Inducing neuroplasticity in the human motor system by transcranial magnetic stimulation: from pathophysiology to a therapeutic option in movement disorders

Dissertation

zur Erlangung des mathematisch-naturwissenschaftlichen
Doktorgrades
„Doctor rerum naturalium“
der Georg-August-Unversität Göttingen

vorgelegt von
Holger Rothkegel
aus Bonn

Göttingen 2009

Doctoral Thesis Committee:

Prof. Dr. med. Walter Paulus (Supervisor, First Referee)
Abteilung Klinische Neurophysiologie
Universitätsmedizin Göttingen
Robert-Koch-Straße 40
37075 Göttingen

Prof. Dr. Stefan Treue (Reviewer)
Abteilung Kognitive Neurowissenschaften
Deutsches Primatenzentrum (DPZ)
Kellnerweg 4
37077 Göttingen

Prof. Dr. Thomas Rammsayer (Reviewer)
Institut für Psychologie
Universität Bern
UniTobler
Muesmattstrasse 45
CH-3000 Bern 9
Schweiz

Date of Submission: 31st December 2009

Date of the oral examination: 16th February 2010

Statement of Originality

I hereby declare that this thesis is my own work and has been written independently with no other sources and aids than quoted in the text, references and acknowledgements.

Göttingen, 31st December 2009

Holger Rothkegel

Table of Contents

Abbreviations

AMT	active motor threshold
D1/D2	dopamine receptor subtypes
DBS	Deep Brain Stimulation
dPMC	dorsolateral premotor cortex
GPe	external segment of the globus pallidus
GPi	internal segment of the globus pallidus
LICI	long-latency intracortical inhibition
MEP	motor evoked potentials
%MSO	percentage of maximum stimulator output
NMDA	N-methyl-D-aspartate
PAS	paired associative stimulation
PD	Parkinson’s Disease
RMT	resting motor threshold
SAI	short-latency afferent inhibition
SICI	short-latency intracortical inhibition
SMA	supplementary motor area
SNc	substantia nigra pars compacta
SNr	substantia nigra pars reticulate
SP	silent period
STN	Subthalamic nucleus
TBS	Theta Burst Stimulation
TES	transcranial electrical stimulation
TMS	transcranial magnetic stimulation
rTMS	repetitive transcranial magnetic stimulation
VNS	vagus nerve stimulation

Chapter 1 - Introduction

Transcranial Magnetic Stimulation (TMS) is a non-invasive technique which allows assessing and modulating the excitability of cortical areas directly targeted by the stimulation as well as remote cortical and subcortical areas connected to the targeted area via projections. Since its introduction in 1985 (Barker et al., 1985) TMS has become a well established method in clinical neurophysiology and a common research tool in neurology and psychiatry (Kobayashi and Pascual-Leone, 2003; Rossini and Rossi, 2007). Beyond that repetitive TMS (rTMS) offers the potential to induce long-lasting changes of cortical excitability which makes it a promising technique for a non-invasive therapeutic approach in neuropsychiatric disorders.

Several studies have already explored the application of rTMS in different movement disorders with promising results in specific motor tasks. Currently major limitations for clinical application of rTMS are that (a) the effect of the rTMS protocols used so far is not yet strong enough to include this method into standard treatment strategies, (b) the mechanisms behind rTMS induced after effects are not fully understood and (c) the effects of non-invasive brain stimulation on cortical areas with pathologically altered excitability cannot be predicted from studies on young healthy subjects alone.

The objective of this dissertation was primarily to better understand rTMS parameters in order to design hypothesis generated protocols based on neurophysiological data that might be more suitable for clinical trials. For this reason the first study was planned to compare in a groups of patients with Parkinson's disease the immediate after effects of conventional rTMS and the recently introduced Theta Burst Stimulation protocol (TBS) (Huang et al., 2005). In the next steps the influence of breaks during high frequency rTMS as well as the effect of TMS pulse configuration and duration were assessed in healthy volunteers in order to improve the theoretical background for designing appropriate protocols. In movement

disorders the influence of concomitant medication or changes in relevant transmitter systems as well as the pathophysiology of movement planning has to be taken into account. The wider objective of these studies was to define strategies for future approaches to apply non-invasive brain stimulation techniques in research and therapeutical trials in movement disorders.

1.1 Plasticity of the Central Nervous System

The term “neuroplasticity” refers to the remarkable ability of the central nervous system to change its functional connectivity and thus enable adaptation to a changing environment or injury and disease. Even though the potential for functional reorganisation is highest during childhood an activity-dependent modification of cortical and subcortical neuronal circuits takes place throughout the lifetime of each individual. These changes are thought to underlie processes like learning and memory formation.

The concept of functional changes in synaptic efficacy had been postulated by Donald Hebb in 1949 (Hebb, 1949), while the idea that plastic changes of brain structure might underlie certain mental processes can already be found in the work of Santiago Ramon y Cajal (DeFelipe, 2006). Activity-dependent alterations of synaptic strength outlasting the time of stimulation were first demonstrated by Bliss and Lømo in 1973 (Bliss and Lomo, 1973). Following this discovery several mechanisms and forms of long-term potentiation (LTP) and long-term depression (LTP) of synaptic strength have been found and studied in vitro in the hippocampus and other brain areas. These processes describe the modification of postsynaptic responses to presynaptic transmitter release as a result of coincident activation. The main form of neocortical LTP of excitatory glutamatergic synapses is mediated by N-methyl-D-aspartate receptors (Feldman, 2009). In addition even forms of structural plasticity such as formation of new synapses, loss of synapses, remodelling of dendritic spines and neurogenesis have been described.

The knowledge about links between cellular plasticity and features of plasticity at the systems level is still in the early stages. While the term neuroplasticity sensu stricto still refers to synaptic plasticity correlates of these processes such as practice-dependent plasticity have been described in animal models and even in awake and behaving human subjects (Meintzschel and Ziemann, 2006). With the help of recently developed imaging and neurophysiological techniques, changes in

regional cerebral blood flow, receptor binding potentials and stimulus-response-relationships to external brain stimulation can be assessed non-invasively. Furthermore non-invasive brain stimulation allows testing the functional relevance of cortical excitability in specific brain areas for various tasks via the induction of virtual lesions during stimulation and changes of cortical excitability outlasting the duration of stimulation. These techniques can be combined with pharmacological approaches in order to further clarify the involved mechanisms and relate the results to the knowledge of plasticity on a cellular level.

1.2 Non-invasive brain stimulation

In recent decades several methods for the assessment of changes in brain activity have been introduced. While functional imaging techniques such as functional magnetic resonance imaging (fMRI) or positron emission tomography (PET) can visualise changes in brain activity with a high spatial resolution or specificity for a certain transmitter system, the neurophysiological techniques such as electroencephalography (EEG) or non-invasive brain stimulation offer a high temporal precision. While functional imaging techniques allow scans of the whole brain neurophysiological approaches are primarily restricted to superficial cortical areas. In addition to observing brain activity stimulation of cortical areas allows interaction with ongoing processes and the induction of neuroplastic changes in itself.

Several methods for non-invasive brain stimulation have been introduced since the 1980s. Transcranial electrical stimulation (TES) (Merton and Morton, 1980) and transcranial magnetic stimulation (TMS) (Barker et al., 1985) were designed to induce action potentials in neuronal tissue using brief high intensity pulses while transcranial direct current stimulation (tDCS) (Nitsche and Paulus, 2000) and the newly introduced forms of transcranial random noise stimulation (tRNS) (Terney et al., 2008) are intended to induce changes in cortical excitability by a sustained polarization or current flow. As all studies included in this dissertation are

based on different forms of TMS, this technique will be explained in more detail in the following sections.

1.2.1 Transcranial magnetic stimulation

TMS was developed as a means of stimulating the human cerebral cortex in a contactless and painless fashion (Barker et al., 1985). The technique is based on the principle of electromagnetic induction and uses a rapidly changing magnetic field to induce an electrical field and thereby an electrical current in conductive tissue. In order to achieve a very short rise time of the magnetic field a capacitor bank is discharged through a magnetic coil which can be placed over the cortical region of interest. Additional technical details will be discussed in chapter 2.4 which deals with the effect of pulse duration for TMS.

The focality of stimulation depends on the coil geometry, size and orientation. The figure-of-eight coil with two wings of opposite current flow direction (Ueno et al., 1988) has evolved into a standard for focal application of TMS. It is important to note that the magnetic field decreases exponentially with distance from the coil which limits the use of TMS to superficial cortical areas.

While TES is thought to activate pyramidal neurons directly TMS preferentially activates pyramidal neurons transsynaptically. Within the volume of neural tissue targeted by TMS a mixture of different types of neurons might be activated depending on their orientation in relation to the induced electrical field. Thus TMS might elicit excitatory and inhibitory effects simultaneously. There are only two brain regions where TMS gives rise to a positive response: Suprathreshold stimulation of motor areas leads to excitation of corticospinal projections and measurable muscle twitches. Subjective visual sensations can be induced by stimulation over the visual cortex. In other cortical areas TMS leads to inhibitory processes or disruption of information processing (“virtual lesion”), which are also present in motor and visual system.

1.2.2 Repetitive transcranial magnetic stimulation

While a single TMS pulse might affect cortical excitability for a few hundred milliseconds a sequence of stimuli can induce bidirectional changes in cortical excitability outlasting the time of stimulation by several minutes up to a few hours. Since the first systematic assessment of rTMS effects using suprathreshold TMS at different intensities and frequencies applied over the motor cortex (Pascual-Leone et al., 1994) an abundance of different stimulation protocols has been introduced. At the starting point of this thesis project the repetition rate of TMS pulses was considered to be the single most important factor determining the direction of the induced after effects (Fitzgerald et al., 2006) with low frequencies of 1 Hz or less leading to inhibition (Chen et al., 1997) and high frequencies of 2 Hz and more leading to facilitation (Peinemann et al., 2004; Quartarone et al., 2005). LTP-/LTD-like plasticity has been proposed to underlie rTMS induced effects based on similar basic properties – associativity of convergent pathways, input specificity, and a similar effect duration of rTMS effects compared to slice experiments (Ziemann et al., 2006). This assumption is supported by pharmacological studies (Thickbroom, 2007). The aspect of input specificity is even clearer than in rTMS when a peripheral electrical stimulation of a sensory nerve is repeatedly paired with a suprathreshold TMS pulse (Stefan et al., 2000; Wolters et al., 2003). This paired associative stimulation (PAS) protocol is capable of inducing facilitatory and inhibitory after effects depending on the interstimulus interval and thus the order of events at the level of the motor cortex resembling the pattern of spike-timing dependent plasticity (Wolters et al., 2005).

Based on electrophysiological protocols commonly used for the induction of LTP in hippocampal or cortical slices Huang and colleagues developed a special rTMS protocol termed Theta Burst Stimulation (TBS) (Huang et al., 2005). This protocol combines high frequency burst (3 pulses at 50/s) with a repetition rate of these bursts at 5/s (which lies in the theta range of the EEG spectrum). The application of this pattern continuously for 40s leads to inhibition while splitting up the same number of pulses in 2s

stimulation blocks followed by 8s breaks leads to facilitation. The major advantage of TBS protocols compared to conventional rTMS protocols seemed to be a stronger and more stable effect following conveniently short low intensity stimulation trains.

However, several other parameters of the stimulation itself as well as the properties of the stimulated brain areas might alter the magnitude and even the direction of the after effects (Helmich et al., 2006). In this context the state of excitation at the time of stimulation and the recent history of activation for the targeted cortical area are of particular interest as processes of homeostatic plasticity might enhance or reverse the expected effects (Iyer et al., 2003; Lang et al., 2004; Siebner et al., 2004).

The resulting changes in cortical excitability following rTMS can be assessed by electrophysiological parameters in the motor system, functional imaging techniques or behavioural parameters and have been interpreted as correlates of neuroplastic processes involving alterations of synaptic efficacy.

While TMS can only target superficial cortical areas directly even remote effects of rTMS on functionally connected brain areas can be observed (Strafella et al., 2001; Strafella et al., 2003; Wassermann et al., 1998). Transcallosal projections between the primary motor cortices can even be demonstrated after single TMS pulses (Ferbert et al., 1992).

1.3 The motor system as a model for neuroplastic mechanisms in man

As stated above the motor system is unique for studies of neuroplasticity in man as its output is readily accessible and can be measured objectively and non-invasively by neurophysiological methods, which also makes it the best characterized system regarding different elements of cortical excitability.

Following suprathreshold stimulation of the primary motor cortex (Barker et al., 1985) and even some frontal non-primary motor areas (Teitti et al., 2008) motor evoked potentials can be recorded from contralateral muscles. Most studies have used the small hand muscles as they have

lowest stimulation thresholds and large representational areas close to the surface, but more proximal arm muscles, leg muscles as well as facial muscles can be targeted as well. Epidural cervical recordings demonstrated that TMS preferentially acts via transsynaptic activation of corticospinal neurons reflected by a dominance of later corticospinal volleys termed I-Waves (indirect) (Di Lazzaro et al., 1998; Kaneko et al., 1996) depending on the direction of the induced electrical current in the brain while TES produces D-Waves (direct).

