to the other measurements (baseline atDCS, baseline stDCS and post stDCS; parameter estimate: 97.57± 2.50). For the parameters 'b', 'c', and 'int_0' no significant influence was found (all, $p > 0.05$). As mentioned earlier, the parameter 'f_0' was set to zero.

2.4.5. Discussion

The present study shows for the first time that a single session of atDCS applied to M1 contralateral to the more severely impaired hand of MS-patients leads to increased CS output and projection strength. This finding is in line with evidence from previous studies in healthy subjects (Nitsche et al., 2005) and stroke patients (Hummel et al., 2005) reporting increased CS excitability after atDCS. Whereas our findings showed no significant difference in the steepness of the slope of the recruitment curves between the pre-and post-TMS sessions in the atDCS group, we did find that CS excitability increased significantly at the higher stimulation intensities after atDCS resulting in a higher plateau. As compared to Hummel et al. (2005) (Hummel et al., 2005) and Nitsche et al. (2005) (Nitsche et al., 2005) who studied tDCS-induced slope changes at TMS intensities between 100 and 150% rMT, our range of intensities was set between 70 and 190% rMT. This range was chosen to ensure that the entire recruitment curve, including the lower and the upper plateau, was measured. Previously, it was reported that higher TMS intensities activate large-diameter myelinated axons that are further remote from the stimulation site (Siebner and Rothwell, 2003). Therefore, a plausible explanation for the increased plateau might be that atDCS lead to an increased activation of these large-diameter myelinated axons at higher distance from the stimulation site. This explanation is supported by evidence from a recent study with resting state fMRI, showing that a 10 min session of atDCS over the scalp's M1 representational field of the right hand in healthy humans enhanced long distance functional communication within M1 (Polania et al., 2012). Consequently, the present observations generally confirmed the hypothesis that a single-session of atDCS over the primary motor cortex in MS patients can increase cortical excitability, as previously reported in healthy individuals. This may have occurred primarily via increasing membrane depolarization (Nitsche and Paulus, 2000, 2001, Nitsche et al., 2005).

Findings from functional magnetic resonance imaging (fMRI) suggest that cortical reorganization in MS patients is reflected by local synaptic reorganization, recruitment of parallel existing pathways, and reorganization at distant sites (Filippi et al., 2004). Furthermore, it has been shown that functional connectivity in the resting-state network underlying sensorimotor functions in MS patients is reported to increase at the early stage of the disease (Faivre et al., 2012). Interestingly, Polania et al (Polania et al., 2012) revealed in healthy subjects that the more distributed the functional architecture of M1 was prior to atDCS, the more efficient the atDCS-induced functional modulations were. As the present intervention with atDCS may possibly have influenced excitability of cortical interneurons at a distance from the stimulation site, we propose that interneural connections within M1 as well as interneural projections from other cortical regions to M1 may have been affected by the intervention.

Failure to affect the steepness of the recruitment curve in MS patients with atDCS could be attributed to weaker inhibition in MS, possibly due to loss of GABAergic interneurons (Clements et al., 2008). Increased excitability is expected to result in recruitment of larger neuronal pools with TMS, causing a ceiling effect around the hotspot. Conversely, the observed increased plateau of the recruitment curve in the intervention group but not in the sham group suggests that atDCS increased the gain of motor output in areas that are distant from the hotspot.

Although the mechanisms underlying the effect of tDCS on M1 excitability in MS remain unclear, it is likely that atDCS also had an effect on GABAergic, adrenergic and glutamatergic processes as the shape of the recruitment curve seems to be sensitive for detecting changes in these processes (Chen, 2000, Di Lazzaro et al., 2003). Additionally, studies using drug administration protocols combined with tDCS reported that the aftereffects depend on several factors, such as membrane polarization (Nitsche et al., 2003), synaptic modulation (Liebetanz et al., 2002, Nitsche et al., 2003) and $GABA_A$ergic interneurons (Nitsche et al., 2004).

The present findings may have significant implications for neural rehabilitation in MS-patients as several studies already indicated that atDCS has the ability to enhance reaction times (Fregni et al., 2006, Hummel et al., 2006), pinch force (Hummel et al., 2006, Tanaka et al., 2011), motor control (Hummel et al., 2005, Madhavan et al., 2011), and motor learning (Galea and Celnik, 2009, Fritsch et al., 2010, Tecchio et al., 2010) in different populations. Although we did not investigate the functional effects of atDCS in the current study, it is reasonable to hypothesize that atDCS on M1 might increase the efficiency of the rehabilitation process in MS. In this respect, a recent study (Mori et al., 2012) reported that a five-day intervention with atDCS applied to the somatosensory cortex of MS-patients ameliorated tactile sensitivity with long-lasting beneficial effects. However, there are currently no studies reporting beneficial effects on motor function after the application of atDCS on M1 in MS.

Even though the present findings are encouraging, some limitations need to be recognized. Firstly, we have to be careful generalizing our results given the relatively small sample size. Secondly, including functional outcome measures might be required to determine the functional relevance of atDCS in MS rehabilitation. Finally, the inclusion of other (inter- and intrahemispheric) TMS measures might increase the understanding of the underlying mechanisms of atDCS-induced CS excitability changes in MS patients.

In summary, we conclude that atDCS can up-regulate CS output and projection strength in MS-patients. Furthermore, our findings may pave the way for application of tDCS as a complementary therapeutic tool for neural rehabilitation in MS.

2.4.6. References

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STUDY 5

A single session of 1mA anodal tDCS-supported motor training does not improve motor performance in patients with multiple sclerosis

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2.5.1. Abstract

Anodal transcranial direct current stimulation (atDCS) has been shown to improve motor learning in healthy subjects and neurodegenerative populations. Until now the effects of atDCS on motor learning in patients with multiple sclerosis (MS) are not examined.

In the current study, a sham controlled double-blind crossover design was used to evaluate the effect of 20 minutes of 1mA atDCS or sham tDCS (stDCS) on a unimanual motor sequence-training task, consisting of sequential finger presses on a computer keyboard with the most impaired hand. Patients received stimulation (atDCS or stDCS) during motor training. tDCS was applied over the primary motor cortex contralateral to the most impaired hand. Motor performance was assessed immediately before, during and 30 minutes after stimulation.

Although we need to be careful with the interpretation of the data due to lack of power, our results showed no significant effect of atDCS on motor performance.

Our findings indicate that atDCS-supported motor training was not able to improve motor performance more than sham-supported motor training. Possibly, the effects of atDCS are mediated by specific MS-related characteristics. Furthermore, increasing atDCS intensity and offering multiple stimulation sessions might be necessary to optimize motor performance resulting from atDCS-supported motor training.

2.5.2. Introduction

Recently, transcranial direct current stimulation (tDCS) has been applied for improving motor function in healthy subjects and patient populations. Studies in stroke (Hummel et al., 2005, Hummel et al., 2006, Madhavan et al., 2011, Tanaka et al., 2011), Parkinson's disease (Fregni et al., 2006) and healthy aging (Hummel et al., 2010) showed that a single session of anodal tDCS (atDCS) over the primary motor cortex (M1) was sufficient to improve motor performance, reaction time (Fregni et al., 2006, Hummel et al., 2006), pinch force (Hummel et al., 2006, Tanaka et al., 2011), motor control (Hummel et al., 2005, Madhavan et al., 2011), and motor learning (Galea and Celnik, 2009, Fritsch et al., 2010, Tecchio et al., 2010) significantly.

Although the underlying mechanisms of tDCS remain largely unclear, previous reports (Nitsche and Paulus, 2000, 2001) revealed that a single-session of direct current stimulation induced sustained (up to 90 minutes) and polarity-dependent cortical excitability changes. Furthermore, atDCS is presumed to influence the resting membrane potential during stimulation; and to modulate GABAergic and glutamatergic synapses within the cortex after stimulation (Stagg and Nitsche, 2011). There is strong evidence that motor training combined with atDCS applied on the primary motor cortex (M1) improves motor performance (Nitsche et al., 2003, Reis et al., 2009, Reis and Fritsch, 2011, Kantak et al., 2012, Lefebvre et al., 2012, Zimerman et al., 2012).

Until now, there is no evidence that the combination of motor training and atDCS improves motor performance in patients with MS. MS is an inflammatory disease in which the myelin sheaths around the axons of the brain and spinal cord are damaged, leading to a disturbed signal transfer between central and peripheral regions. Despite of this dysfunctional signal transfer, evidence from a recent magnetic resonance imaging (MRI) study (Tomassini et al., 2011) confirmed that the potential to learn new motor skills is preserved in MS patients, provided that the potential for functional reorganization remains relatively unimpaired (Schoonheim et al., 2010).

The aim of the current study was to evaluate the effect of a single atDCS session combined with a unimanual sequence-training task on motor performance in patients with mild to moderate MS. We hypothesize that atDCS-supported motor training leads to superior motor performance as compared to sham-supported motor training.

2.5.3. Methods

2.5.3.1. Subjects

Thirty-one patients with MS (9 men and 22 women) aged 27 to 65 years (mean ± SD: 48.16 ± 10.13 years) participated in this double-blinded crossover design (see Table I for patient characteristics). Expanded Disability Status Scale (EDSS) scores ranged between 1.5 and 6.5 (mean ± SD 3.15 ± 1.22). Patients were recruited at REVAL Research Institute in Diepenbeek and the Multiple Sclerosis and Rehabilitation Hospital in Overpelt. Experimental procedures were approved by the Ethical Committee of the University of Hasselt according to the Declaration of Helsinki. All patients gave their written consent prior to the study. Handedness was assessed with the Edinburgh Handedness Inventory (Oldfield, 1971). Twenty-nine patients were right-handed (mean LQ ± SD = 89.43 ± 18.74) and two were left-handed (mean LQ ± SD = -58.35 ± 58.90). Patients showed no cognitive deficits (score ≥ 26 on the Montreal Cognitive Assessment Test, mean ± SD: 28.00 ± 1.34) and exhibited stable MS, showing no relapse for at least 3 months prior to the study. Before inclusion, patients were screened for other pathologies associated with peripheral and/or central sensory dysfunction, psychotropic or antiepileptic medication intake and contra-indications for tDCS.