The excitability of the motor cortex is commonly characterised by measuring resting (RMT) and active motor threshold (AMT), stimulus intensity–response–curves or MEP amplitudes at a certain stimulus intensity. Motor threshold is the lowest stimulus intensity that produces discernable MEPs and is believed to reflect mainly axon excitability regulated by voltage gated sodium channels (Ziemann, 2003). MEPs reflect the synaptic strength and balance of excitatory and inhibitory processes acting at the corticospinal output neurons (as well as spinal excitability) and are the most sensitive parameter for changes in cortical neurotransmission. In addition inhibitory intracortical mechanisms can be specified by measuring cortical silent period (SP) following a single TMS pulse or using conditioning pulse – test pulse paradigms such as short latency intracortical inhibition (SICI) (Kujirai et al., 1993), long-latency intracortical inhibition (LICI) (Roick et al., 1993), transcallosal inhibition (Ferbert et al., 1992) or short-latency afferent inhibition (SAI) (Tokimura et al., 2000).

Thus neuroplastic changes in the motor system following rTMS, pharmacological interventions or motor learning can be assessed both on a neurophysiological level as well as on a behavioural level. Most rTMS protocols have been tested and characterised in the motor system first before they were applied to other brain regions.

1.4 Application of non-invasive brain stimulation in movement disorders

Pathologically altered cortical excitability has been found in a number of neuropsychiatric disorders such as movement disorders, stroke, chronic pain, migraine, epilepsy and depression (Rossini and Rossi, 2007; Wassermann and Lisanby, 2001). In addition to being a direct consequence of a primary lesion these changes reflect adaptive (and in part even maladaptive) processes involving neuroplastic mechanisms.

Thus it has been hypothesised that externally induced plasticity aiming to compensate for altered cortical excitability, enhance beneficial adaptations or prevent maladaptive processes would be a valuable therapeutic option. This approach is supported by experience with implantable electrical stimulation devices such as Deep Brain Stimulation (DBS) in Parkinson's Disease, epidural stimulation in chronic pain or vagus nerve stimulation (VNS) in epilepsy, which are established treatment options.

As the motor system is probably the best characterized system in humans regarding externally induced neuroplastic changes it is not surprising that the second largest group of clinical rTMS studies has focused on movement disorders outnumbered only by depression.

1.4.1 Parkinson's Disease – Clinical Features and Pathology

Parkinsonism is defined as a movement disorder showing the typical clinical symptoms of akinesia in combination with rigidity, resting tremor or postural instability. Additional symptoms may include sensory signs, vegetative disorders, cognitive impairment and psychic symptoms, depression in particular (Diener and Putzki, 2008). The most common etiology for Parkinsonism accounting for about 75% of all cases is Parkinson's Disease (PD) while it also occurs as part of other neurodegenerative diseases such as multisystem atrophy, progressive supranuclear palsy or corticobasal degeneration and in monogenetically

inherited forms. Even vascular lesions or medication can cause the clinical signs of (secondary) Parkinsonism.

Parkinson's Disease is a one of the most common neurodegenerative diseases with a prevalence of 100-200/100.000 in the general population in Germany, which increases to 1.800/100.000 in the population aged > 65 years (Diener and Putzki, 2008).

The pathological process underlying motor symptoms in PD is a progressive loss of dopaminergic neurons in the substantia nigra pars compacta projecting to the striatum. At present it is still unclear what causes this rather selective loss of a specific subset of neurons. Recently a model of a spreading affection of vulnerable neurons starting in the olfactory bulb, anterior olfactory nucleus, and dorsal motor nucleus of the vagus nerve has been proposed based on post-mortem examinations (Braak et al., 2003). According to this model a yet unknown pathogen causes progressive neuronal loss in 6 stages. As alternative explanation for the loss of dopaminergic neurons excitotoxic effects have been proposed.

According to classical models of basal ganglia function (Albin et al., 1989; Alexander and Crutcher, 1990; DeLong, 1990) the loss of dopaminergic neurons leads to complex alterations in the cortex – basal ganglia – cortex loop. In the indirect pathway (putamen – external segment of the globus pallidus (GPe) – subthalamic nucleus (STN) – internal segment of the globus pallidus (GPi)/substantia nigra pars reticulata (SNr)) reduced activation of putaminal D2 receptors leads to excessive activation of the inhibitory output nuclei which is paralleled by disinhibition of the GPi/SNr via reduced activation of D1 receptors on neurons which are part of the direct pathway (putamen – GPi/SNr). This in turn leads to an increased inhibition of the ventrolateral thalamus and a consecutively reduced excitatory drive to cortical premotor areas which has been associated with bradykinesia.

In the initial stages of PD a sufficient control of motor symptoms can be achieved by intake of dopaminergic drugs. However, with disease progress the therapeutic range of these drugs shrinks and patients develop motor fluctuations and treatment-induced dyskinesias (Watts,

1997). In addition gait disorder, on-freezing and postural stability as well as non-motor symptoms do not respond sufficiently well to dopaminergic treatment. For patients with severe motor fluctuations Deep Brain Stimulation (DBS) has been introduced in recent years which is believed to reduce the pathologically increased activity of the STN by high-frequency stimulation (functional lesion).

1.4.2 Electrophysiological Findings in PD

Despite the huge variability in clinical presentation and predominant symptoms in PD patients and different inclusion criteria for TMS studies there are some consistent findings in the literature regarding electrophysiological measures in PD (review in (Lefaucheur, 2005)). As expected from models of PD pathophysiology the corticospinal tract itself is not affected in PD as shown by normal central conduction times to direct stimulation using TES (Dick et al., 1984) or transsynaptic stimulation using TMS (Cantello et al., 1991; Ellaway et al., 1995). However, the response to TMS differs considerably when measured at rest or under tonic contraction. Using TMS over the primary motor cortex lower motor threshold (Cantello et al., 1991) and higher MEP-amplitudes at rest with a decreased facilitation by tonic contraction (Valls-Sole et al., 1994) have been observed reflecting an increased excitability at rest combined with an impaired voluntary drive. This pattern resembles the clinical features of rigidity and bradykinesia respectively.

The assessment of inhibitory mechanisms in the primary motor cortex has shown a shortened cortical silent period (Cantello et al., 1991) reflecting decreased GABA-B receptor mediated inhibition, which tends towards normal values after L-DOPA intake (Priori et al., 1994). A reduced GABA-A receptor mediated SICI has been shown in PD patients OFF medication, which is partly restored ON medication (Ridding et al., 1995). The contribution of striatal and cortical dopamine receptors for these observations remains open.

In addition studies using functional imaging demonstrated a reduced activation of the rostral supplementary motor area (SMA) and prefrontal

areas in PD patients while performing a simple movement task (Buhmann et al., 2003; Sabatini et al., 2000).

1.4.3 Externally induced neuroplasticity in PD

The rationale behind the application of non-invasive brain stimulation in PD is to induce changes in cortical excitability which can compensate for alterations caused by the primary pathology or to enhance adaptive and prevent maladaptive plasticity. So far the primary motor cortex has been the target region for most studies as it a) is the final output regions of the motor system, b) has been shown to be affected by alterations in cortical excitability and c) can be reached easily by non-invasive stimulation. In addition it has been found that rTMS over the primary motor cortex is capable of inducing increased dopamine release in the striatum (Strafella et al., 2003).

At the starting point of this thesis project a number of rTMS-studies had already been conducted in Parkinson's disease. Positive effects on bradykinesia had been reported following a variety of different stimulation intensities and repetition rates (Khedr et al., 2003; Lefaucheur et al., 2004; Siebner et al., 1999; Siebner et al., 2000; Sommer et al., 2002). A study using high frequency stimulation targeting the SMA yielded a worsening in complex movements (Boylan et al., 2001). Other studies did not confirm a therapeutical effect on a movement task during or following rTMS (Ghabra et al., 1999; Tergau et al., 1999). Because of the high variability in patient selection, stimulation parameters and rather modest clinical effects it is not possible to draw firm conclusions from these studies what the optimal stimulation parameters for rTMS in PD might be. In that context a more reliable, conveniently short and highly effective protocol as proposed for TBS would be a promising option for clinical trials (Huang et al., 2005; Paulus, 2005).

Furthermore non-invasive brain stimulation could be useful to better define which patients might benefit from DBS, which is a technically challenging operation associated with the risk of brain surgery.

1.5 Aims

The general objective of this thesis project was to re-evaluate the therapeutic potential of rTMS in Parkinson's Disease and identify stimulation parameters which could be optimised in order to facilitate a clinically meaningful application of non-invasive brain stimulation. While the first study focused on aspects of rTMS induced after effects on motor function in PD, the following studies were designed to explore the relevance of specific rTMS parameters and the mechanisms behind movement timing. These studies were done in the motor system of healthy subjects as the impact of breaks during rTMS and pulse duration for single pulse TMS had not been investigated previously.

The specific aims of the following studies were

- to compare the potential of the newly introduced TBS protocol and conventional rTMS protocols as a therapeutic option in Parkinson's Disease,
- to clarify the role of concomitant dopaminergic medication on rTMS induced after effects
- to explore the role of breaks during pronged rTMS trains
- to explore the effect of pulse duration in TMS
- to clarify the role of the dorsolateral premotor cortex in movement timing.

Chapter 2 – Original Articles

The following articles will be presented in this chapter:

I. Rothkegel H, Sommer M, Rammsayer T, Trenkwalder C, Paulus W. Training Effects Outweigh Effects of Single-Session Conventional rTMS and Theta Burst Stimulation in PD Patients. Neurorehabil Neural Repair 2009; 23: 373-81.
The main study design was given by a DFG proposal by Dr. M. Sommer. H. Rothkegel was responsible for the choice of motor tasks and the selection of the two conventional rTMS protocols. All experiments were performed and analysed by H. Rothkegel. Statistical analysis was done by H. Rothkegel and Dr. M. Sommer. The manuscript was prepared by H. Rothkegel with contributions of all authors.

II. Lang N, Speck S, Harms J, Rothkegel H, Paulus W, Sommer M. Dopaminergic potentiation of rTMS-induced motor cortex inhibition. Biol Psychiatry 2008; 63: 231-3.
The study was designed by Dr. N. Lang. H. Rothkegel was involved in the execution of the experiments, statistical analysis and writing of the manuscript.

III. Rothkegel H, Sommer M, Paulus W. Breaks during 5Hz rTMS are essential for facilitatory after effects. Clin Neurophysiol. [Epub ahead of print, available online 16 Dec 2009]
The idea for this study was developed by H. Rothkegel and Dr. M. Sommer. H. Rothkegel developed the study design and scripts for rTMS timing, carried out all experiments and performed data and statistical analysis. The manuscript was written by H. Rothkegel with contributions of Prof. W. Paulus and Dr. M. Sommer.

IV. Rothkegel H, Sommer M, Paulus W, Lang N. Impact of pulse duration in single pulse TMS. [submitted]

The idea for this study was developed by H. Rothkegel and Dr. N. Lang. Study design, experiments, script programming for offline EMG measurements, data and statistical analysis were done by H. Rothkegel. the manuscript was prepared by H. Rothkegel with contributions by Prof. W. Paulus, Dr. N. Lang and Dr. M. Sommer.

V. Pollok B, Rothkegel H, Schnitzler A, Paulus W, Lang N. The effect of rTMS over left and right dorsolateral premotor cortex on movement timing of either hand. Eur J Neurosci 2008; 27: 757-64.

The study was designed by Dr. B. Pollok and Dr. N. Lang with contributions of H. Rothkegel. The main test program was provided by Dr. B. Pollok, additional programming for randomized TMS control was done by H. Rothkegel. Experiments were performed by Dr. B. Pollok, Holger Rothkegel and Dr. N. Lang. Data analysis, statistical evaluation and preparation of the manuscript were done by Dr. B. Pollok. All authors contributed in writing the manuscript.

2.1 Training Effects Outweigh Effects of Single-Session Conventional rTMS and Theta Burst Stimulation in PD Patients

Previous studies using conventional rTMS have shown positive effects on motor symptoms in PD patients. The results have so far been rather modest and inconsistent between studies. The reasons for this might be the differing selection of patients in different stages of the disease, the state of medication and the use of various stimulation protocols. At the starting point of this thesis a new rTMS protocol had been introduced, which was designed to transfer theta burst stimulation, a commonly used pattern for induction of LTP or LTD in cell physiology, to experimental conditions in humans. TBS promised to produce stronger and more reliable after effects (Huang et al., 2005; Paulus, 2005).

The main objective of this study was to compare short-term effects of TBS with those of conventional rTMS as assessed by several motor tasks in PD patients. As the antiparkinsonian medication (mainly dopaminergic drugs) or the lack of dopamine compared to healthy subject might interfere with the expected rTMS induced effects we included a group of patients ON and OFF medication respectively.

Surprisingly the major finding of this study was a strong and prolonged motor learning in the patients ON medication in tasks which were derived from standard clinical tests. This effect was not observed in the patients OFF medication. Neither the group ON medication nor the group OFF medication showed any effects on motor function which could be clearly attributed to the rTMS protocol as there was no difference compared to sham stimulation. These results demonstrate that a lack of dopamine in PD leads to impaired motor learning. The lack of rTMS induced effects might be explained by interactions with previous motor learning in the ON group and by the impaired neuroplastic capacity in the OFF groups.

Neurorehabilitation and Neural Repair
Volume 23 Number 4
May 2009 373-381

10.1177/1545968308322842
http://nnr.sagepub.com

Training Effects Outweigh Effects of Single-Session Conventional rTMS and Theta Burst Stimulation in PD Patients

Holger Rothkegel, Martin Sommer, MD, Thomas Rammsayer, PhD, Claudia Trenkwalder, MD, and Walter Paulus, MD

Background. Focal single-session repetitive transcranial magnetic stimulation (rTMS) of the primary motor cortex has been claimed to be capable of improving motor function in Parkinson's disease. *Objective*. The authors sought to determine which type of rTMS protocol holds the highest potential for future therapeutic application. *Methods*. Twenty-two patients with Parkinson's disease received 5 different rTMS protocols on 5 consecutive days in a pseudorandomized and counterbalanced order either in the defined OFF condition or with their usual medication. The protocols tested in the present study included 2 conventional rTMS protocols (0.5 and 10 Hz) as well as the recently introduced theta burst stimulation (cTBS, iTBS) and a sham condition. Cortical excitability, motor performance (pointing movement, pronation-supination, Purdue Pegboard Test, walking), and mood were assessed before and after each session. *Results*. The authors observed motor training from days 1 to 4, particularly in the group on dopaminergic medication. None of the rTMS paradigms excelled placebo stimulation. The only exception was the Purdue Pegboard Test, in which all active stimulation paradigms yielded slightly stronger effects than sham stimulation. *Conclusions*. Within a single session, no clinically relevant difference in the rTMS protocols could be detected. Training effects outweigh and may have masked rTMS effects, particularly in the group on dopaminergic mediation.

Keywords: *Parkinson's disease; Single-session repetitive transcranial magnetic stimulation (rTMS); Theta burst stimulation (TBS).*

Dopaminergic drugs are a highly effective treatment in the initial stage of Parkinson's disease (PD). However, gait disorder, on-freezing, and postural instability do not respond well to dopaminergic treatment. With disease progression, the dopaminergic drug effects shrink, with response fluctuations and akinesia on one side and sometimes painful and disabling dyskinesias on the other side. Therefore, a number of alternative, nonpharmacological procedures have been suggested.

Repetitive transcranial magnetic stimulation (rTMS) is a noninvasive technique that is capable of inducing alterations of neuronal network excitability in the area directly targeted by the stimulation coil as well as in connected areas outlasting the time of stimulation.[1,2] Even with focal stimulation over the motor cortex, remote areas such as the basal ganglia can be affected.[3] Therefore, rTMS has been assumed as a tool possibly restoring pathologically altered excitability of cerebral motor areas in movement disorders. A multitude of different stimulation paradigms varying in frequency, intensity, configuration, or location of rTMS has already been tested in PD patients. However, the results so far have been modest and inconsistent[4-7] (for review, see Fregni et al,[8] Helmich et al,[9] and Sommer and Paulus[10]). None of the protocols so far has made its way into standard therapy.

Recently, theta burst stimulation (TBS) has been adapted as a new TMS protocol.[11] Theta burst stimulation seems to have a higher potential of inducing stronger and more reliable aftereffects than conventional rTMS, thus possibly making it a better option for treatment studies in movement disorders.[12] Because it is not possible to predict the effects of neuroplasticity-inducing protocols on cortical areas with pathologically altered excitability from studies on young healthy subjects alone,[4,13] we have studied TBS effects in PD patients.

The aims of this study were to assess whether (1) a single session of TBS was able to improve motor performance in PD immediately after stimulation, (2) TBS was more effective than conventional rTMS, (3) unilateral stimulation of the hand motor area had differential effects on contralateral hand muscles compared with other muscle groups, (4) dopaminergic medication was necessary to achieve these effects, and (5) effects on motor performance were associated with changes in cortical excitability.

From the Department of Clinical Neurophysiology, University of Göttingen, Göttingen, Germany (HR, MS, WP); Paracelsus Elena-Klinik, Kassel, Germany (CT); Georg-Elias-Müller Institute for Psychology, Göttingen, Germany (TR); and University of Bern, Institute for Psychology, Bern, Switzerland (TR). Address correspondence to W. Paulus, MD, Department of Clinical Neurophysiology, University of Göttingen, Robert-Koch-Straße 40, 37075 Göttingen, Germany. E-mail: wpaulus@med.uni-goettingen.de.

Table 1
Clinical Features and Antiparkinsonian Medication

	Gender	Age, y	Duration	Symptoms	UPDRS III	L-Dopa, mg	Intakes Per Day	Agonists	LED, mg	Other
OFF-group										
1	M	66	25	ART	28	575	7	Pram 1.5 mg	750	Ent, Aman
2	F	69	38	ART	28	350	9	Cab 4 mg, Pram 2.1 mg	800	Ent, Aman
3	F	64	8	ART	14	350	6	Cab 6 mg	650	
4	M	52	3	ART	18	300	4	Rop 20 mg	800	
5	M	65	6	ART	52	825	8	Pram 2.45 mg	1115	Tol, Aman
6	F	75	7	AR	35	925	9	Pram 2.1 mg	1175	Tol, Aman
7	M	75	6	AR	51	600	5	Pram 0.72 mg	685	Ent
8	M	65	3	ART	23	400	4	Cab 6 mg	700	
9	M	67	7	ART	17	500	5	Cab 4.5 mg	725	
10	F	66	7	ART	20	675	8	Pram 2.45 mg	965	Ent, Aman, Ras
11	F	73	4	AR	11	800	9	—	800	Ent
Mean	F/M:	67.0	10.4		27.0	572.7	6.7		833.2	
SD	5/6	6.4	11.0		13.9	214.3	2.0		175.7	
ON-group										
12	F	74	12	ART	26	800	8	Pram 1.75 mg	1005	Tol, Aman
13	F	76	11	AR	39	700	9	Rop 24 mg	1300	Ent, Aman
14	F	67	8	ART	21	350	6	Pram 1.05 mg	475	
15	M	48	1	AR	15	100	2	Pram 1.4 mg	265	Ras, Aman
16	M	65	13	AR	38	650	8	Rop 6 mg	800	Ent
17	M	59	8	AR	18	1100	9	Cab 4 mg, Pram 1.62 mg, Apo 4 mg (2 days)	1490 (1590)	Ent, Ras, Aman
18	F	63	17	ART	46	1000	9	Pram 2.1 mg	1350	Ent, Aman
19	M	34	6	ART	21	0	0	Cab 6 mg, Pram 2.1 mg	550	Ras, Aman
20	F	65	7	AR	21	350	8	Cab 3 mg	500	
21	M	56	5	ART	17	575	8	Pram 2.8 mg	905	Ras
22	M	65	8	ART	32	675	6	Pram 1.75 mg	880	Bud
Mean	F/M:	61.1	8.7		26.7	572.7	6.6		865.5	
SD	5/6	11.9	4.3		10.4	345.8	3.0		398.2	
Overall Mean	F/M:	64.0	9.5		26.9	572.7	6.7		849.3	
SD	10/12	9.8	8.2		12.0	280.7	2.5		300.8	

Abbreviations: M, male; F, female; duration, duration since disease onset in years. Symptoms, dominant symptoms of Parkinson's Disease; AR, akinetic-rigid; ART, akinetic-rigid-tremor; UPDRS III, Unified Parkinson's Disease Rating Scale part III motor score. Dopamine agonists and daily dose: Pram, pramipexol; Cab, cabergoline; Rop, ropinirol; Apo, apomorphine; LED, L-Dopa equivalent dose. Other antiparkinsonian drugs: Ent, entacapone; Tol, tolcapone; Ras, rasagiline; Aman, amantadine; Bud, budipine.

Material and Methods

Subjects

Twenty-two patients were studied while inpatients at the Paracelsus-Elena-Klinik, Kassel. All patients fulfilled the UK Parkinson's Disease Brain Bank Criteria for PD and were in Hoehn & Yahr stages II to IV. No antiparkinsonian medication was newly introduced or stopped during the trial week, and slight changes of the dosage were allowed according to the patients' needs. Patients with severe motor fluctuations, dementia, or any contraindication against TMS (metal or electronic implants, cerebral ischemia, epilepsy, instable psychiatric or internal diseases, pregnancy, drug or alcohol abuse) were excluded. Depression, a frequent comorbidity in this population, was evaluated using the Beck Depression Inventory[14] with 5 patients in the OFF group and 4 patients in the ON group, yielding scores higher than 11. None of the subjects had ongoing psychosis or hallucinations at the time of the study.

All patients gave written informed consent to the study protocol, which had been approved by the ethics committees of the University of Göttingen and the Landesärztekammer Hessen and was in accordance with the Declaration of Helsinki.

General Study Design

To study the influence of dopaminergic medication, we decided to randomize patients to receive rTMS either in the defined OFF condition in the morning (ie, after a 12-hour overnight withdrawal of antiparkinsonian medication[15]; OFF-group) or as add-on intervention while continuing to take their normal medication (ON-group). The 2 groups did not differ significantly in gender, age, duration, and dominant PD symptom (tremor or hypokinesia), dose of L-dopa, or L-dopa equivalent dose (see Table 1), as shown by 2-tailed unpaired *t* tests or chi-square tests where appropriate (SPSS 12.0 for Windows, SPSS Inc, Chicago, Illinois). A P value of $<.05$ was considered significant for all statistical tests. L-dopa equivalent dose was calculated according to the guidelines of the

German Parkinson-Network (www.kompetenznetz-parkinson.de/Parkinson/leittherapie.html, retrieved July 11, 2006: L-dopa equivalent dose = L-dopa + apomorphine/4 * 100 + cabergoline/2 * 100 + pramipexole/0.85 * 100 + ropinirole/4 * 100 [daily doses]).

All patients had one training session encompassing all clinical tests before entering the study to prevent strong learning effects in the motor tasks and to familiarize them with the procedures of TMS. Five different rTMS protocols, including sham stimulation, were tested on 5 consecutive days in a pseudorandomized order at the same time of day for each subject. Patients were told that different rTMS protocols were about to be studied in the search of the optimal clinical effect, but no details were given about the different types of stimulation or about the presence of a sham condition.

Intervention

rTMS was generated by a Medtronic MagPro X100 + MagOption stimulator in the biphasic mode with reversed current direction (initially posterior-anterior current flow in the brain, as originally described for TBS[11]). A slightly bent figure-of-8 coil (Medtronic MC-B70) was held perpendicular to the head over the optimal representation of the target muscle (see assessment of corticospinal excitability) with the handle pointing posteriorly and 45 degrees laterally.

We chose 0.5 Hz (continuously, 600 pulses at an intensity of 80% resting motor threshold [RMT]) and 10 Hz (20 trains of 100 pulses, 50-second intertrain interval, 80% RMT) as conventional rTMS protocols, as published by Lefaucheur et al.[5] For TBS, we used the continuous (cTBS, 600 pulses, 80% active motor threshold [AMT]) and intermittent (iTBS, 20 trains of 30 pulses, 8-second intertrain interval) pattern, as described by Huang et al.[11] In brief, TBS stimulation consists of triplets of pulses at a high frequency (50 Hz) repeated with a lower frequency in the theta range (5 Hz). For sham intervention, we used the iTBS protocol with the coil tilted at 90 degrees so that only the edge of the coil touched the head.[16]

Assessment

Immediately before the first and after the last session, patients were assessed using the motor section of the Unified Parkinson's Disease Rating Scale (UPDRS),[17] and they completed the Beck Depression Inventory (BDI)[14] to test for changes during the week of experiments.