Table I. Patient Characteristics

							FUNCTIONAL SYSTEM SCORES							
ID	AGE	SEX	FIRST SYMPTOM	DIAGNOSIS	MS TYPE	VISUAL	BRAINSTEM	PYRAMIDAL	CEREBELLAR	SENSORY	BLADDER/BOWEL	MENTAL	EDSS
1	51	F	aug-01	okt-06	SPMS	0	0	2	2	2	3	1	4.5
2	32	M	jan-08	may-04	RRMS	0	2	2	2	2	0	0	3
3	57	M	dec-10	jun-11	RRMS	0	0	1	1	3	1	0	3
4	60	F	jan-98	jan-98	SPMS	0	0	1	1	2	1	0	2
5	58	M	jan-96	jan-96	RRMS	1	0	3	3	2	1	2	4
6	34	F	nov-13	nov-14	RRMS	0	0	1	2	2	1	0	2.5
7	27	F	jun-13	jul-13	RRMS	0	0	1	1	2	0	1	2
8	61	M	jan-00	jul-01	SPMS	0	0	2	2	2	1	0	3
9	51	F	dec-93	dec-93	SPMS	0	2	2	3	3	1	1	4
10	44	F	jan-93	feb-93	RRMS	0	0	2	2	2	1	1	3
11	52	F	aug-04	sep-04	RRMS	1	0	1	2	2	1	1	2.5
12	42	F	jan-07	feb-07	RRMS	1	0	2	2	2	1	1	3
13	60	F	jan-04	jan-05	SPMS	0	1	2	2	2	1	2	5.5
14	58	F	aug-11	mrt-13	RRMS	0	1	2	1	2	1	0	2.5
15	48	F	sep-09	sep-09	RRMS	0	0	2	1	2	1	1	2.5
16	46	F	oct-00	nov-04	RRMS	0	0	2	1	1	1	1	2
17	54	F	jan-84	jan-91	SPMS	0	2	2	3	3	1	0	6.5
18	61	F	jan-73	jan-87	SPMS	0	0	2	2	2	1	1	3
19	38	F	jan-13	oct-10	RRMS	0	1	2	1	1	1	1	2
20	65	M	jan-05	jan-06	PPMS	0	1	0	1	1	2	2	2.5
21	49	M	jan-92	jan-12	RRMS	0	0	3	2	2	1	1	3.5
22	55	F	feb-12	feb-12	RRMS	0	1	1	1	2	1	0	2
23	45	F	apr-92	apr-02	SPMS	0	2	3	3	2	1	1	4
24	43	F	jan-09	may-09	RRMS	0	1	2	1	0	1	1	2
25	41	M	jan-10	jan-11	SPMS	0	1	2	3	3	1	0	4
26	54	F	dec-08	dec-08	RRMS	0	1	2	1	2	1	1	2.5
27	43	M	jul-11	jul-11	RRMS	0	1	1	0	2	0	0	2
28	28	M	jan-12	may-08	RRMS	0	0	1	1	0	0	0	1.5
29	55	F	jan-02	feb-02	PPMS	0	1	3	2	3	1	0	6
30	43	F	sep-12	mar-10	RRMS	0	0	3	1	2	0	1	3.5
31	38	F	jan-08	feb-08	RRMS	0	1	3	1	2	1	1	3.5

2.5.3.2. Experimental design

Prior to the experiment, the Nine-hole Peg Test was administered to assess motor performance of each hand separately to determine the most impaired hand (called the 'intervention hand'). The mean time required to perform the test was 25.16 (± 7.20 SD) seconds for the intervention hand and 21.53 (± 5.38 SD) seconds for the least impaired hand ($p < 0.0001$; paired t-test). Subsequently, patients moved on to a double-blind (both the experimenter applying the stimulation and the patient were blinded for the intervention) crossover procedure. In two pseudo-randomized, counterbalanced sessions separated by at least a week, patients received either atDCS or sham tDCS (stDCS) on M1 contralateral to the intervention hand while performing a unimanual sequence-training task.

2.5.3.3. Motor training

Patients were instructed to perform a unimanual sequence-training task (Cuypers et al., 2013) consisting of sequential finger presses using the intervention hand (see Fig. 1). They were seated in front of a computer screen and were instructed to press the key corresponding to the number on the screen with one of the four fingers (2nd - 5th) as quickly and as accurately as possible. In a single session patients performed a total of 26 blocks. Motor performance was measured prior (baseline, 3 blocks), during training (20 blocks) and 30 minutes after the end of the training (post-intervention, 3 blocks). In a single block, sequences were initiated in 30-second time frame. Each block was terminated after completion of the last sequence. Patients were instructed to perform as many correct sequences as possible; therefore the amount of sequences provided during each block depended on the speed of the patient. Each time a key was pressed a black dot appeared beneath the corresponding number. No feedback about the correctness of the performance was provided. The sequences were pseudo-randomized and counterbalanced over the sessions and had the same level of difficulty. The sequences were [4 2 1 3 4 2 3 2] and [2 4 3 1 2 3 2 4] (1 = index finger, 2 = middle finger, 3 = ring finger and 4 = little finger).

Fig 1. Subjects were instructed to perform a 8-element finger sequence with the dominant hand by pressing different keys, each corresponding to one of the four fingers (2nd - 5th).

2.5.3.4. Non-invasive cortical stimulation

During motor training patients received either atDCS (HDCstim, Newronika, Italy) or stDCS on M1 contralateral to the intervention hand. The anode (surface 25cm^2) was centered on the cortical representation field (hotspot) of the First Dorsal Interosseous (FDI) as determined by transcranial magnetic stimulation (TMS). The cathode (surface 50cm^2) was fixed on the contralateral supraorbital region. By increasing the size of the cathode this electrode the current density (0.02 mA/cm^2) and consequently, the efficacy of this electrode will be reduced, since the efficacy of tDCS seem to depend on the current density under the electrode (Nitsche et al., 2007). Stimulation was delivered with a current intensity of 1mA for 20min. In the stDCS condition the same current intensity was delivered but only during the first 12 seconds.

2.5.3.5. Psychophysical assessment

In each session visual analogue scales (VAS) were provided to assess the level of attention, fatigue, and pain/discomfort during the experiment. In addition, sleep duration and sleep quality (VAS) was also assessed.

2.5.3.6. Data analysis

Advanced linear models applications (SAS 9.2, SAS institute Inc., Cary, NC) were used for statistical analysis. Prior to analysis, scores for the compound measures [percentage correct sequences/mean inter tap interval (ITI) and percentage correct key presses/mean ITI], were normalized (%) to baseline for each subject separately.

To evaluate the effect of tDCS during motor training over time, a mixed model including fixed effects for condition (atDCS vs. stDCS), time (20 training blocks) and their interaction, was used to estimate the rate of change (i.e. slope-analysis) of motor performance. More specifically, the following parameters were tested: percentage correct sequences/mean ITI, percentage correct key presses/mean ITI, percentage correct sequences, percentage correct key presses, mean ITI, and mean number of correct sequences in the performance interval.

To reveal the effect of tDCS-induced motor training on motor performance at post-intervention, paired t-tests were applied to evaluate the evolution of motor performance within conditions and between conditions. In addition, a power analysis was performed on the current data to calculate the minimum sample size required to detect an effect of a given size. The significance level was set at $p < 0.05$.

2.5.4. Results

2.5.4.1. Baseline motor performance

At baseline, paired t-tests revealed no significant differences in performance between the different stimulation conditions for none of the parameters (all, $p > 0.05$). The results for each parameter are illustrated in Figure 2.

2.5.4.2. Motor performance during tDCS-supported training

The slope analysis revealed no significant effects for condition and for the interaction between condition and time during motor training for none of the parameters (all, $p > 0.05$), indicating that atDCS did not significantly contribute to motor performance. With respect to the effect of time, the slope analysis revealed significant effects for percentage correct sequences/mean ITI ($p < 0.001$), percentage correct key presses/mean ITI ($p < 0.001$), percentage correct sequences ($p < 0.001$), percentage correct key presses ($p < 0.045$), and mean ITI ($p < 0.001$). The mean number of correct sequences in the performance interval did not significantly change over time ($p > 0.05$).

2.5.4.3. Motor performance at post-intervention

Effects of tDCS on motor performance

At post-intervention, no significant differences in motor performance between the atDCS and stDCS condition were found, indicating that there was no additional effect of the intervention over time. (all, $p > 0.05$). For the parameter mean number of correct sequences in the performance interval, a marginal trend was found for the atDCS condition ($p = 0.077$).

A power analysis showed insufficient power for all parameters (see Table II).

Table II. Power Analysis

PARAMETER	POWER (%)	SUBJECTS REQUIRED FOR A POWER OF 80%
percentage correct sequences/mean ITI	17.7	211
percentage correct key presses/mean ITI	11.8	386
percentage correct sequences	12.1	368
percentage correct key presses	5	89000
mean ITI	6	1554
mean correct sequences in the performanc	42.5	73

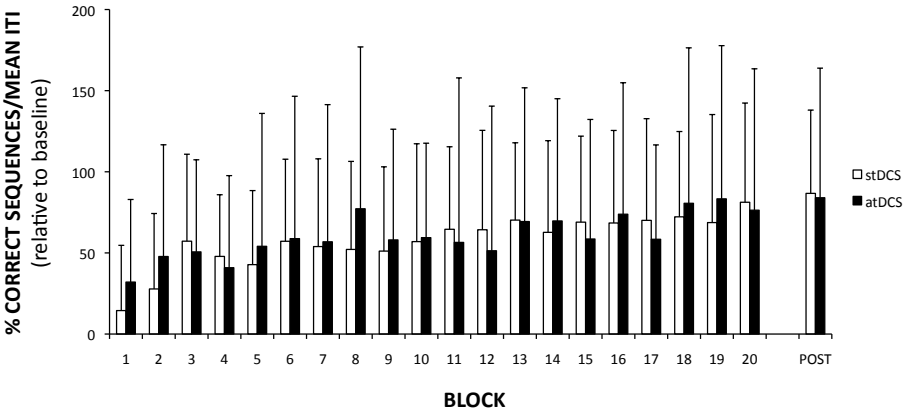

Fig 2a. Evolution of the percentage correct sequences/intertab interval (ITI) during motor learning and at post-intervention (relative to baseline) for the atDCS and stDCS condition.