Corticospinal excitability, several motor tasks, and a behavioral self-rating scale were assessed in the following order starting 5 minutes after intervention and, for practical reasons, in reversed order for baseline measurements:

1. Corticospinal excitability (RMT, AMT, motor evoked potential [MEP], background electromyographic [EMG] activity)
2. Rapidly alternating arm movements (pointing task, pronation-supination)
3. Purdue Pegboard Task (PPT)
4. Mood self-rating scale
5. Gait (time, number of steps)

(1) Corticospinal excitability. Surface EMG was recorded with Ag/AgCl cup electrodes in a belly-tendon montage from the abductor digiti minimi (ADM) of the more affected hand. Signals were amplified with a Toennies Electromyograph II (Toennies, Würzburg, Germany) using a bandpass filter of 1.6 to 1000 Hz, sampled with a CED Micro 1401 mk II (Cambridge Electronic Design, Cambridge, England) at a rate of 5 kHz and stored on a lab computer for offline analysis. Single-pulse TMS was applied using the Medtronic stimulator with the same settings as for the interventions (biphasic pulses, reversed current direction). The coil was moved over the assumed location of the primary motor cortex contralateral to the more affected side of the body. The point where maximum responses in the ADM were observed was defined as the optimal cortical representation of this muscle and was used for single-pulse and repetitive stimulation.

Resting motor threshold was determined as the minimum intensity at which at least 5 out of 10 consecutive TMS pulses induced MEPs of >50 μV in amplitude with the subject at rest. Active motor threshold was measured under tonic contraction of the target muscle of about 20% of maximum EMG activity, as monitored by visual feedback. The minimum intensity at which at least 5 out of 10 TMS pulses induced MEPs of >200 μV in amplitude was considered AMT.[18]

For assessment of MEP amplitudes, 20 single pulses were applied every 4 ± 0.4 seconds. The intensity of the magnetic pulses was adjusted to induce MEPs of about 1 mV at baseline and kept constant for the measurement after intervention. Peak-to-peak amplitudes were measured offline.

Mean baseline EMG activity was measured in the 80 ms preceding the TMS stimulus. Pearson's correlation coefficients were calculated to investigate the relations between baseline EMG activity, motor thresholds, and overall rigidity, as well as rigidity of the more affected arm (ie, contralateral to the stimulated hemisphere), as assessed by the respective UPDRS score on the first day of experiments.

(2) Rapidly alternating arm movements. Two types of rapidly alternating arm movements were recorded with an ultrasound-based 3D motion analysis system (zebris CMS-HS using customized WinArm Software, zebris Medical GmbH, Isny im Allgäu, Germany).

First, an arm-hand pointing movement between 2 targets 30 cm apart was performed according to the Core Assessment Program for Surgical Interventional Therapies in Parkinson's Disease (CAPSIT-PD[15]) and recorded at a sampling rate of 100 Hz.

Second, a forearm pronation/supination movement was assessed using the predefined item of the WinArm Software (sampling rate 80 Hz).

We recorded 2 trials for either hand at each time point. Motion trajectories were analyzed offline for average frequency and amplitude of 8 full-movement cycles, leaving out the first 2

cycles (starting phase). As the frequency of rapid, alternating movements depends on the amplitude, we chose movement speed (ie, product of mean frequency and mean amplitude) as a more reliable parameter. Results for each sample were normalized to the individual baseline of each experiment.

(3) Purdue Pegboard Task. As a test for fine motor skills and complex upper limb movements, we tested performance with the PPT (Lafayette Instrument Co. Europe, Loughborough, UK) for both hands separately and bimanually. Patients were instructed to pick up pins from a cup and place them in holes in the board starting with the top hole as fast as possible for 30 seconds. Two trials for either hand and for the bimanual task were performed, and the number of pins placed correctly was counted.

(4) Mood self-rating scale. Before and after each session, subjects completed a self-rating questionnaire containing the following items as 7-point scales ranging from –3 to +3, adapted and translated from Strafella et al[3]: comfort, anxiety, fatigue, mood, irritation, attention, and pain. Positive values represent positive feelings, whereas negative values represent negative feelings. After stimulation, subjects were asked whether they felt the respective protocol to be effective for them and whether they felt better, worse, or the same as before.

(5) Gait performance. Patients were asked to perform the walking test of the CAPSIT-PD.[15] We measured the duration and counted the number of steps for walking 7 meters forth and back, including turning (2 trials at each time point). All values were normalized to the individual baseline of each experiment.

Statistical Analysis

For UPDRS and BDI scores, repeated-measures analyses of variance (ANOVAs) were calculated with the 2 time points (before/after the week of stimulation) as the within-subjects factor and group (ON/OFF) as the between-subjects factor. We also tested for baseline differences between the 2 groups using 2-tailed unpaired *t* tests.

To test for different effects of the 5 intervention protocols (0.5 Hz, 10 Hz, iTBS, cTBS, sham), we performed repeated-measures ANOVAs (Statview 5.0, SAS Institute Inc, Cary, North Carolina) for all tests separately with intervention protocol and group (ON/OFF) as between-subjects factors. Within-subjects factors were time (pre/post), trial (for all motor tasks), and hand (only for arm movement tasks). For motor thresholds, the level of activation was used as an additional within-subjects factor. The intraindividual variance of MEP amplitudes was too high to allow for meaningful statistical analysis, which we therefore omitted. Results of the Purdue Pegboard Task were entered into separate repeated-measures ANOVAs for the unimanual and the bimanual tasks.

The 7-point self-rating scales were tested for changes after intervention or over the week of experiments by ANOVAs as described above for the motor tasks. The 2 items, efficacy and general change, were tested using chi-square tests for cross-table relationships.

To further address the question of training effects over the week of experiments, we performed ANOVAs on the nonnormalized values of each test with group as the between-subjects factor and day (+ trial and hand, where applicable) as within-subjects factors.

Results

The behavioral measures showed a remarkable training effect, with gradual performance improvement from day 1 to day 4. This training effect was particularly pronounced in the group of patients "on" dopaminergic medication.

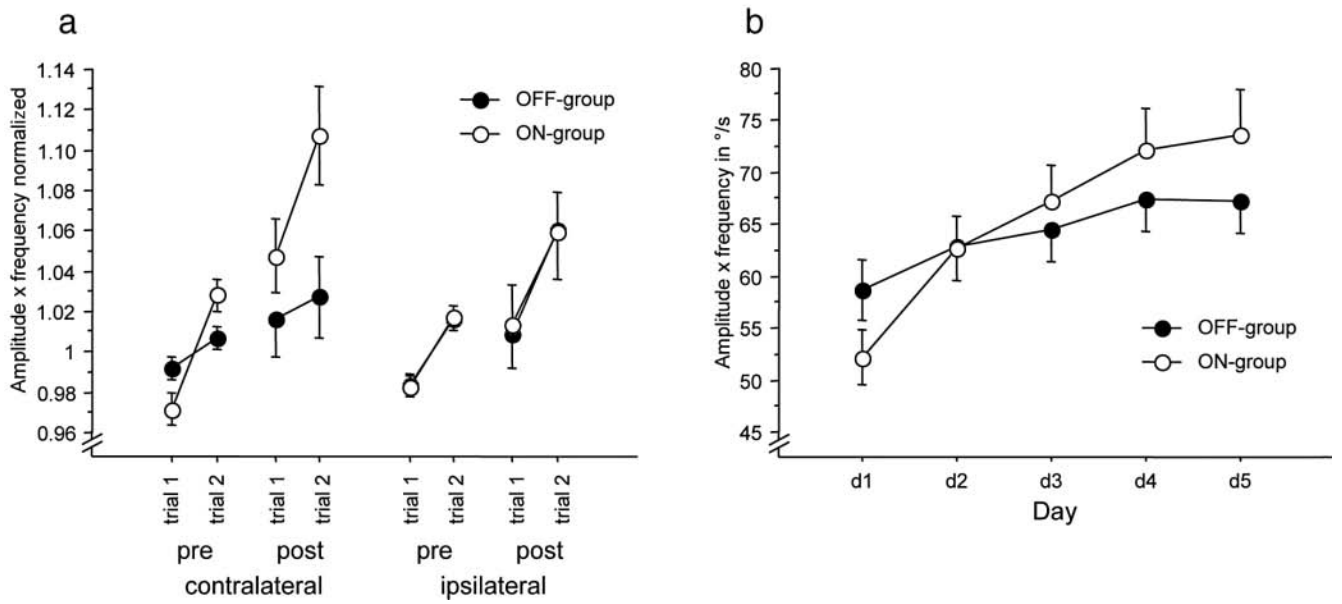
Rapidly alternating movements. In both groups, performance in the arm-hand pointing task improved in either hand after intervention and from trial 1 to trial 2 (ANOVA, effect of time, $F(1, 100) = 11.050$, $P = .0012$; effect of trial, $F(1, 100) = 35.087$, $P < .0001$). This improvement was particularly pronounced for the more affected side in the ON-group (Interaction Side × Group, $F(1, 100) = 6.912$, $P = .0099$; Interaction Side × Time × Group, $F(1, 100) = 6.862$, $P = .0102$; Interaction Side × Trial × Group, $F(1, 100) = 7.225$, $P = .0084$; see Figure 1a).

Analysis of baseline raw values revealed increasing performance during the first 4 days of the study week for both groups (ANOVA, effect of day, $F(4, 80) = 16.544$, $P < .0001$), which was more pronounced in the ON-group (Interaction Day × Group, $F(4, 80) = 2.800$, $P = .0313$; see Figure 1b). Performance in trial 2 was generally better than in trial 1 (effect of trial, $F(1, 20) = 11.160$, $P = .0033$), with the steepest increase for the more affected hand in the ON-group (Interaction Side × Trial × Group, $F(1, 20) = 4.374$, $P = .0495$).

For the forearm pronation/supination task in the ON-group but not in the OFF-group, we found an improved performance after intervention, as measured by the product of frequency and amplitude normalized to baseline (ANOVA, effect of group, $F(1, 100) = 4.281$, $P = .0411$; effect of time, $F(1, 100) = 4.784$, $P = .0311$; Interaction Time × Group, $F(1, 100) = 3.920$, $P = .0505$). Performance in the second trial compared with the first trial was reduced in the ON-group (effect of trial, $F(1, 100) = 11.873$, $P = .0008$; Interaction Trial × Group, $F(1, 100) = 5.596$, $P = .0199$) for both the stimulated and nonstimulated sides before and after intervention (no interaction of trial with side or time), whereas performance in the OFF-group was rather constant in both trials (see Figure 2a).

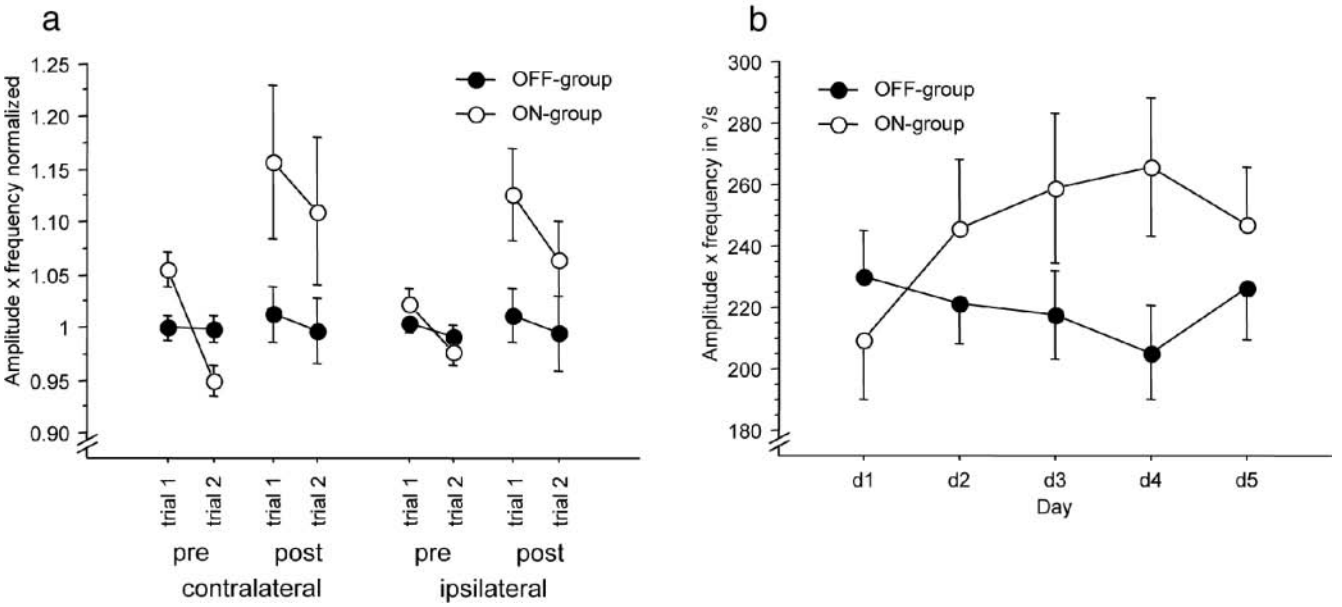
Over the week of experiments, the ON-group's baseline performance improved during the first days, whereas there was a slight decrease in performance for the OFF-group (ANOVA for baseline raw values, Interaction Day × Group, $F(4, 80) = 5.113$, $P = .0010$; see Figure 2b). Trial 2 was generally worse than trial 1 (effect of trial, $F(1, 20) = 7.279$, $P = .0138$), only for the first day, this pattern was reversed (Interaction Day × Trial, $F(4, 80) = 2.649$, $P = .0392$).