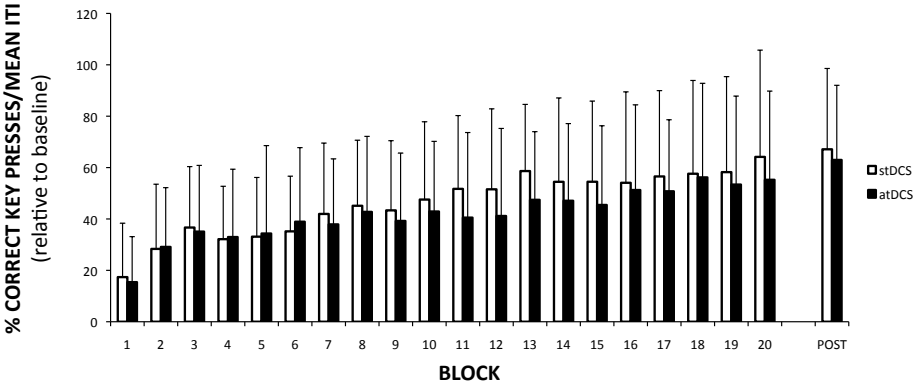

Fig 2b. Evolution of the percentage correct sequences/intertab interval (ITI) during motor learning and at post-intervention (relative to baseline) for the atDCS and stDCS condition.

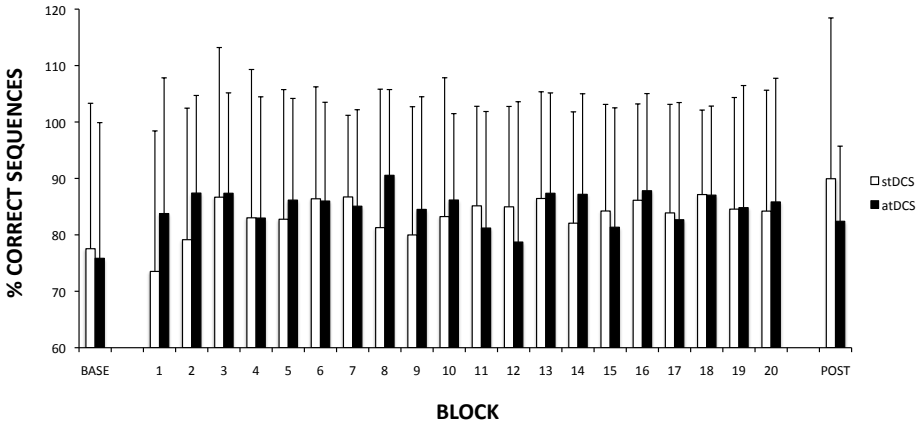

Fig 2c. Evolution of the percentage correct sequences at baseline, during motor learning and at post-intervention for the atDCS and stDCS condition.

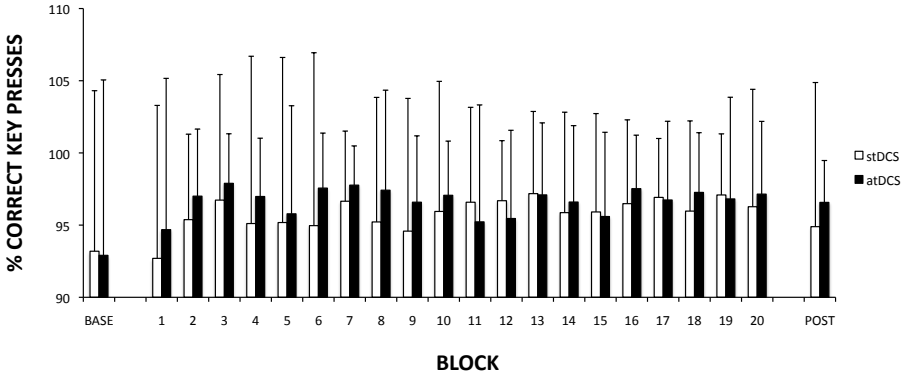

Fig 2d. Evolution of the percentage correct key presses at baseline, during motor learning and at post-intervention for the atDCS and stDCS condition.

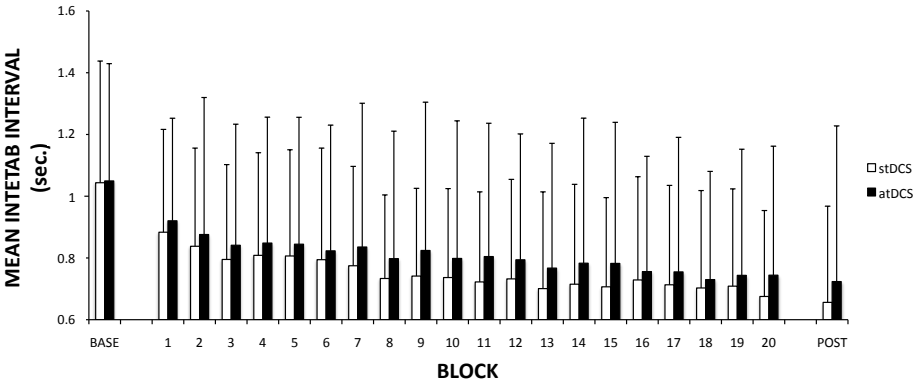

Fig 2e. Evolution of the mean intertab interval at baseline, during motor learning and at post-intervention for the atDCS and stDCS condition.

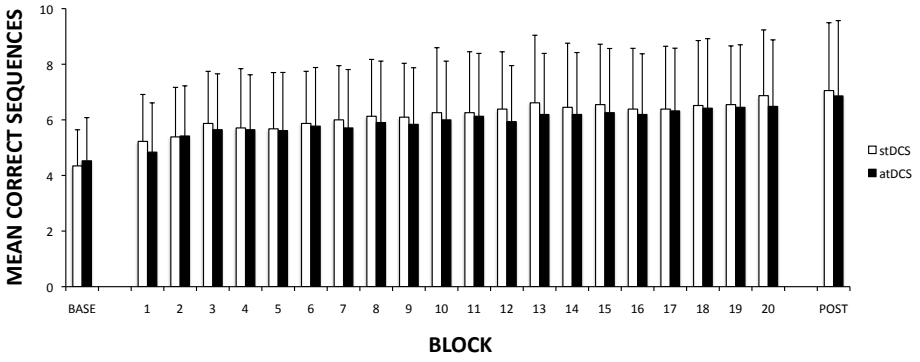

Fig 2f. Evolution of the mean number of correctly performed sequences at baseline, during motor learning and at post-intervention for the atDCS and stDCS condition.

Overall training effects

The following parameters improved after motor training (at post-intervention) for the atDCS condition: percentage correct sequences/mean ITI ($p < 0.0001$), percentage correct key presses/mean ITI ($p < 0.0001$), mean ITI ($p < 0.0001$), mean number of correct sequences in the performance interval ($p < 0.0001$)

For the stDCS condition, the percentage correct sequences/mean ITI ($p < 0.0001$), percentage correct key presses/mean ITI ($p < 0.0001$), percentage correct sequences ($p = 0.010$), mean ITI ($p < 0.0001$), mean number of correct sequences in the performance interval ($p < 0.0001$) improved after motor training.

All other parameters did not change significantly (all, $p > 0.05$).

2.5.4.4. Psychophysical assessment

Paired sample t-tests revealed no significant differences for the level of attention, fatigue, pain/discomfort, sleep duration and sleep quality (all, $p > 0.05$; see Table III).

Table III. Psychophysical assessment. The amount of sleep (±StDev) is reported. Visual analog scales scores (±StDev) are shown for sleep quality (1=bad sleep quality; 10=excellent sleep quality), attention (1=no attention; 10=highest level of attention), fatigue (1=highest level of fatigue; 10= no fatigue) and pain/discomfort (1=no pain/discomfort; 10= maximal level of pain/discomfort).

		VISUAL ANALOG SCALE SCORE			
condition	sleep (hours)	sleep quality	attention	fatigue	pain/discomfort
atDCS	7.61 (1.63)	7.10 (2.04)	8.16 (1.32)	3.06 (2.82)	0.84 (1.98)
stDCS	7.63 (1.08)	6.90 (2.19)	7.97 (1.50)	2.93 (2.70)	0.70 (1.70)

2.5.5. Discussion

The present study is the first to address the question whether a single session of anodal tDCS stimulation on M1 contralateral to the target hand was able to improve motor performance in MS patients. Based on the findings reported in other neurodegenerative populations (Hummel et al., 2005, Fregni et al., 2006, Hummel et al., 2006, Madhavan et al., 2011, Tanaka et al., 2011), we hypothesized that atDCS-supported training will lead to superior motor performance as compared to sham-supported training.

Our results indicated that atDCS-supported motor training was not able to improve motor performance more than sham-supported motor training. This result is in contrast with findings in stroke (Hummel et al., 2005, Hummel et al., 2006, Madhavan et al., 2011, Tanaka et al., 2011) and healthy aging (Hummel et al., 2010) indicating that a single session of tDCS during motor training was sufficient to significantly improve motor performance as compared to sham-supported motor training. Our results can be explained in several ways.