Figure 1
Arm-Hand Pointing Movement



Note: Higher values indicate better performance. Results of the different types of intervention are pooled because analysis of variance (ANOVA) did not show any main effect or interaction for this factor. (a) Amplitude × Frequency normalized to individual mean baseline values (pretrials 1 and 2); (b) Amplitude × Frequency, mean baseline values over the days of the experiment, both sides pooled; error bars indicate ±1 SEM.

Figure 2
Forearm Pronation-Supination Movement



Note: Higher values indicate better performance. Results of the different types of intervention are pooled because analysis of variance (ANOVA) did not show any main effect or interaction for this factor. (a) Amplitude × Frequency normalized to individual mean baseline values (pretrials 1 and 2); (b) Amplitude × Frequency, mean baseline values over the days of the experiment, both sides pooled; error bars indicate ±1 SEM.

PPT. Performance in the unimanual tasks improved after intervention (ANOVA, effect of time, $F(1, 95) = 5.161$ $P = .0254$; see rTMS-related effects). Performance also improved from trial 1 to trial 2 (effect of trial, $F(1, 95) = 49.414$, $P < .0001$) in the ON-group, especially at baseline (Interaction Time × Trial × Group, $F(1, 95) = 5.121$, $P = .0259$; Time × Trial, $F(1, 95) = 4.067$, $P = .0466$).

For the bimanual task, performance improved after intervention and from trial 1 to trial 2 (ANOVA, effect of time, $F(1, 95) = 7.355$, $P = .0079$; effect of trial, $F(1, 95) = 12.047$, $P = .0008$).

Baseline values in the unimanual task improved during the week of experiments (ANOVA, effect of day, $F(4, 76) = 9.363$, $P < .0001$), from 9.24 ± 2.00 (mean ± SD) pins to 10.41 ± 1.86 pins on the more affected side and from 10.29 ± 1.88 to 11.29 ± 1.76 pins on the less affected side (effect of side, $F(1, 19) = 10.075$, $P = .0050$). Performance also improved from trial 1 to trial 2 (effect of trial, $F(1, 19) = 24.845$, $P < .0001$). In the bimanual task, we found improved performance during the week of experiments (ANOVA, effect of day, $F(4, 76) = 6.673$, $P = .0001$) but no significant effect of trial.

One patient in the ON-group was not able to complete the pegboard task with the less affected hand or bimanually because of a ruptured flexor tendon in the thumb of the less affected hand.

Gait performance. There were no significant main effects or interactions concerning number of steps or total duration needed to complete the task.

rTMS-Related Effects

None of the rTMS protocols was able to induce marked changes in any measure after a single session. Only for the unimanual PPT did we find differential effects for either hand depending on the intervention protocol (Interaction Side × Intervention Protocol, $F(4, 95) = 2.802$, $P = .0301$; Interaction Side × Time × Intervention Protocol, $F(4, 95) = 2.802$, $P = .0301$), with improved performance of the more affected side after any active rTMS protocol but not after sham (see Figure 3).

Statistical analysis did not show significant main effects or interactions with the intervention protocol for any other task, indicating that there was no difference of any active rTMS protocol compared with sham stimulation.

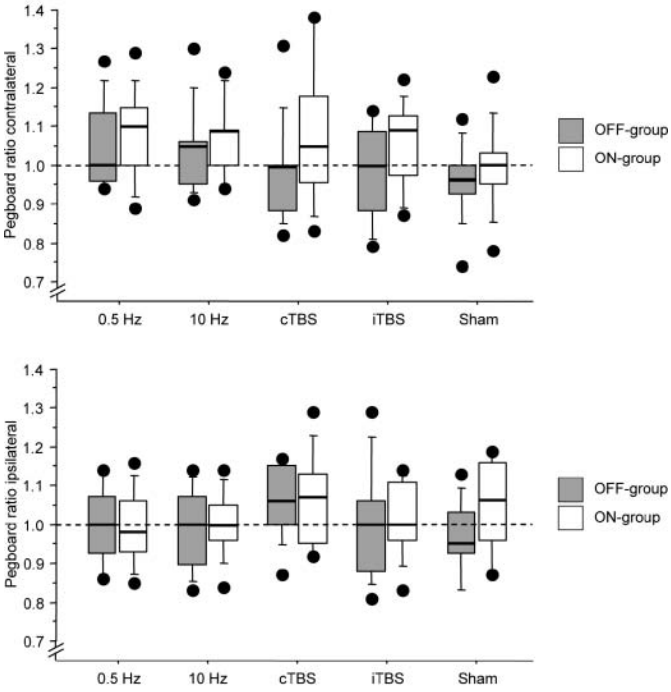
Analysis of baseline raw values showed only that the OFF-group needed less steps on day 1 compared with the other days (Interaction Group × Day, $F(4, 80) = 2.547$, $P = .0456$).

Corticospinal Excitability

As expected, RMT was higher than AMT (see Figure 4; ANOVA: effect of level of activation, $F(1, 100) = 401.772$, $P < .0001$). We found higher RMT and lower AMT in the ON-group compared with the OFF-group (interaction of Level of Activation × Group, $F(1, 100) = 15.002$, $P = .0002$) with slightly decreased RMT and increased AMT after stimulation for both groups (Level of Activation × Time, $F(1, 100) = 7.850$, $P = .0061$). In the control ANOVA, for baseline values, we did not find any effect or interaction of day.

No correlation was found between motor thresholds, baseline EMG activity, and overall or limb rigidity.

Figure 3
Boxplots for the Purdue Pegboard Task (PPT): Performance on the Unimanual Tasks for the Different Types of Intervention Separately for Either Group



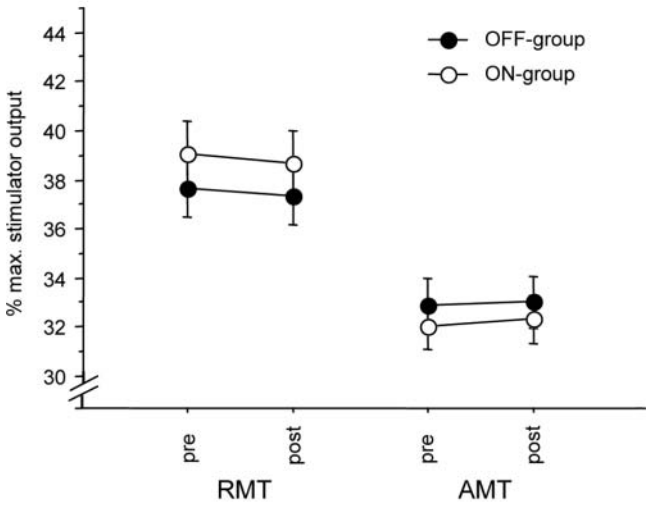
Note: The box represents all values within the 75th percentile with a horizontal bar at the position of the median, error bars at the 90th percentile, and single dots all values >90th percentile. Values higher than 1 indicate improved performance after repetitive transcranial magnetic stimulation (rTMS) intervention. cTBS, continuous theta burst stimulation; iTBS, intermittent theta burst stimulation.

Mood Scale

Mean values for the mood scale are summarized in Table 2. All patients reported decreased attention after the experiment (ANOVA, effect of time, $P = .0013$). The OFF-group reported more often that the stimulation had been effective than the ON-group (Chi-Square Test Efficacy × Group, $P = .045$). However, this was not related to the stimulation protocol. All other parameters did not show any significant effects.

Baseline values did not differ between groups or over the week of experiments.

Figure 4
Effect of Group (OFF/ON) on RMT and AMT for the ADM of the More Affected Side in Percentage of Maximum Stimulator Output



Note: Error bars indicate ±1 SEM. As there was no significant effect of type of intervention, data of all 5 sessions have been pooled. RMT, resting motor threshold; AMT, active motor threshold; ADM, abductor digiti minimi.

UPDRS, BDI

No significant main effects or interactions were found for UPDRS motor scores or BDI scores (see Table 3).

Side Effects

There were no serious side effects of TMS. Two patients reported transient headache following the experiments, and 1 patient reported nausea.

Discussion

The pivotal positive finding of this study is the marked and prolonged behavioral training effect of PD patients in the ON state, extending over the first 4 days of study. Dopamine is involved in motor planning,[19] and motor learning can be enhanced by dopaminergic medication, particularly in the elderly.[20] It is used for this purpose in some rehabilitative settings.[21] Neurophysiological studies on direct-current stimulation and on paired associative stimulation have shown that dopaminergic input facilitates synapse-specific plasticity and reduces unspecific plasticity, thus focusing facilitation.[22] However, the high degree of short-term (within 1 session) and long-term (from day to day) training effects in the arm motor tasks (pointing, pronation-supination, PPT) was unexpected as all tests were based on standard clinical tests. We are not aware of a study that has shown this extent and this duration of learning present over several days of training, even with rather conventional tasks.

We therefore did not include a group of patients who only received sham treatment for 5 consecutive days. It is hard to say whether the observed improvement of motor function reflects an unspecific rTMS effect or purely motor training. However, the marked and prolonged training effect is obviously dependent on dopaminergic input and has to be carefully considered for future clinical trials.

The pivotal negative finding of this study is that none of the rTMS protocols was strong enough to induce remarkable effects after a single session.

Against the background of several studies reporting beneficial effects of rTMS on motor performance in PD, some shortcomings in our study design have to be considered for the interpretation of these negative findings. The high degree of training and placebo effects might have masked subtle differential effects of the rTMS protocols used in this study. Moreover, there might have been interactions between the stimulation protocols as the experiments were conducted on consecutive days. We can therefore only comment on immediate short-term responses, whereas other authors stimulated patients with the same protocol in a sequence of several days or 4 weeks.[23,24]

In addition, it has been shown that PD patients are highly susceptible to chronic placebo effects[25-27] and even show bilateral dopamine release in the dorsal and ventral striatum in response to placebo rTMS.[28] However, a recent study on short-term placebo effects in PD found an improvement in UPDRS scores after sham rTMS of about 21%, which did not reach statistical significance, but there was a much more pronounced increase in subjective self-evaluation of motor function using a visual analog scale.[29] Even mood changes might interfere with motor performance, and rTMS has been shown to be effective in the treatment of depression.[30-32] Patients in the present study often reported after the experiment that they felt "somehow more at ease," but this could not be confirmed by the results of the mood scale and was attributed as a placebo effect. Interestingly, the OFF-group reported more often than the ON-group that they had found the stimulation effective, even if there was no change in motor performance. This might be explained by a "greater drive for symptom relief."[28]

Another important aspect is a potentially shared neuronal network responsible for motor learning as well as rTMS effects. On one hand, this might preclude distinct rTMS effects in patients who show impaired motor learning, as the OFF-group did in the present study. This is in line with a recent study showing that facilitating after effects of paired associative stimulation (PAS) could be found only in PD patients on medication but not in the same patients off medication.[33] Thus, a dopaminergic pretreatment might be necessary for an effective rTMS. On the other hand, such a shared network could be a limit to neuroplastic changes, and intensive

Table 2
Behavioral Self-Rating Scale, Mean Values

	OFF		ON	
	Pre	Post	Pre	Post
Comfort	–0.09 ± 1.53	0.22 ± 1.27	0.49 ± 1.78	0.64 ± 1.65
Anxiety	0.49 ± 1.20	0.53 ± 1.09	0.58 ± 1.45	0.76 ± 1.31
Fatigue	0.18 ± 1.44	–0.29 ± 1.07	0.20 ± 1.53	0.24 ± 1.60
Mood	0.44 ± 1.00	0.49 ± 0.81	0.62 ± 1.23	0.75 ± 1.25
Irritation	0.80 ± 1.00	0.80 ± 1.00	0.89 ± 1.26	0.62 ± 1.37
Attention	0.24 ± 1.32	–0.13 ± 1.14*	0.47 ± 1.53	–0.02 ± 1.60*
Pain	1.04 ± 1.89	1.02 ± 1.98	1.58 ± 1.67	1.53 ± 1.60

*Analysis of variance, effect of time, $P = .0013$.

Table 3
Mean UPDRS and BDI Values

	UPDRS Day 1	UPDRS Day 5	BDI Day 1	BDI Day 5
OFF	27.0 ± 13.9	30.3 ± 11.1	11.8 ± 5.0	11.5 ± 5.4
ON	26.7 ± 10.4	27.3 ± 9.9	9.8 ± 7.6	7.5 ± 2.3
All	26.7 ± 12.0	28.8 ± 10.4	10.8 ± 6.4	9.5 ± 5.7

Abbreviations: BDI, Beck Depression Inventory; UPDRS, Unified Parkinson's Disease Rating Scale.

motor training might prevent the effects of rTMS applied directly after the training session. This has already been shown for PAS in healthy subjects.[34,35] Thus, the intensive assessment of motor performance in the present study potentially constitutes a confounding factor, especially for the ON-group, which showed clear training effects.

Recently, it has been shown that repeated sessions of rTMS at a frequency of 25 Hz are capable of inducing stronger and longer lasting effects on motor performance than single-session rTMS.[23,24] In addition, in these studies, several motor areas were stimulated in each session. This presumably indicates that more intensive rTMS protocols, together with sufficient times for consolidation of the effects, are crucial to overcome mechanisms of maladaptive plasticity or simply an impaired susceptibility to the external induction of neuroplastic changes to reach a therapeutic value.

Conclusions

This study shows a high degree of training effects even in standard clinical assessment methods that outweigh possible effects of a single session of rTMS over the hand motor area. This emphasizes the importance of a negative control condition for TMS (sham stimulation) as well as careful and prolonged pretraining of motor tasks to avoid interference with rTMS interventions. The high hopes that were connected with the introduction of the TBS protocol could not be confirmed for short-term effects following a single session. For future studies with a therapeutic intention, a design of repeated rTMS sessions seems most promising.

References

1. Siebner HR, Peller M, Willoch F, et al. Lasting cortical activation after repetitive TMS of the motor cortex: a glucose metabolic study. *Neurology*. 2000;54:956-963.
2. Siebner HR, Rothwell J. Transcranial magnetic stimulation: new insights into representational cortical plasticity. *Exp Brain Res*. 2003;148:1-16.
3. Strafella AP, Paus T, Fraraccio M, Dagher A. Striatal dopamine release induced by repetitive transcranial magnetic stimulation of the human motor cortex. *Brain*. 2003;126(pt 12):2609-2615.
4. Sommer M, Kamm T, Tergau F, Ulm G, Paulus W. Repetitive paired-pulse transcranial magnetic stimulation affects corticospinal excitability and finger tapping in Parkinson's disease. *Clin Neurophysiol*. 2002;113:944-950.
5. Lefaucheur JP, Drouot X, Von Raison F, Menard-Lefaucheur I, Cesaro P, Nguyen JP. Improvement of motor performance and modulation of cortical excitability by repetitive transcranial magnetic stimulation of the motor cortex in Parkinson's disease. *Clin Neurophysiol*. 2004;115:2530-2541.
6. Pascual-Leone A, Valls-Sole J, Brasil-Neto JP, Cammarota A, Grafman J, Hallett M. Akinesia in Parkinson's disease: II. Effects of subthreshold repetitive transcranial motor cortex stimulation. *Neurology*. 1994;44:892-898.
7. Tergau F, Wassermann EM, Paulus W, Ziemann U. Lack of clinical improvement in patients with Parkinson's disease after low and high frequency repetitive transcranial magnetic stimulation. *Electroencephalogr Clin Neurophysiol Suppl*. 1999;51:281-288.
8. Fregni F, Simon DK, Wu A, Pascual-Leone A. Non-invasive brain stimulation for Parkinson's disease: a systematic review and meta-analysis of the literature. *J Neurol Neurosurg Psychiatry*. 2005;76:1614-1623.
9. Helmich RC, Siebner HR, Bakker M, Munchau A, Bloem BR. Repetitive transcranial magnetic stimulation to improve mood and motor function in Parkinson's disease. *J Neurol Sci*. 2006;248(1-2):84-96.
10. Sommer M, Paulus W. Pulse configuration and rTMS efficacy: a review of clinical studies. *Suppl Clin Neurophysiol*. 2003;56:33-41.
11. Huang YZ, Edwards MJ, Rounis E, Bhatia KP, Rothwell JC. Theta burst stimulation of the human motor cortex. *Neuron*. 2005;45:201-206.
12. Paulus W. Toward establishing a therapeutic window for rTMS by theta burst stimulation. *Neuron*. 2005;45:181-183.
13. Jancke L, Steinmetz H, Benilow S, Ziemann U. Slowing fastest finger movements of the dominant hand with low-frequency rTMS of the hand area of the primary motor cortex. *Exp Brain Res*. 2004;155:196-203.
14. Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J. An inventory for measuring depression. *Arch Gen Psychiatry*. 1961;4:561-571.
15. Defer GL, Widner H, Marie RM, Remy P, Levivier M. Core assessment program for surgical interventional therapies in Parkinson's disease (CAPSIT-PD). *Mov Disord*. 1999;14:572-584.
16. Lisanby SH, Gutman D, Luber B, Schroeder C, Sackeim HA. Sham TMS: intracerebral measurement of the induced electrical field and the induction of motor-evoked potentials. *Biol Psychiatry*. 2001;49:460-463.

17. Fahn S, Elton R, Committee MotUD. Unified Parkinson's Disease Rating Scale. In: Fahn S, Marsden CD, Calne DB, Goldstein M, eds. *Recent Developments in Parkinson's Disease*. Vol. 2. Florham Park, NJ: Macmillan Health Care Information; 1987:153-304.
18. Rothwell JC, Hallett M, Berardelli A, Eisen A, Rossini P, Paulus W. Magnetic stimulation: motor evoked potentials. The International Federation of Clinical Neurophysiology. *Electroencephalogr Clin Neurophysiol Suppl*. 1999;52:97-103.
19. Garraux G, Peigneux P, Carson RE, Hallett M. Task-related interaction between basal ganglia and cortical dopamine release. *J Neurosci*. 2007;27:14434-14441.
20. Floel A, Vomhof P, Lorenzen A, Roesser N, Breitenstein C, Knecht S. Levodopa improves skilled hand functions in the elderly. *Eur J Neurosci*. 2008;27:1301-1307.
21. Scheidtmann K, Fries W, Muller F, Koenig E. Effect of levodopa in combination with physiotherapy on functional motor recovery after stroke: a prospective, randomised, double-blind study. *Lancet*. 2001;358:787-790.
22. Kuo MF, Paulus W, Nitsche MA. Boosting focally-induced brain plasticity by dopamine. *Cereb Cortex*. 2008;18:648-651.
23. Lomarev MP, Kanchana S, Bara-Jimenez W, Iyer M, Wassermann EM, Hallett M. Placebo-controlled study of rTMS for the treatment of Parkinson's disease. *Mov Disord*. 2006;21:325-331.
24. Khedr EM, Rothwell JC, Shawky OA, Ahmed MA, Hamdy A. Effect of daily repetitive transcranial magnetic stimulation on motor performance in Parkinson's disease. *Mov Disord*. 2006;21:1311-1316.
25. Goetz CG, Leurgans S, Raman R, Stebbins GT. Objective changes in motor function during placebo treatment in PD. *Neurology*. 2000;54:710-714.
26. McRae C, Cherin E, Yamazaki TG, et al. Effects of perceived treatment on quality of life and medical outcomes in a double-blind placebo surgery trial. *Arch Gen Psychiatry*. 2004;61:412-420.
27. de la Fuente-Fernandez R, Ruth TJ, Sossi V, Schulzer M, Calne DB, Stoessl AJ. Expectation and dopamine release: mechanism of the placebo effect in Parkinson's disease. *Science*. 2001;293:1164-1166.
28. Strafella AP, Ko JH, Monchi O. Therapeutic application of transcranial magnetic stimulation in Parkinson's disease: the contribution of expectation. *Neuroimage*. 2006;31:1666-1672.
29. Fregni F, Boggio PS, Bermpohl F, et al. Immediate placebo effect in Parkinson's disease: is the subjective relief accompanied by objective improvement? *Eur Neurol*. 2006;56:222-229.
30. Chistyakov AV, Kaplan B, Rubichek O, et al. Antidepressant effects of different schedules of repetitive transcranial magnetic stimulation vs. clomipramine in patients with major depression: relationship to changes in cortical excitability. *Int J Neuropsychopharmacol*. 2005; 8(2):223-233.
31. George MS, Nahas Z, Molloy M, et al. A controlled trial of daily left prefrontal cortex TMS for treating depression. *Biol Psychiatry*. 2000; 48(10):962-970.
32. Fregni F, Santos CM, Myczkowski ML, et al. Repetitive transcranial magnetic stimulation is as effective as fluoxetine in the treatment of depression in patients with Parkinson's disease. *J Neurol Neurosurg Psychiatry*. 2004;75:1171-1174.
33. Ueki Y, Mima T, Kotb MA, et al. Altered plasticity of the human motor cortex in Parkinson's disease. *Ann Neurol*. 2006;59:60-71.
34. Stefan K, Wycislo M, Gentner R, et al. Temporary occlusion of associative motor cortical plasticity by prior dynamic motor training. *Cereb Cortex*. 2006;16:376-385.
35. Ziemann U, Ilic TV, Pauli C, Meintzschel F, Ruge D. Learning modifies subsequent induction of long-term potentiation-like and long-term depression-like plasticity in human motor cortex. *J Neurosci*. 2004;24: 1666-1672.

2.2 Dopaminergic potentiation of rTMS-induced motor cortex inhibition

Dopaminergic neuromodulation plays an important role in various cognitive functions and has been associated with NMDA-receptor dependent neuroplasticity. It has also been shown that practice-dependent plasticity in the human motor cortex can be enhanced by the D2 agonist cabergolin while it is blocked by the D2 antagonist haloperidol (Meintzschel and Ziemann, 2006).

In the following study we found that a single dose of pergolide potentiated and prolonged the inhibitory effect of an inhibitory 1 Hz rTMS protocol applied over the left primary motor cortex in healthy human subjects. Pergolide is a combined D1/D2 receptor agonist which also acts on serotonin receptors and ion channels. In a parallel study using tDCS (Nitsche et al., 2006) a prolongation of the inhibitory after effects of cathodal tDCS was found after intake of pergolide, while the inhibitory effect was prevented by the D2 receptor antagonist sulpirid even in the combination with pergolide (resulting in predominantly D1 activation). Therefore it seems most likely that the enhancement of inhibitory neuroplastic effects is mediated by D2 receptor activation.

For clinical application of externally induced neuroplasticity these findings imply that stronger and more stable effects might be achieved not only by optimizing the stimulation protocol itself but also by combination of brain stimulation with a pharmacological intervention. On the other hand conditions with reduced dopaminergic neuromodulation such as Parkinson's Disease or neuroleptic medication might be associated with a reduced potential for neuroplastic changes.

Dopaminergic Potentiation of rTMS-Induced Motor Cortex Inhibition

Nicolas Lang, Sascha Speck, Jochen Harms, Holger Rothkegel, Walter Paulus, and Martin Sommer

Background: Experiments in animal models suggest that neuronal plasticity can be enhanced by dopaminergic receptor activation. The present study tested whether stimulation-induced plasticity of human motor cortex after low-frequency repetitive transcranial magnetic stimulation (rTMS) could be potentiated by a single oral dose of the combined D1/D2 receptor agonist pergolide.

Methods: In a randomized, double-blind, placebo-controlled cross-over design, nine healthy young volunteers received .125 mg pergolide or placebo 2 hours before 1 Hz rTMS was applied for 20 min to the left primary motor cortex. In a control experiment 7 subjects received .125 mg pergolide 2 hours before sham rTMS. We used single-pulse TMS at rest to assess corticospinal excitability before and up to 24 min after rTMS.

Results: Suppression of corticospinal excitability by 1 Hz rTMS was more pronounced after pergolide intake compared with placebo and lasted approximately 20 min after pergolide but only 5 min after placebo. No change of corticospinal excitability could be observed when sham rTMS was performed after pergolide intake.

Conclusions: The results suggest a possible role for dopaminergic potentiation of rTMS-induced neuroplasticity in experimental or therapeutic applications and should be considered when rTMS is applied in patients under medication with dopamine agonists or antagonists.

Key Words: D1, D2, pergolide, primary motor cortex, receptor, repetitive transcranial magnetic stimulation, stimulation-induced plasticity

Changes in cortical excitability can be induced in humans non-invasively with repetitive transcranial magnetic stimulation (rTMS) (1,2). Although the mechanisms of these changes are not fully understood, analogies to long-term potentiation (LTP) and long-term depression (LTD) of individual synapses are apparent. When rTMS is given with constant interpulse-intervals the direction of after-effects can be controlled by the frequency of stimulation: lower frequencies, in the range of 1 Hz, can produce LTD-like inhibition of motor cortical excitability (3,4), whereas frequencies of ≥5 Hz can induce LTP-like facilitation (5,6).