Firstly, we have to be aware that the statistical power in this study was low, making the interpretation of the current results difficult. Although the statistical analysis did not reveal any significant effect of the intervention for the different parameters, we cannot conclude that there was no effect (due to lack of power). According to the power analysis more subjects are required to reach acceptable statistical power (80%).

Secondly, it is possible that performance improvements are limited (Morgen et al., 2004) or occur slower in MS patients. In this respect, Hatzitaki et al. (2006) reported that visuo-motor learning occurred at a lesser extent in patients with MS as compared to healthy controls (Hatzitaki et al., 2006). Additionally, it was reported that motor performance in MS patients was highly variable. This variability could be attributed to the widespread and unpredictable nature of demyelization of the central nervous system affecting motor performance in MS (Hatzitaki et al., 2006). Additionally, Casadio et al. (2008) showed that MS patients achieved close-to-normal motor function by performing a greater proportion of micro-adjustments to compensate for partly incorrect descending commands (Casadio et al., 2008). Although we chose to train the most impaired hand from a therapeutically point of view, it might be argued that (based on symptom severity) more variability would be expected when training this hand. However, as we did not train and/or collected sequence-training data of the least impaired hand in the current study, we cannot discuss this issue. Based on the findings mentioned above, we can assume that if individual motor performance variability is too high, as a result of MS, the contribution of atDCS-induced motor performance might be washed out.

Third, nonetheless a recent study of our group reported that 20 min of 1mA atDCS is sufficient to increase corticospinal excitability in a

comparable group (age, symptoms, EDSS) of MS patients (Cuypers et al., in press), it might be possible that atDCS induces excitability changes on the cortical level in absence of sufficient/efficient signal transfer to the peripheral level, required for optimal motor performance. As mentioned earlier it is reported that the signal transfer between central and peripheral regions is disturbed. Studies using TMS showed significant correlations between disability and TMS abnormalities in MS patients (Sahota et al., 2005, Thickbroom et al., 2005, Kale et al., 2009). More specifically, parameters such as MEP amplitude, MEP latency and central motor conduction time were abnormal as compared to healthy controls.

A fourth explanation is that tDCS intensity might be too low to induce atDCS-supported training effects in a single session. Recently, our group (Cuypers et al., 2013) reported that stimulation intensity plays an important role in obtaining the desired results. Furthermore, it was reported that 20 minutes of atDCS-supported motor training at 1.5mA significantly improved online and offline motor performance in healthy subjects as compared to sham-supported motor training. Between atDCS-supported motor training at 1mA and sham no significant differences were reported.

Fifth, it might be reasonable that a single session was not sufficient to obtain the desired therapeutic result and that multiple sessions are required. Recently, Mori et al. (2012) evaluated the effect of atDCS on tactile sensation in MS. Although they did not found any beneficial effects after the first stimulation session, they reported that a 5-day course of atDCS was sufficient to ameliorate tactile sensory loss with long-lasting beneficial effects (Mori et al., 2012). In line with this finding, Reis et al. (2009) found that atDCS enhanced skill acquisition in healthy subjects after 5 consecutive atDCS-supported motor training sessions (Reis et al., 2009). Interestingly, they reported no differences in online skill acquisition between the atDCS and the stDCS conditions. Instead, the atDCS-supported learning effect was mediated by beneficial offline effects referred to as 'motor consolidation'.

In summary, our findings indicate that atDCS-supported motor training was not able to improve motor performance more than sham-supported motor training. Possibly, effects of atDCS are mediated by specific MS-related characteristics. Furthermore, increased atDCS intensity and multiple stimulation sessions might be necessary to optimize motor performance resulting from atDCS-supported motor training.

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STUDY 6

Is motor learning mediated by tDCS intensity?

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2.6.1. Abstract

Although tDCS has been shown to improve motor learning, previous studies reported rather small effects. Since physiological effects of tDCS depend on intensity, the present study evaluated this parameter in order to enhance the effect of tDCS on skill acquisition.

The effect of different stimulation intensities of anodal tDCS (atDCS) was investigated in a double blind, sham controlled crossover design. In each condition, thirteen healthy subjects were instructed to perform a unimanual motor (sequence) learning task.

Our results showed (1) a significant increase in the slope of the learning curve and (2) a significant improvement in motor performance at retention for 1.5mA atDCS as compared to sham tDCS. No significant differences were reported between 1mA atDCS and sham tDCS; and between 1.5mA atDCS and 1mA atDCS.

2.6.2. Introduction

Recently, transcranial direct current stimulation (tDCS) has been shown to be effective for improving motor learning (Kang and Paik 2011;Reis et al. 2009) and enhancing motor recovery in healthy subjects and patients suffering from neurological diseases such as stroke (Hummel et al. 2005;Hummel et al. 2006;Tanaka et al. 2011) and Parkinson's disease (Fregni et al. 2006).

Electrophysiological data suggest that direct current stimulation elicits polarity-dependent and long-lasting cortical excitability changes outlasting the stimulation period by up to 90 min (Nitsche and Paulus 2000;Nitsche and Paulus 2001). Furthermore, tDCS is presumed to strengthen synaptic connections through a mechanism similar to long-term potentiation (LTP), a cellular mechanism that underlies learning (Cheeran et al. 2008;Stagg and Nitsche 2011). Fritsch et al. (2010) proposed that tDCS might improve motor skill learning through augmentation of synaptic plasticity within the primary motor cortex (M1). Previous work demonstrated that M1 participates in both fast on-line learning (Karni et al. 1995;Ungerleider et al. 2002) and in early stages of consolidation in motor sequence learning (Muellbacher et al. 2002).

Whereas several studies reported clinically meaningful beneficial effects of a single session of 1mA anodal tDCS (atDCS) in patient populations (Hummel et al. 2005;Hummel et al. 2006;Tanaka et al. 2011;Fregni et al. 2006), less strong effects are reported in studies conducted in healthy subjects. Until now, a current intensity of 1mA for anodal tDCS (atDCS) was applied during motor learning experiments. Optimizing strategies to enhance the efficacy of tDCS are needed. Previous electrophysiological (Nitsche and Paulus 2000) and cognitive studies in patients (Boggio et al. 2006a) and healthy humans (Iyer et al. 2005;Teo et al. 2011) suggest that increasing stimulation intensity might be a valuable approach, since the efficacy of stimulation seems to depend on intensity. Therefore, the present study aims to reveal the effects of increasing stimulation intensity on motor learning in healthy subjects under the hypothesis that higher stimulation intensity leads to enhanced skill acquisition.

2.6.3. Methods

2.6.3.1. Subjects

Thirteen healthy subjects (mean age of 19.92 ± 1.12 years; 7 males) participated in this double-blinded crossover study. Eleven subjects were right-handed (mean lateralization quotient: 79.58 ± 20.84) and 2 were left-handed (mean lateralization quotient: -80.00 ± 28.28) according to the Edinburgh Handedness inventory (Oldfield 1971). Subjects provided written informed consent and experimental procedures were approved by the Central Ethics Committee of UZ Leuven and the local Ethics Committee of the University of Hasselt. The study conforms to the principles stated in the Declaration of Helsinki.

2.6.3.2. Experimental design

In three pseudo-randomized, counterbalanced sessions separated by at least 3 days, subjects received either atDCS (HDCstim, Newronika, Italy) with an intensity of 1.5mA, 1mA or sham tDCS for 20 min on the primary motor cortex (M1) contralateral to the dominant hand while performing a unimanual motor learning task. In the sham condition, the electrode montage was identical to the real stimulation conditions and electrodes were also attached for 20 min, however subjects only received current during the first 26 sec. More specifically, the current was ramped-up for 7 sec, followed by 12 sec of 1mA atDCS and then ramped-down for 7 sec. The anode (surface: 25cm^2, current density of 0.04 mA/cm^2 for 1mA atDCS and 0.06 mA/cm^2 for 1.5mA atDCS) was centered at the hotspot of the first dorsal interosseous muscle, as determined by transcranial magnetic stimulation. The cathode size (surface: 50cm^2, current density of 0.02 mA/cm^2 for 1mA atDCS and 0.03 mA/cm^2 for 1.5mA atDCS) was increased to make the electrode functionally inert (Nitsche et al. 2007) and was fixed over the supraorbital region of the other hemisphere. Subjects were instructed to perform a finger sequence task with the dominant hand by pressing different keys (see, Fig. 1), each corresponding to one of the four fingers (2nd - 5th). The sequences were [4 2 1 3 4 2 3 2], [2 4 2 1 3 2 3 4] and [2 4 3 1 2 3 2 4] (1 = index, 2 = middle, 3 = ring and 4 = little finger). The practiced sequence was displayed on the screen and a black dot appeared on the screen whenever a key was pressed. No feedback about the correctness of the performance was provided. Button presses were recorded using E-Prime (E-prime v2.0, Psychology Software Tools Inc., PA, USA]. In a single block sequences were initiated within a 30 second time frame, followed by a 30 second resting period. Each block was terminated after completion of the last sequence.

Subjects were instructed to perform as many sequences as possible. The amount of sequences provided during each block depended on the speed of the subject. In other words, when a subject performed faster in a block, a larger amount of sequences was provided within that block. Each session consisted of 26 blocks. 3 blocks (baseline, 3 min) were provided before application of the stimulation, followed by 20 training blocks (20 min) under atDCS/sham tDCS; and finally 3 blocks (post-intervention, 3 min) were administered 30 min after the stimulation. All sequences initiated during the 30 sec practice block were considered for analysis and a motor performance score was calculated by dividing the percentage of correct sequences by the mean inter tap interval (= the average time between two successive key presses), thus considering both the speed and accuracy requirements. After each session, the level of attention, fatigue and perceived discomfort during the session was rated by the visual analogue scale (VAS). Furthermore subjects were asked to report the amount (hours) of previous night's sleep and sleeping quality (VAS).

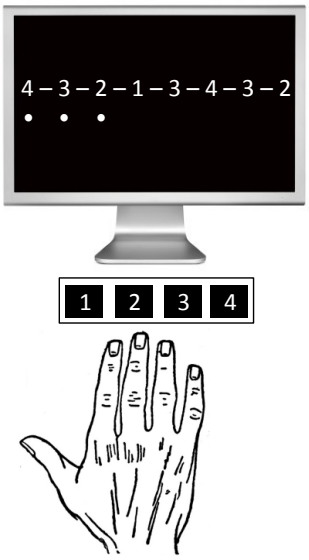

Fig 1. Subjects were instructed to perform a 8-element finger sequence with the dominant hand by pressing different keys, each corresponding to one of the four fingers (2nd - 5th).

2.6.3.3. Data analysis

Advanced linear models applications (SAS 9.2, SAS institute Inc., Cary, NC) were used for statistical analysis. Prior to analysis, the motor performance score was normalized (%) to baseline for each subject separately. To analyze performance differences between conditions at a single (time) point, a paired t-test was applied. The bonferroni correction was used to correct for multiple comparisons. To evaluate the effect of stimulation intensity during motor learning over time, a mixed model including fixed effects for INTENSITY (1mA atDCS, 1.5mA atDCS and sham), and TIME (20 training blocks) and their interaction was used to estimate the rate of change (i.e. slope-analysis) of motor performance. Statistical power and sample size calculations were carried out for the evolution of the slope and at post-intervention. The significance level was set at $p < .05$.

2.6.4. Results

At baseline, paired t-tests revealed no significant differences in motor performance between the different stimulation conditions (all, $p > 0.05$).

During motor learning (20 blocks), a significant INTENSITY × TIME interaction was reported ($F = 4.32$, $p = 0.014$). This result indicates that the slopes are significantly different (see, Fig. 2) for the different tDCS intensities. The slope was significantly steeper for 1.5mA atDCS as compared to sham condition [Difference in slope estimates for 1.5mA atDCS as compared to sham: 7.22 (StDev. = 2.49), $p = 0.004$], indicating that motor learning occurred faster during 1.5mA atDCS. No significant difference in slope was reported between 1.5mA and 1mA [Difference in slope estimates for 1.5mA atDCS as compared to 1mA atDCS: 3.93 (StDev. = 2.34), $p = 0.092$]; and between 1mA atDCS and sham [Difference in slope estimates for 1mA atDCS as compared to sham: 3.29 (StDev. = 2.54), $p = 0.20$).

At post-intervention (see, Fig. 2), a paired t-test revealed a significant difference in motor performance for 1.5mA atDCS as compared to sham ($p = 0.044$). No significant difference was found between 1.5mA and 1mA atDCS ($p = 0.08$) and between 1mA atDCS and sham ($p = 0.34$).

The results mentioned above should be interpreted in combination with the power and sample size calculations as shown in Table I.

No significant differences in the amount of previous night's sleep, sleep quality, level of attention, level of fatigue and level of discomfort were reported between conditions (all, $p > 0.05$; see Table II).

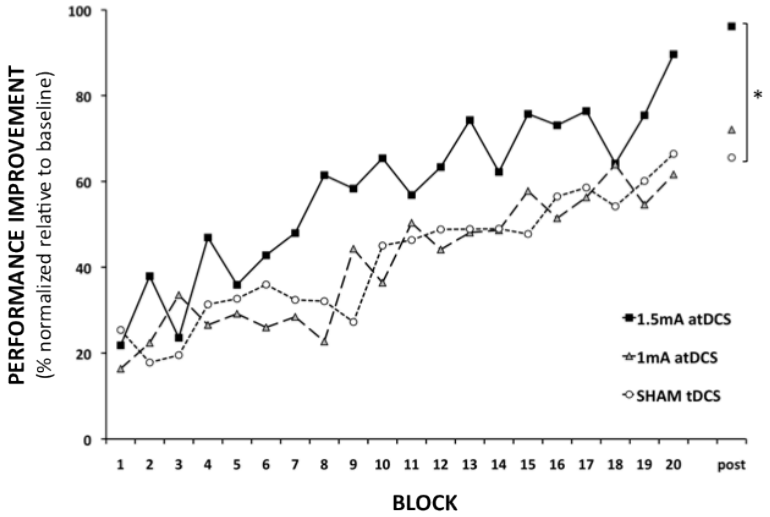

Fig 2. Evolution of motor performance during motor learning and at post-intervention (% normalized relative to baseline) for the 1.5mA atDCS, 1mA atDCS and sham tDCS condition.

Table I. For all contrasts the values for the effect size, power and the required sample size to reach a power of 0.80 are reported. Results for the evolution of the slopes and at post-intervention are shown. Note that the effect size is defined as the absolute value of the mean difference between two groups.

	Slope			Post-intervention		
	Effect Size	Power	Sample size (Power = 0.80)	Effect Size	Power	Sample size (Power = 0.80)
1.5mA atDCS vs. 1mA atDCS	28	0.29	63	24	0.40	39
1.5mA atDCS vs. SHAM tDCS	23	0.43	35	30	0.67	18
1mA aTDCS vs. SHAM tDCS	4.5	0.10	593	6.5	0.11	445

Table II. Mean (StDev) sleep (duration and quality) and level (0 = low, 10 = high) of attention, fatigue, and discomfort perceived during each session (SHAM tDCS, 1mA atDCS and 1.5mA atDCS). No significant differences were reported between sessions (all, $p > 0.05$)

	SHAM tDCS	1mA atDCS	1.5mA atDCS
Sleep (hours)	7.46 (1.42)	7.96 (1.03)	7.23 (1.13)
Sleep (quality)	6.85 (2.58)	7.08 (1.55)	8.00 (1.35)
Attention	7.08 (1.26)	7.08 (1.60)	7.62 (0.65)
Fatigue	3.31 (2.66)	2.92 (2.79)	2.69 (2.36)
Discomfort	1.61 (1.56)	2.15 (2.38)	0.92 (0.86)

2.6.5. Discussion

The present study reveals that a combination of motor learning and 1.5mA atDCS over M1 contralateral to the (dominant) hand performing the motor task leads to a significant improvement of motor performance as compared to sham stimulation in healthy subjects. Remarkably, this effect was seen both during motor training and at post-intervention (30 min after stimulation). Although the effects of a single session of atDCS on motor learning in healthy individuals have been studied previously (Boggio et al. 2006b;Nitsche et al. 2003), this is the first study evaluating the stimulation intensity-dependent effects of atDCS intensity on motor learning.

Our results are in line with Boggio et al (2006a) who reported a significant improvement in working memory performance in Parkinson's disease patients when applying 2mA atDCS on the left dorsolateral prefrontal cortex, whereas 1mA atDCS or sham stimulation did not result in significant effects. In contrast, Nitsche et al. (2003) showed a significant shortening of absolute reaction time during a serial reaction time task (SRTT) after a single session of 15min of 1mA atDCS over M1 as compared to sham stimulation in healthy subjects. Similar results were also reported by a recent study of Kantak et al. (2012) reporting decreased reaction time in a SRRT during (online) and after (offline) atDCS in healthy adults. In contrast, our results showed no performance differences between 1mA atDCS and sham after motor learning. Whereas Nitsche et al. (2003) and Kantak et al. (2012) used a protocol evaluating reaction time, the current results are obtained using a compound measurement assessing performance as function of both accuracy and speed. Since both parameters influence each other, the current protocol does not allow disentangling accuracy and speed and therefore we cannot attribute performance to these parameters independently. The absence of a performance difference between 1mA atDCS and sham in the present study is in line with Boggio et al. (2006b), who showed no significant effect of sham or 1mA atDCS over the dominant M1 on fine motor skill performance in healthy subjects. Although we expected to find a significant improvement of motor performance during 1.5mA atDCS as compared to the 1mA atDCS condition, only a non-significant trend was reported. This finding is probably due to the relative small sample size.

In the current study we did not evaluate the physiological changes underlying changes in motor performance. Previous findings provide evidence that increased stimulation intensity will lead to increased excitability of the area (M1) under the anode during and after atDCS (Nitsche et al. 2005). Furthermore, Nitsche & Paulus (2000) reported that the size and endurance of excitability changes after atDCS depended on stimulation duration and current intensity. More specifically, increasing either intensity or duration led to prolonged and larger after effects. Therefore it might be speculated that larger current intensity leads also to increased

strengthening of learning-related synaptic connections, thus resulting in improved performance. On the contrary, Antal et al. (2007) reported decreased excitability after atDCS when atDCS was associated with motor excercise, showing that tDCS-induced plasticity is highly dependent on the state of the subject during stimulation. Future studies are needed to clarify these findings.

In conclusion, this study demonstrates (1) a significant improvement in online and (2) offline performance for 1.5mA atDCS as compared to sham tDCS. No significant effects were reported between 1mA atDCS and sham tDCS; and between 1.5mA atDCS and 1mA atDCS. Although only a trend was reported between 1.5mA atDCS and 1mA atDCS, our results indirectly support the hypothesis that stimulation intensity plays an important role in obtaining the desired result. Increasing the sample size and/or current intensity (for example 2mA or more) might lead to increased effects between conditions.

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Chapter III:

General discussion and conclusions

General discussion

This work provides novel insights into the functional changes and the underlying mechanisms resulting from non-invasive electrical current applications in both MS-patients and healthy subjects. Two easy accessible and patient friendly therapeutic interventions (TENS and tDCS) were evaluated in order to optimize neurorehabilitation. Because the combinations of adjustable parameters were almost unlimited, it was necessary to focus on well-defined stimulation protocols. The main finding of this work is that long-term (TENS) therapy induces long-term effects on both the functional and neural (corticospinal) level. Moreover, long-term cortical reorganization and increased sensitivity was reported in MS. Besides the evaluation of long-term TENS, this work also assessed the effects of short-term tDCS in MS patients for the first time. However, even though a single session of tDCS leads to increased corticospinal output and projection strength in MS patients, short-term tDCS-supported motor training did not result in improvement of motor performance. Therefore, it might be necessary to extend the stimulation duration into multiple tDCS sessions applied over consecutive days.