In recent years, rTMS has attracted considerable interest as a therapeutic tool in neuropsychiatry. The method has been used in numerous clinical trials to improve a variety of brain diseases, such as Parkinson's disease, epilepsy, major depression, and schizophrenia (7,8). Because clinical effects have often been subtle and variable, the therapeutic potential of rTMS is still open to debate, and methodological considerations to enhance rTMS efficiency by optimizing stimulation pattern (9,10) or sensitizing cortical areas with preconditioning (11–13) are developing.

Another approach of potentiating rTMS effects might be achieved by pharmacological interventions with dopaminergic drugs. There is evidence that dopaminergic mechanisms are involved in N-methyl-D-aspartate (NMDA) receptor-dependent neuroplasticity. In animal models, LTP and LTD can be modified by D1 or D2 receptor activation: D1 receptor activity can have enhancing effects on the induction and consolidation of LTP (14–17) but can also facilitate LTD induction (17,18). Reports on the effects of plasticity as a consequence of D2 receptor activation are less consistent: with regard to LTP it has been described as enhancing (19), suppressing (20), or without effect (16). Long-term depression has been shown to be enhanced by D2 receptor activation (21,22) but also to be inhibited (18).

The present study was designed to explore the effects of pergolide, a combined D1/D2 receptor agonist, on motor cortex excitability changes induced by low-frequency rTMS in a randomized, double-blind, placebo-controlled crossover design. The preponderance of evidence suggests that pergolide, as a D1/D2 agonist, would enhance LTD, although studies specifically examining the impact of this drug on LTD are lacking.

Methods and Materials

Altogether 14 healthy human subjects (8 women and 6 men, ages 21–44 years, median age 25 years) gave their informed consent before participating in the experiments. Experimental procedures had the approval of the Ethics Committee of the University of Goettingen and were performed according to the ethical standards laid down in the Declaration of Helsinki.

In the main experiment nine participants (six women and three men, ages 21–26 years, median age 24 years) underwent two rTMS sessions on different days separated by at least 1 week. One session was done after pergolide intake and one after placebo intake. A uniform capsule, containing .125 mg pergolide or placebo, was orally administered 2 hours before each experiment. The order of intake (pergolide or placebo) was pseudo-randomized and balanced, and subjects and TMS examiners were not informed about it. At the end of each session subjects were interviewed about possible adverse effects. The rTMS was given at a rate of 1 Hz over 20 min (i.e., 1200 pulses) with an intensity of 90% of the individual resting motor threshold (RMT) to the left primary motor cortex (M1). Corticospinal excitability was studied over a period of 8 min before rTMS (baseline) and again for 24 min after rTMS with single pulse TMS. Here TMS was given to the left M1 at a rate of .25 Hz, and the intensity was adjusted to yield baseline motor evoked potentials (MEP) in the relaxed right first dorsal interosseus muscle (FDI) with mean peak-to-peak amplitudes of approximately 1 mV. This intensity

From the Department of Neurology (NL), Christian-Albrechts University, Kiel, Germany; and Department of Clinical Neurophysiology (SS, JH, HR, WP, MS), Georg-August University, Goettingen, Germany.

Address reprint requests to Dr. Nicolas Lang, Department of Clinical Neurophysiology, Georg-August-University, Robert-Koch-Strasse 40, 37075 Goettingen, Germany; E-mail: nlang@gwdg.de.

Received November 21, 2006; revised April 11, 2007; accepted April 12, 2007.

0006-3223/08/$34.00
doi:10.1016/j.biopsych.2007.04.033

(SI_{1mV}) was individually determined at the beginning of each experiment and held constant throughout the session.

An additional control experiment was performed in seven subjects (four women and three men, ages 21–44 years, median age 24 years) with sham rTMS given 2 hours after a single oral dose of .125 mg pergolide in order to exclude an unspecific effect of pergolide intake on cortical excitability. For sham rTMS, the coil placed over the motor hot spot was disconnected from the stimulator during rTMS. The TMS stimulator was then discharged through a second coil, which was fixed to a coil holder positioned approximately 50 cm behind the subject's head. This provides a similar noise compared with real rTMS and can serve as a reasonable method to provide sham rTMS (11). All other procedures were kept identical to the main experiment.

TMS was performed with a Medtronic MagPro stimulator and a figure-of-eight–shaped Medtronic MC-B70 coil (Medtronic Functional Diagnostics, Skovlunde, Denmark). The coil was held tangentially to the skull over the optimal cortical representation of the right FDI with the handle pointing posterolaterally at a 45-degree angle to the sagittal plane. Stimuli were biphasic, and the first phase of the stimulus elicited an anterior–posterior current in the brain.

In each subject rTMS experiments were done at identical times during the day with the participant comfortably seated in a reclining chair with head and arm rests. Surface electromyogram (EMG) was recorded from the right FDI through a pair of silver–silver chloride (Ag-AgCl) surface electrodes in a belly-tendon montage. Raw signals were amplified, band-pass filtered (3Hz–3kHz), digitized with a micro 1401 AD converter (Cambridge Electronic Design, Cambridge, United Kingdom) controlled by Signal Software (Cambridge Electronic Design, version 2.13), and stored on a personal computer for offline analysis. Complete relaxation was controlled through auditory and visual feedback of EMG activity.

Mean MEP values of each individual were calculated for baseline recordings and for 6 time bins of 4-min duration each, covering a 24-min period after rTMS. For the main experiment these values were entered into a two-way repeated-measures analysis of variance (ANOVA) with "drug" (two levels: pergolide and placebo) and "time" (seven levels: before rTMS and time bins 1–6 after rTMS) as within-subject factors. For the control experiment we performed a one-way ANOVA with "time" (seven levels: before rTMS and time bins 1–6 after rTMS) as within-subject factor. Conditional on a significant F value, we performed follow-up one-way ANOVAs and post hoc paired-samples two-tailed t tests to characterize the effects revealed by the main ANOVA. Two-tailed t tests were also used to test for differences in RMT and SI_{1mV} at baseline between drug conditions. A p value of $< .05$ was considered significant for all statistical analyses. All results are given as mean and SEM.

Results

Three subjects reported some mild and transient adverse effects after the intake of pergolide, such as dizziness and nausea; however this did not interfere with the ability of the subjects to complete the study. None of the subjects reported adverse effects after placebo intake. Side effects from pergolide might have partially unblinded the investigators and therefore weakened the double-blinded study design. However, participants did not make explicit statements to the investigators about adverse events until the end of each session.

In the main experiment ANOVA on mean MEP revealed significant main effects of the factors "drug" [$F(1,8) = 11.31$, $p = .01$] and "time" [$F(6,48) = 5.92$, $p = .003$] and a significant "time" × "drug" interaction [$F(6,48) = 2.38$, $p = .042$]. Follow-up one-way ANOVAs separately performed on each drug condition showed significant effects for the factor time in both conditions [placebo: $F(6,48) = 2.77$, $p = .021$; pergolide: $F(6,48) = 4.50$, $p = .01$]. Post hoc analyses showed that MEPs were more strongly suppressed after pergolide intake compared with the placebo condition within the first 20 min after rTMS (post hoc t tests; all $p < .05$). After placebo intake a significant inhibition compared with baseline values could only be seen within the first 4 min (1 time bin) after rTMS (post hoc t test; $p = .039$), whereas it lasted for 20 min (5 time bins) after pergolide (post hoc t tests; all $p < .05$). At the last time bin (21–24 min after rTMS) mean MEP values in both drug conditions were not different from baseline (Figure 1).

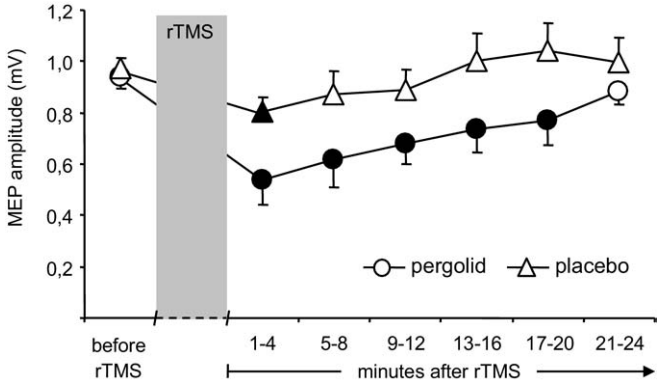


Figure 1. Low-frequency repetitive transcranial magnetic stimulation (rTMS)–induced motor cortex inhibition 2 hours after intake of .125 mg pergolide or placebo. Filled symbols indicate significant differences of mean motor evoked potential (MEP) amplitudes compared with before rTMS (post hoc t test; $p < .05$, error bars indicate SEM).

Mean values (± SEM) in the pergolide and placebo condition, respectively, were: for baseline MEP .94 ± .04 and .97 ± .04 mV, for RMT 41 ± 2 and 41 ± 2% maximum stimulator output, and for SI_{1mV} 50 ± 2 and 49 ± 2% maximum stimulator output. Analyses of baseline MEP values, RMT, and SI_{1mV} data did not reveal significant differences between drug conditions for these data (t tests; all $p > .5$).

In the control experiment with sham rTMS after pergolide intake no significant effect on "time" could be observed [$F(6,36) = 1.336$, $p = .267$].

Discussion

The present study confirms previous studies (3,4,23) that continuous 1-Hz rTMS to the human motor cortex can induce a transient decrease in corticospinal excitability. Extending previous work, we show that this effect can be potentiated by a single dose of the combined D1/D2 receptor agonist pergolide: suppression of corticospinal excitability was more pronounced after pergolide compared with placebo and lasted approximately 20 min after pergolide, whereas it ceased within 5 min after placebo. No change of corticospinal excitability could be observed when sham rTMS was performed after pergolide intake. These findings are in line with results obtained from animal experiments that show that dopamine receptor activation can enhance LTD (17,24). However, it should be considered that our data has been obtained in motor cortex, and it cannot be assured that these conditions can be applied identically to other cortical areas. Moreover, it has been shown that pergolide—at least on a peripheral level—not only interacts with dopamine receptors but also with serotonin (5-HT) receptors and ion channels (25,26). Therefore, it cannot be ruled out completely that the observed central nervous system effects could partially be mediated by mechanisms other than dopaminergic receptor activation.

Our results parallel a recent study that tested effects of D1/D2 receptor agonists and antagonists on excitability changes induced by transcranial direct current stimulation (tDCS) (27). Like rTMS, tDCS can be used to alter cortical excitability bi-directionally and non-invasively in humans (28), and its pharmaco-physiological properties suggest that activity-dependent synaptic plasticity, such as LTP and LTD, mediates the effects induced by tDCS (29). With tDCS, combined D1/D2 receptor activation by pergolide also enhanced LTD-like effects after inhibitory cathodal tDCS, whereas D2 antagonism with sulpiride alone or D1 activation alone (achieved by combining pergolide and sulpiride) prevented inhibition. This was taken as an explanation that D2 receptor activation has a consolidation-enhancing effect on LTD-like cortical excitability changes.

Although not tested in the present study, it might be questioned whether only LTD-like plasticity induced by rTMS could be potentiated by dopaminergic receptor activation or whether LTP-like plasticity, such as the facilitation of excitability by 5-Hz rTMS, would be similarly enhanced. In the aforementioned tDCS study no enhancement or prolongation of LTP-like effects after facilitatory anodal tDCS could be observed with pergolide (27). However, with another non-invasive stimulation protocol that also induces LTP- and LTD-like after-effects in motor cortex (i.e., paired associative stimulation [PAS]), it was demonstrated that D2/D3 activation with cabergoline enhanced LTP-like plasticity after PAS, whereas application of the dopamine receptor antagonist haloperidol suppressed PAS-induced LTP-like processes (30). Could it be that dopaminergic receptor activation accompanying stimulation-induced plasticity can generally be used for "gating" of LTP- and LTD-like processes in motor cortex? Further experiments will be necessary to clarify this question.

In conclusion, the present study demonstrates that LTD-like motor cortex inhibition induced by rTMS in humans can be enhanced by a single dose of the D1/D2 receptor agonist pergolide. This suggests a possible role for dopaminergic potentiation of rTMS-induced neuroplasticity in experimental or therapeutic applications and should be considered when rTMS is applied in patients under medication with dopamine agonists or antagonists.

Dr. Sommer was supported by the Deutsche Forschungsgemeinschaft (DFG grants So-429/1 and So-429/2).

The authors deny any potential conflict of interest as it relates to the subject of this report.

We wish to thank Dr. Matthias Bohn, Goettingen University Hospital Pharmacy, for his support and the preparation of the study medication.