3.1. Long-term TENS stimulation induces long-lasting functional and neuroplastic changes in MS.

In contrast with most TENS studies which focused on the short-term effects, this work evaluated the effect of a long-term TENS intervention. In study 1, 2 and 3 the same stimulation protocol was applied. The following parameters were used; a biphasic symmetrical rectangular pulse-wave with a frequency of 100 Hz and a pulse width of to 250μs. Stimulation was applied to the median nerve region. The stimulation intensity was above sensory but below the motor threshold and the stimulation duration was fixed to 60min per day for 21 consecutive days.

A long-term stimulation paradigm was used in order to obtain long-lasting effects following stimulation. Whereas a single session of TENS leads to neuroplastic changes and/or functional effects of several minutes to a few hours (Mima et al., 2004; Tinazzi et al., 2005; Tinazzi et al., 2006), we showed that multiple sessions are necessary to extend these effects to several days/weeks. This work provides evidence that long-term TENS has the ability to induce long-term cortical reorganization (> 24h) in both healthy subjects (study 1) and patients with MS (study 3). Furthermore, TENS was able to increase tactile sensitivity with long-term aftereffects in MS patients with sensory deficits (study 2).

To evaluate the effects of TENS on neural plasticity, a TMS mapping protocol was used. TMS mapping of the motor cortex was performed to

assess changes in excitability of the corticospinal projections and/or functional reorganization. Using this technique we showed that long-term TENS induced long-lasting neuroplastic changes in cortical motor representations. Remarkably, the effect of TENS was not similar in healthy subjects and in patients with MS. Whereas in healthy subjects the map area was significantly increased, a significant decrease was reported in MS-patients.

Although TENS is mainly used to relieve pain in patients with nerve injury (Engholm and Leffler, 2010; Hole and Berge, 1981), study 2 showed that a long-term TENS therapy was able to restore tactile sensitivity in MS patients with sensory deficits. Remarkably, the level of sensitivity improved to a level that was comparable to that of age- and gender-matched healthy controls. In line with our findings, Chitsaz et al. (2009) reported that an 8-week treatment course of either nortriptyline (a drug that is mainly used to treat depression) or self-applied daily TENS were effective in reducing both pain and/or sensory complaints in the upper-extremities of MS-patients (Chitsaz et al., 2009). The latter study did not implement any follow-up measurements after the end of the intervention, as we did. In contrast with long-term applications, a single session (30 min, 90Hz, submotor) of TENS showed to decrease tactile sensitivity in healthy subjects, indicating that a single session of TENS has an inhibitory effect on the sensory system (Mima et al., 2004). Furthermore, Mima et al. (2004) argue that the elevation of the sensory motor threshold was due to post-TENS paraesthesias caused by ongoing activity in peripheral nerve fibers and stimulation induced refractoriness in central synaptic relays (Burke and Applegate, 1989). Kowalewski et al. (2012) reported that 2-consecutive days of TENS (30 minutes, 20Hz) stimulation did not affect tactile sensitivity, however tactile discrimination improved significantly in a group of healthy young adults (Kowalewski et al., 2012). Other recent findings provide evidence that long-term peripheral stimulation can lead to long-lasting improvements in sensitivity. For example, Kalisch et al. (2010) found that repetitive electrical stimulation using TENS (for 4 weeks, 2 sessions of 30 minutes a week) significantly improved tactile acuity over a period of 2 weeks after the end of the intervention in older subjects (Kalisch et al., 2010). Recently, Kattenstroth et al. (2012) evaluated the effects of long-term (up to 76 weeks) sensory stimulation in patients with chronic cerebral lesions (Kattenstroth et al., 2012). They reported significant long-lasting improvements in tactile and sensorymotor function.

Based on previous findings it might be possible that TENS affected reorganization on both the peripheral and the central level. As our findings showed that TENS did not only improve sensitivity in the stimulated muscle (innervated by the median nerve) but also in a muscle innervated by a different (ulnar) nerve, it might be possible that reorganization occurred at the peripheral level as there is evidence for ulnar to median nerve communication (Kimura et al., 1983). Besides the hypothesis that

reorganization occurred at the peripheral level, our results can also be explained by central (subcortical and cortical) mechanisms responsible for reorganization. At the subcortical level, Nakatsuka et al. (1999) reported that inflamed animals received more direct Aβ inputs in the dorsal horn (Nakatsuka et al., 1999). It might be possible that there was plasticity at this level (at least for the MS group), as the TENS modality we applied targeted Aβ afferents. In contrast with short-term high frequency TENS applications, which showed an increase of GABA in the dorsal horn (leading to inhibitory processes responsible for pain reduction), we expect that long-term TENS triggers other processes. However, as there is currently no evidence available on this topic, it is not possible to define which pathways, neurotransmitters and/or chemical substances are involved with respect to the current findings. At the cortical level, our findings provide evidence for reorganization. Interestingly, reorganization was reported at the primary motor cortex. It is suggested that sensory input from Aβ afferents projects to the dorsal horn, the primary sensory cortex and subsequently to the primary motor cortex (Zarzecki et al., 1978). We speculate that reorganization at the cortical level is mainly due to GABAergic changes at the level of the interneurons as a result of sensory input (Jacobs and Donoghue, 1991). It is possible that MS and healthy might react in a different (opposite) way to TENS due to differences in the GABAergic system (Clements et al., 2008). MAP expansion can be induced by true reorganization of the connectivity patterns in the cortex and/or an increased excitability of (inhibitory or excitatory) connections that were already present but not detected by TMS. On the contrary a map decrease may have been due to the formation of new GABAergic inhibitory connections that were previously impaired by the MS pathology (Dutta et al., 2006).

Although this work did not report findings with respect to changes in motor functionality after TENS, several studies provide evidence that peripheral stimulation at submotor threshold intensity results in beneficial effects on motor performance (Celnik et al., 2007; Fraser et al., 2002; Pomeroy et al., 2006; Sorinola et al., 2012). To the best of our knowledge, there are no studies reporting effects of TENS on motor performance in MS. However other TENS-induced behavioral effects are reported in MS. In this respect, a limited number of studies have demonstrated that several weeks of TENS treatment was effective in MS-related spasticity. For example, Shaygannejed et al., 2013 reported that spasticity decreased significantly after 4-weeks of self-applied TENS (Shaygannejad et al., 2013). Furthermore, they reported that TENS was more effective than Baclofen, a drug used to treat spasticity symptoms. Similar findings, showing that TENS was effective in treating spasticity, were also reported by other studies (Armutlu et al., 2003; Sluka and Walsh, 2003; Tjon Eng Soe et al., 2009). Besides rehabilitation of spasticity in MS, TENS has also been successfully used to treat neurogenic urinary disorders in MS-patients. For example, De

Seze et al. (2011) showed a significant clinical improvement of overactive bladder symptoms in 82.6% of the treated patients after 30 days of daily (20 min a day) TENS on the posterior tibial nerve (de Seze et al., 2011).

Nonetheless studies evaluating the effect of TENS on motor performance in MS are generally lacking. The effectiveness of TENS on motor performance in stroke patients and other populations has been described previously. For example, Tyson et al. (2013) reported that a single session of TENS delivered via a sock electrode was sufficient to significantly improve balance, gait speed, plantar flexion strength and proprioception of plantar flexion in patients with chronic stroke (Tyson et al., 2013). Another recent study (Cho et al., 2013) reported that 60 minutes of TENS on the gastrocnemius lead to significant improvements in spasticity and balance in chronic stroke patients. Previously, Ng et al. (2009) showed that TENS can improve the effectiveness of task-related excercise for increasing walking capacity in hemiparetic stroke survivors (Ng and Hui-Chan, 2009).

With respect to long–term applications, Conforto et al. (2010) reported that multiple sessions of repetitive peripheral nerve stimulation could facilitate motor recovery in stroke patients (Conforto et al., 2010). Interestingly, they found that stimulation intensity was crucial to obtain the desired result. Moreover, motor function (measured with the Jebsen Taylor Hand Function Test) improved only when lower stimulation intensity (subsensory: 83.0 ± 3.0% of the sensory threshold) was applied. No changes were observed when higher intensity stimulation (suprasensory: 207.3 ± 23.9% of the sensory threshold) was provided.

In healthy elderly, Kalisch et al. (2010) reported that repetitive electrical stimulation using TENS significantly improved haptic (haptic object recognition test) and motor performance (square pegboard test) over a period of at least 2 weeks after the end of the intervention (Kalisch et al., 2010). In line with these findings, Sorinola et al. (2012) also reported that TENS lead to improved motor performance in young healthy subjects after a single session (2 hours, 10Hz, supramotor)(Sorinola et al., 2012). Remarkably, in parallel with improved motor performance, the authors reported a reduction of muscle performance (grip strength). They explain this reduction by fatigue induced by prolonged somatosensory stimulation.

In summary, we can conclude that both our results and findings from previous studies indicate that TENS can (1) lead to cortical reorganization and (2) modulate changes in sensorimotor function; and (3) that long-term stimulation is necessary to induce long-term effects. In addition our work showed for the first time that long-term TENS leads to (1) a decrease in cortical motor representation and (2) to long-term improvement of sensory function in MS patients.

3.2. Evaluation of tDCS as an adjuvant therapy for MS rehabilitation

To evaluate the effect of tDCS on neuroplastic and functional changes in MS-patients, we applied a commonly used and well-studied protocol [current intensity: 1mA, duration: 20 min, anode: over M1 (surface: 25 cm^2), cathode: over the supraorbital region of the other hemisphere (surface: 50 cm^2)]. Although this protocol has already been successfully used in healthy subjects and in neurodegenerative populations such as stroke and Parkinson's disease, only few studies reported findings in MS (Mori et al., 2010; Mori et al., 2012). Additionally, the effects of intensity was evaluated in healthy subjects because previous electrophysiological (Nitsche and Paulus, 2000) and cognitive studies in patients (Boggio et al., 2006) and healthy humans (Iyer et al., 2005; Teo et al., 2011) suggest that increasing stimulation intensity might be a valuable approach, since the efficacy of stimulation seems to depend on current intensity.

Although the mechanisms underlying tDCS-induced neural plasticity are still partly unclear, tDCS is able to modulate cortical excitability in a polarity dependent manner (Nitsche and Paulus, 2000). More specifically, anodal tDCS is able to increase corticospinal excitability. Evidence gathered with different neuroimaging techniques tried to unravel the neurophysiology underlying neuroplastic changes. A recent resting state fMRI study reported that tDCS applied at rest was able to reorganize the intrinsic functional architecture of the human primary cortex. More specifically, it was reported that cathodal tDCS boosted local connectedness, while anodal tDCS enhanced long distance communication within M1 (Polania et al., 2012). Previously, a positron emission tomography (PET) study (Lang et al., 2005) revealed that the regional cerebral blood flow (rCBF) in M1, the somatosensory cortex (S1) and the frontal cortical regions increased after 20 min of both, anodal and cathodal tDCS, as compared to sham. Evidence from TMS studies revealed that excitability increased after anodal tDCS and diminished after cathodal tDCS (Nitsche and Paulus, 2000; Nitsche and Paulus, 2001; Nitsche et al., 2005). The duration of the aftereffects depended on the duration of the stimulation (Nitsche and Paulus, 2001). Neurophysiological data revealed that the aftereffects of anodal tDCS seem to depend on the modulation of both GABAergic and glutamatergic synapses, whereas the aftereffects of cathodal tDCS depend only on the modulation of glutamatergic synapses. Furthermore, tDCS-induced changes are suggested to occur at the level of the intracortical interneurons (Stagg and Nitsche, 2011). Stagg & Nitsche (2011) suggest that long-lasting excitability changes can be established with tDCS if the correct stimulation parameters are determined.

Until now it was not investigated whether tDCS was able to modulate neural plasticity in patients with MS. Our findings (study 4) revealed that atDCS can cause increased CS output and projection strength in the motor cortex of MS patients. Based on the shape of the TMS recruitment curves, it

was speculated that atDCS increased the activation of large-diameter myelinated axons at higher distance from the stimulation site (Siebner and Rothwell, 2003). In addition it is likely that tDCS had an effect on GABAergic, adrenergic and glutamatergic processes as the shape of the recruitment curve seems to be sensitive for detecting changes in these processes (Chen, 2000; Di Lazzaro et al., 2003). We can only speculate about the exact mechanisms underlying tDCS-induced aftereffects. Nonetheless, studies using drug administration protocols combined with tDCS reported that the aftereffects depend on several factors, such as membrane polarization (Nitsche et al., 2003), synaptic modulation (Liebetanz et al., 2002; Nitsche et al., 2003) and $GABA_A$ergic interneurons (Nitsche et al., 2004).

Although we did not study the effects of tDCS on tactile sensitivity in this work, recent studies have provided evidence that tDCS over the somatosensory cortex can optimize tactile perception in healthy subjects (Ragert et al., 2008) as well as in MS patients with sensory disturbances (Mori et al., 2012). In both studies tactile perception was measured using the graded orientation task (GOT). Moreover subjects were asked to indicate the orientation of the gratings surfaces. Whereas Ragert et al. (2008) reported beneficial effects after 20 minutes of anodal tDCS (lasting for 40 minutes), Mori et al. (2012) even found effects lasting for 2 weeks after the end of the intervention following anodal tDCS (2mA for 20min) over 5 consecutive days.

Studies in both healthy subjects and in patients suffering from neurodegenerative disease have indicated that tDCS could be used for neural rehabilitation as tDCS can improve motor learning and motor performance. More specifically, tDCS can enhance reaction times (Fregni et al., 2006; Hummel et al., 2006), pinch force (Hummel et al., 2006; Tanaka et al., 2011), motor control (Hummel et al., 2005; Madhavan et al., 2011), and motor learning (Fritsch et al., 2010; Galea and Celnik, 2009; Tecchio et al., 2010). Since motor learning is a key component for successful neural rehabilitation, we evaluated the effects of anodal tDCS combined with training of a fixed finger-sequencing task in patients with MS (study 5). Unfortunately, we were not able to replicate tDCS-related improvements in motor learning in MS-patients. The absence of these improvements might be explained by several factors, such as the nature of the disease (degradation of a broader network), the duration of the stimulation (one session vs. multiple sessions), the intensity of the current (the higher the better?), etc. To investigate if current strength was a mediator for motor leaning, the same experiment was repeated in a group of healthy subjects (study 6). Our results showed that intensity was indeed a factor that mediated motor learning in the latter group. Moreover higher tDCS intensity led to increased motor performance during and after tDCS-supported motor learning.

In summary, we can conclude that (1) a single session of 1mA of anodal tDCS increased CS output and projection strength in the motor cortex

of MS patients; (2) a single session of 1mA anodal tDCS-supported motor practice did not improve motor performance in MS patients; (3) that tDCS intensity might play a crucial role in obtaining the desired therapeutic effect; and (4) that previous studies indicate stronger and long-lasting effects after several consecutive days of tDCS.

3.3. Limitations and future perspectives

3.3.1. Limitations of the current studies

A first limitation is the relatively low number of subjects included in the studies. Particularly in the MS population recruitment was not always evident due to various reasons. First, subjects had to use their own transportation or public transportation to participate in the experiments in our lab in Diepenbeek. This was not always evident for MS-patients with motor restrictions or other symptoms such as pain, fatigue, etc. We tried to compensate for this problem by moving our setup to the hospital. However this was not always evident given the fact that space is limited in hospital settings. Additionally some setups were not easily transportable. Second, given the amount of variability in MS symptoms and the variability of TMS itself, we used strict inclusion/exclusion criteria. For example we did only test subjects with low EDSS scores, tremor was an exclusion criterion, the TMS motor threshold had to be under a predetermined value to avoid discomfort during the measurements, etc. Third, when long-term experiments with a crossover design where performed (study 3), it was not easy to keep subjects motivated to complete the entire study. Additionally, there is a higher chance of drop-out in long-term studies due to relapse or other circumstances (medical problems, private problems, transportation problems, etc.). To deal with these issues and particularly when subjects participated in a long-term home-based training program, we contacted the subjects on a regular basis to ask them if they experienced problems performing the program. Furthermore, they had the opportunity to contact the principal investigator if any problems would occur.

A second limitation is the restricted set of stimulation parameters used throughout the experiments. We chose to further elaborate on well-defined parameters based on previous studies. However, other parameter combinations might lead to better/different results. Furthermore, different pathologies may require different parameter combinations. For example when a brain area needs to be more activated to achieve beneficial rehabilitation results anodal tDCS can be used, for example in stroke. In contrast cathodal tDCS might also lead to beneficial rehabilitation in pathologies requiring a decrease of cortical activation, such as attentional deficits (Weiss and Lavidor, 2012).

A third limitation is that functional changes and mechanisms underlying these changes were not evaluated within the same study. Therefore, it was impossible to correlate these measurements, making the observed findings less strong. However, because our interventions were not well studied yet in patients with MS and evaluation of these interventions consumed much energy of the patients, we chose to use simple, well-defined and patient-friendly protocols.

3.3.2. Future perspectives

In order to establish long-lasting neuroplastic changes and behavioral improvements during the rehabilitation process, we believe that long-term experimental studies are needed to optimize the therapeutic application of central and peripheral electrical stimulation. In this respect we reported that long-term TENS has the ability to improve sensitivity and neural plasticity over a significant time period. With respect to tDCS, long-term tDCS application also seems to be required to achieve long-lasting rehabilitation benefits, as was indicated by recent studies (Mori et al., 2010; Mori et al., 2012; Reis et al., 2009). In MS, the group of Mori et al. already reported beneficial effects of tDCS on pain (Mori et al., 2010) and sensitivity (Mori et al., 2012) after five consecutive days of stimulation. Therefore future protocols should focus on the effects of long-term application of tDCS on motor learning and performance in MS.

Since the underlying mechanisms of the techniques used throughout our studies remain mainly unclear, it might be recommended to take a step back and return to more fundamental research. To obtain a better understanding of what tDCS is exactly doing, it might be useful to combine TMS with other imaging techniques such as EEG and MRI, which might provide more detailed information about the localization of neuroplastic changes and changes in brain network activity. In addition, pharmacologic interventions combined with stimulation and imaging might also increase insights into the working mechanisms (Nitsche et al., 2012). Furthermore, a better understanding of the underlying mechanisms is required to figure out why some individuals respond to a therapy and others do not. Accordingly, unraveling these mechanisms will be necessary to provide individualized therapy programs with a high success rate.

Another challenge is to combine both peripheral and central electrical stimulation techniques and comparing the effects with single-locus stimulation techniques. Findings of Celnik et al. (2009) in stroke already confirmed that the combination of both techniques resulted in significant training improvements on motor impairments as compared to single applications (Celnik et al., 2007). Another study of Boggio et al. (2009) also reported significantly better effects of coupled tDCS-TENS stimulation as compared to single application in the treatment of patients with chronic pain (Boggio et al., 2009). Based on these findings a tDCS-TENS intervention

may possibly be superior compared with a single application. Therefore it is recommended to evaluate this application on motor performance and pain management in MS-patients.

3.4. Conclusions

In summary we can conclude that both peripheral and central electrical stimulation applications are feasible and carry potential to improve the rehabilitation process in patients with MS. Long-lasting changes in sensitivity and neural plasticity were reported after long-term TENS in MS-patients. With respect to tDCS, we can conclude that tDCS leads to increased corticospinal excitability and projection strength over the stimulated region. However, no significant behavioral changes were reported in MS-patients. Moreover, it is argued that increased current intensity and repeated epochs of stimulation are required to establish significant functional improvements in this population.

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Chapter IV:

Nederlandstalige samenvatting

Het doel van dit doctoraatsproefschrift was om na te gaan in welke mate centrale en/of perifere elektrische stimulatie kan leiden tot veranderingen in neuroplasticiteit en of deze stimulatie het functieherstel van spieren en/of zenuwen kan bevorderen bij personen met neurodegeneratieve aandoeningen. Bij patiënten met multiple sclerose (MS) verloopt de revalidatie vaak moeizaam. Naast psychologische klachten zijn de voornaamste symptomen: vermoeidheid, verlies van kracht, spasticiteit, gevoelsstoornissen en motorische coördinatieproblemen. Vanuit therapeutisch perspectief zijn we vooral geïnteresseerd in een eenvoudige en betaalbare revalidatietherapie die minimaal belastend is voor de patiënt en die leidt tot positieve langetermijneffecten. Elektrostimulatietherapie wordt reeds jaren gebruikt voor allerlei doeleinden, gaande van het verminderen van chronische pijn tot het actief houden van spieren die niet meer worden gebruikt (vb. bij coma patiënten). Tot op vandaag weten we zeer weinig over de effecten van (langdurige) elektrotherapie bij patiënten met MS. MS wordt gekenmerkt door een verminderde elektrische signaaloverdracht van de zenuwen ten gevolge van de aantasting van de myelineschede. Het idee van dit doctoraatproefschrift bestaat erin dat we met behulp van elektrotherapie de abnormale impulsgeleiding weer tot een optimaal/normaal niveau willen brengen. Vanuit voorgaand onderzoek weten we immers dat er een verband is tussen een aangetaste impulsgeleiding en sensorimotorische klachten bij MS patiënten. We verwachten dan ook dat wanneer de therapie 'werkt' deze klachten zullen verminderen. Daarnaast zijn we vooral geïnteresseerd in wat de onderliggende mechanismen zijn die deze sensorimotorische veranderingen teweegbrengen. Op basis van veelbelovende resultaten uit voorgaande studies kwamen twee types van elektrotherapie in aanmerking om de impulsgeleiding te optimaliseren, namelijk Transcutaneous Electrical Nerve Stimulation (TENS) en Transcranial Direct Current Stimulation (tDCS). Bij zowel TENS als tDCS wordt er een nauwelijks voelbare elektrische stroom opgewekt tussen twee elektroden die zijn aangebracht op de huid. Het verschil tussen beide interventies is dat de elektroden bij TENS op de aangetaste spier/zenuw worden aangebracht en bij tDCS op de schedellocatie waaronder zich het hersengebied bevindt dat gekoppeld is aan de aangetaste spier. Onderzoek toonde aan dat beide therapieën (stroomvorm, frequentie, amplitude, etc.) de prikkelbaarheid van het centrale zenuwstelsel kunnen beïnvloeden indien de juiste parameters worden gebruikt. Met andere woorden, ze kunnen ervoor zorgen dat een zenuwcel makkelijker kan geactiveerd worden en bijgevolg makkelijker een elektrisch signaal kan doorgeven. Met TENS kunnen we de sensorische zenuwcellen stimuleren. Via deze sensorische zenuwcellen verkrijgen we informatie over allerhande prikkels, bijvoorbeeld over de vorm, de textuur of de grootte van een object. Vervolgens kunnen we op basis van deze sensorische informatie een actie ondernemen zoals bijvoorbeeld het aansturen van bepaalde spieren via motorische zenuwcellen om het object

vast te pakken. Het vergemakkelijken van het aansturen van motorische zenuwcellen kunnen we beïnvloeden met tDCS.

In de eerste 3 studies beschreven in dit doctoraatproefschrift werden de effecten van een lange-termijn TENS therapie (stimulatie gedurende 3 weken, 1 uur per dag) op de handspieren onderzocht. Uit deze studies kunnen we concluderen dat TENS de gevoeligheid kon verbeteren bij MS patiënten met sensorische problemen. De patiënten konden met een grotere gevoeligheid prikkels waarnemen. Bovendien verbeterde de gevoeligheid tot op een niveau dat vergelijkbaar was met dat van gezonde leeftijdsgenoten en was het effect nog steeds aanwezig tot minstens 3 weken na het beëindigen van de therapie. Opmerkelijk was dat niet alleen de gevoeligheid van de gestimuleerde spier/zenuw verbeterde, maar ook deze van de naburige spieren. Naast het onderzoeken van deze functionele veranderingen zijn we behulp van transcraniële magnetische stimulatie (TMS) nagegaan welke neuroplastische veranderingen werden vastgesteld na TENS. Met TMS kunnen we het activiteitspatroon (facilitatie/inhibitie) van de motorische hersengebieden in kaart brengen. Je krijgt als het ware een 'map' van hersenactiviteit. Verschillende interventies zoals bijvoorbeeld leren, immobilisatie en sensorische stimulatie kunnen leiden tot veranderingen van deze maps. Onze resultaten toonden aan dat de maps na TENS groter werden bij gezonde personen en kleiner werden bij MS patiënten in vergelijking met de maps die werden gemeten vóór de TENS behandeling. Dit doet ons vermoeden dat de richting van neuroplastische veranderingen gestuurd wordt door het al dan niet aanwezig zijn van een bepaalde pathologie. Uit voorgaand onderzoek bleek dat hersenactiviteit bij MS patiënten een uitgesproken globaal patroon vertoont, dat mogelijk veroorzaakt wordt door de aantasting van inhibitorische connecties in de hersenen. Mogelijk zorgt TENS voor een normalisatie van deze hersenactiviteit door in te grijpen op deze inhibitorische connecties en leidt dit proces bijgevolg tot een normalisatie van de hersenactiviteit in het betreffende hersengebied. Deze verklaring is echter voorbarig en dient verder onderzocht te worden.

Naast de evaluatie van een langdurige interventie met TENS werd het effect van anodale tDCS op neuroplastische veranderingen (studie 4) en motorische prestatie (studie 5) nagegaan bij MS patiënten. Tot op heden werden bij MS patiënten enkel effecten van tDCS op pijnklachten en sensorische klachten geëvalueerd, met positieve resultaten tot gevolg. In dit doctoraatsproefschrift hebben we aangetoond dat een 20 minuten durende interventie met anodale tDCS leidde tot neuroplastische veranderingen in de motorische cortex van MS patiënten. Meer specifiek werd er een toename van de corticospinale output vastgesteld na anodale tDCS. Dit wil zeggen dat er globaal gezien minder energie nodig is om dit hersengebied te activeren na tDCS. Gebaseerd op de bevindingen van gelijkaardige onderzoeken uitgevoerd bij andere neurodegeneratieve populaties zouden we kunnen verwachten dat anodale tDCS motorische prestaties en/of leertaken zal

faciliteren. Deze hypothese hebben we getoetst in studie 5, waarin we het effect van tDCS gekoppeld met een motorische leertaak op motorische prestatie zijn nagegaan bij MS patiënten. Uit onze resultaten bleek dat een sessie van tDCS gekoppeld aan training van een taak echter niet leidde tot een verhoogde motorische prestatie. Verder onderzoek is aangewezen om na te gaan wat de factoren zijn die de uitkomst van de therapie bepalen. We vermoeden dat zowel factoren die eigen zijn aan de patiënt (vb. anatomische en genetische factoren), stimulatieparameters (herhaaldelijke sessies, stimulatie intensiteit, elektroden plaatsing) en hun interactie een belangrijke rol spelen. In studie 6 hebben we de parameter stimulatieintensiteit dieper onderzocht. Onze resultaten toonden bij gezonde personen aan dat de stimulatie intensiteit een cruciale rol speelde in het faciliteren van een motorische leertaak. Meer bepaald, stelden we een significante verbetering vast van de motorische prestatie wanneer een motorische leertaak werd gekoppeld met tDCS aan een hogere intensiteit (studie 6).

Aan de hand van dit doctoraatsproefschrift kunnen we besluiten dat zowel perifere als centrale elektrostimulatie de revalidatie van neurodegeneratieve aandoeningen zoals MS kan faciliteren. Naast veranderingen in de organisatie van corticale netwerken en het verhogen van de prikkelbaarheid van de gestimuleerde hersenstructuren, hebben we kunnen vaststellen dat zowel perifere als centrale stimulatie tot significante veranderingen in sensorimotorische prestatie kan leiden. Toekomstig onderzoek zal moeten uitwijzen welke (combinatie van) factoren bepalend zijn voor het slagen van de therapie. Onze resultaten tonen alvast aan dat een langdurige therapie aangewezen is voor het induceren van langetermijneffecten en dat intensiteit van de stimulatie cruciaal is voor het bekomen van deze effecten.

Curriculum Vitae

Koen Cuypers was born in Beringen (Belgium) on April 14th 1981. In 1999, he studied Physical Education and Kinesiology at KU Leuven and obtained his master in 2003. Subsequently, he decided to continue his education and entered the Psychology program in 2003 at the same university. He obtained a master degree in Clinical Psychology in 2007. In October 2007, he started to work at the Provinciale Hogeschool Limburg as a research associate under the supervision of Prof. Dr. Raf Meesen. In October 2008, he started his PhD at the REVAL Rehabilitation Research Center, supported by the special research fund (BOF) of the University of Hasselt. During the past years, he was also affiliated as free research associate with the Movement Control and Neuroplasticity Group at KU Leuven.

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