1. Hallett M (2000): Transcranial magnetic stimulation and the human brain. *Nature* 406:147–150.
2. Siebner HR, Rothwell J (2003): Transcranial magnetic stimulation: New insights into representational cortical plasticity. *Exp Brain Res* 148:1–16.
3. Lang N, Harms J, Weyh T, Lemon RN, Paulus W, Rothwell JC, *et al.* (2006): Stimulus intensity and coil characteristics influence the efficacy of rTMS to suppress cortical excitability. *Clin Neurophysiol* 117:2292–2301.
4. Chen R, Classen J, Gerloff C, Celnik P, Wassermann EM, Hallett M, *et al.* (1997): Depression of motor cortex excitability by low-frequency transcranial magnetic stimulation. *Neurology* 48:1398–1403.
5. Peinemann A, Reimer B, Löer C, Quartarone A, Münchau A, Conrad B, *et al.* (2004): Long-lasting increase in corticospinal excitability after 1800 pulses of subthreshold 5 Hz repetitive TMS to the primary motor cortex. *Clin Neurophysiol* 115:1519–1526.
6. Quartarone A, Bagnato S, Rizzo V, Morgante F, Sant'angelo A, Battaglia F, *et al.* (2005): Distinct changes in cortical and spinal excitability following high-frequency repetitive TMS to the human motor cortex. *Exp Brain Res* 161:114–124.
7. Sommer M, Paulus W (2003): Pulse configuration and rTMS efficacy: A review of clinical studies. *Suppl Clin Neurophysiol* 56:33–41.
8. Wassermann EM, Lisanby SH (2001): Therapeutic application of repetitive transcranial magnetic stimulation: a review. *Clin Neurophysiol* 112: 1367–1377.
9. Huang YZ, Edwards MJ, Rounis E, Bhatia KP, Rothwell JC (2005): Theta burst stimulation of the human motor cortex. *Neuron* 45:201–206.
10. Thickbroom GW, Byrnes ML, Edwards DJ, Mastaglia FL (2006): Repetitive paired-pulse TMS at I-wave periodicity markedly increases corticospinal excitability: A new technique for modulating synaptic plasticity. *Clin Neurophysiol* 117:61–66.
11. Lang N, Siebner HR, Ernst D, Nitsche MA, Paulus W, Lemon RN, *et al.* (2004): Preconditioning with transcranial DC stimulation sensitizes the motor cortex to rapid-rate TMS and controls the direction of after-effects. *Biol Psychiatry* 56:634–639.
12. Siebner HR, Lang N, Rizzo V, Nitsche MA, Paulus W, Lemon RN, *et al.* (2004): Preconditioning of low-frequency repetitive transcranial magnetic stimulation with transcranial direct current stimulation: evidence for homeostatic plasticity in the human motor cortex. *J Neurosci* 24:3379–3385.
13. Iyer MB, Schleper N, Wassermann EM (2003): Priming stimulation enhances the depressant effect of low-frequency repetitive transcranial magnetic stimulation. *J Neurosci* 23:10867–10872.
14. Bach ME, Barad M, Son H, Zhuo M, Lu YF, Shih R, *et al.* (1999): Age-related defects in spatial memory are correlated with defects in the late phase of hippocampal long-term potentiation in vitro and are attenuated by drugs that enhance the cAMP signaling pathway. *Proc Natl Acad Sci U S A* 96:5280–5285.
15. Bailey CH, Giustetto M, Huang YY, Hawkins RD, Kandel ER (2000): Is heterosynaptic modulation essential for stabilizing Hebbian plasticity and memory? *Nat Rev Neurosci* 1:11–20.
16. Gurden H, Takita M, Jay TM (2000): Essential role of D1 but not D2 receptors in the NMDA receptor-dependent long-term potentiation at hippocampal-prefrontal cortex synapses in vivo. *J Neurosci* 20:RC106.
17. Huang YY, Simpson E, Kellendonk C, Kandel ER (2004): Genetic evidence for the bidirectional modulation of synaptic plasticity in the prefrontal cortex by D1 receptors. *Proc Natl Acad Sci U S A* 101:3236–3241.
18. Chen Z, Ito K, Fujii S, Miura M, Furuse H, Sasaki H, *et al.* (1996): Roles of dopamine receptors in long-term depression: Enhancement via D1 receptors and inhibition via D2 receptors. *Receptors Channels* 4:1–8.
19. Manahan-Vaughan D, Kulla A (2003): Regulation of depotentiation and long-term potentiation in the dentate gyrus of freely moving rats by dopamine D2-like receptors. *Cereb Cortex* 13:123–135.
20. Frey U, Hartmann S, Matthies H (1989): Domperidone, an inhibitor of the D2-receptor, blocks a late phase of an electrically induced long-term potentiation in the CA1-region in rats. *Biomed Biochim Acta* 48:473–476.
21. Otani S, Blond O, Desce JM, Crepel F (1998): Dopamine facilitates long-term depression of glutamatergic transmission in rat prefrontal cortex. *Neuroscience* 85:669–676.
22. Spencer JP, Murphy KP (2000): Bi-directional changes in synaptic plasticity induced at corticostriatal synapses in vitro. *Exp Brain Res* 135:497–503.
23. Sommer M, Lang N, Tergau F, Paulus W (2002): Neuronal tissue polarization induced by repetitive transcranial magnetic stimulation? *Neuroreport* 13:809–811.
24. Otani S, Daniel H, Roisin MP, Crepel F (2003): Dopaminergic modulation of long-term synaptic plasticity in rat prefrontal neurons. *Cereb Cortex* 13:1251–1256.
25. Newman-Tancredi A, Cussac D, Quentric Y, Touzard M, Verriele L, Carpentier N, *et al.* (2002): Differential actions of antiparkinson agents at multiple classes of monoaminergic receptor. III. Agonist and antagonist properties at serotonin, 5-HT(1) and 5-HT(2), receptor subtypes. *J Pharmacol Exp Ther* 303:815–822.
26. Hong Z, Smith AJ, Archer SL, Wu XC, Nelson DP, Peterson D, *et al.* (2005): Pergolide is an inhibitor of voltage-gated potassium channels, including Kv1.5, and causes pulmonary vasoconstriction. *Circulation* 112:1494–1499.
27. Nitsche MA, Lampe C, Antal A, Liebetanz D, Lang N, Tergau F, *et al.* (2006): Dopaminergic modulation of long-lasting direct current-induced cortical excitability changes in the human motor cortex. *Eur J Neurosci* 23:1651–1657.
28. Lang N, Nitsche MA, Paulus W, Rothwell JC, Lemon RN (2004): Effects of transcranial direct current stimulation over the human motor cortex on corticospinal and transcallosal excitability. *Exp Brain Res* 156:439–443.
29. Liebetanz D, Nitsche MA, Tergau F, Paulus W (2002): Pharmacological approach to the mechanisms of transcranial DC-stimulation-induced after-effects of human motor cortex excitability. *Brain* 125:2238–2247.
30. Ziemann U, Meintzschel F, Korchounov A, Ilic TV (2006): Pharmacological modulation of plasticity in the human motor cortex. *Neurorehabil Neural Repair* 20:243–251.

2.3 Breaks during 5Hz rTMS are essential for facilitatory after effects

The repetition rate of TMS pulses has so far been regarded as the single most important factor responsible for the direction of after effects of rTMS protocols. However, for most prolonged high-frequency protocols the total number of stimuli has been split up in short trains of stimulation separated by breaks of several seconds up to one minute.

The aim of the following study was to elucidate the functional relevance of these breaks in high-frequency rTMS. For this purpose a clearly subthreshold protocol was used in order to avoid any safety risk associated with high-frequency stimulation at suprathreshold intensities (Wassermann, 1998). The major finding of this study is that the presence of breaks is essential for facilitatory after effects, while a continuous application of the same number of pulses tends toward inhibition. These results might be explained by a different time course of excitatory and inhibitory processes which are activated simultaneously. Alternatively homeostatic mechanisms might play a role. The optimal relationship of stimulation frequency, stimulation duration and breaks needs to be clarified in further studies.

Clinical Neurophysiology xxx (2009) xxx–xxx

Contents lists available at ScienceDirect

Clinical Neurophysiology

journal homepage: www.elsevier.com/locate/clinph

Breaks during 5 Hz rTMS are essential for facilitatory after effects

H. Rothkegel *, M. Sommer, W. Paulus

Department of Clinical Neurophysiology, University of Göttingen, Robert-Koch-Straße 40, 37075 Göttingen, Germany

ARTICLE INFO

Article history:
Accepted 13 November 2009
Available online xxxx

Keywords:
Conventional repetitive transcranial magnetic stimulation
Current direction
Neuroplasticity

ABSTRACT

Objective: Stimulation frequency has been considered the most important factor in conventional repetitive transcranial magnetic stimulation (rTMS) for determining the direction of after effects on corticospinal excitability. Here, we examined the functional relevance of breaks during high-frequency subthreshold rTMS for the induction of facilitatory after effects.

Methods: The after effects on corticospinal excitability of a standard 5 Hz rTMS protocol in a block design were compared to a continuous rTMS protocol using the same number of pulses. In addition the effect of current direction both for rTMS and single pulse TMS was included in the study design.

Results: While 5 Hz rTMS in a standard block design induces facilitatory after effects on corticospinal excitability, the continuous protocol does not induce facilitation but rather inhibition. In our study only rTMS using an initially posterior–anterior current direction in the brain leads to significant neuroplastic effects at all.

Conclusions: Breaks during conventional high-frequency rTMS are a crucial factor determining the direction of induced neuroplastic changes.

Significance: These results contribute to the understanding of rTMS-induced neuroplasticity and are important for the design of rTMS protocols both for experimental and clinical studies.

1. Introduction

The application of repetitive transcranial magnetic stimulation (rTMS) over various cortical areas has become a widely accepted tool to induce neuroplastic changes outlasting the duration of stimulation for minutes or even hours (for a review see (Ziemann et al., 2008)).

Direction, magnitude and duration of these after effects depend on a complex set of extrinsic factors such as frequency or intensity of stimulation and intrinsic factors such as the functional state of cortical neurons before or during stimulation (Ziemann et al., 2008). So far stimulation frequency of conventional rTMS seems to be the key parameter which determines the direction of after affects. It is widely accepted that low-frequency rTMS (1 Hz or less) produces inhibitory after effects while high-frequency rTMS (2 Hz or more) produces facilitatory after effects (Fitzgerald et al., 2006).

Interestingly high-frequency rTMS protocols are usually applied either as a single short train of pulses or several trains with different intertrain intervals. Prolonged continuous stimulation has been applied mainly to inhibitory 1 Hz paradigms (Chen et al., 1997), but to our knowledge there are only few studies using prolonged high frequency continuous stimulation and none of these examined after effects on corticospinal excitability of the stimulated motor cortex. So far a continuous subthreshold 5 Hz stimulation was only used in one study, which found increased corticospinal excitability contralateral to the stimulated motor cortex as assessed by MEP amplitudes, but did not measure the effects on the stimulated hemisphere (Gorsler et al., 2003).

Two main reasons account for the introduction of intervals resulting in periods of stimulation: (1) safety issues, namely the risk of induction of epileptic seizures which has been observed during and after suprathreshold high frequency protocols. Safety guidelines were established for suprathreshold rTMS protocols only (Wassermann, 1998), while subthreshold rTMS so far seems to be safe and no limitations regarding stimulus frequency or number of pulses in a train have been considered so far. (2) In addition the introduction of breaks during stimulation reduces excessive coil heating by allowing active cooling of the coil in the interval or simply allowing a passive reduction of heating before the next part of the intervention.

The aim of the present study was to determine the functional relevance of breaks during 5 Hz subthreshold rTMS for the induction of facilitatory after effects. For this purpose we compared the excitability of the corticospinal system as assessed by amplitudes of motor evoked potentials following a standard protocol in a block design with a continuous protocol using the same intensity and total number of pulses. Previous studies have shown that

* Corresponding author. Tel.: +49 551 39 66 50; fax: +49 551 39 81 26.
E-mail address: hrothke@gwdg.de (H. Rothkegel).

1388-2457/$36.00 © 2009 International Federation of Clinical Neurophysiology. Published by Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.clinph.2009.11.016

Please cite this article in press as: Rothkegel H et al. Breaks during 5 Hz rTMS are essential for facilitatory after effects. Clin Neurophysiol (2009), doi:10.1016/j.clinph.2009.11.016

rTMS induced neuroplastic changes depend on the pulse configuration and direction of the induced electric field in the brain (Sommer et al., 2006). Therefore current direction for the test stimulus and the rTMS protocol were included as additional factors.

2. Material and methods

2.1. Subjects

Fourteen healthy human subjects (6 women and 8 men, age range 19–28 years) participated in the experiment after giving informed consent. Experimental procedures had the approval of the Ethics Committee of the University of Göttingen and were performed according to the ethical standards laid down in the Declaration of Helsinki.

2.2. Assessment of corticospinal excitability

Surface EMG was recorded with Ag/AgCl cup electrodes in a belly-tendon montage from the abductor digiti minimi (ADM) of the right hand. Signals were band-pass filtered (2–3000 Hz) and amplified using a Digitimer D360 amplifier (Digitimer Ltd., Welwyn Garden City, Hertfordshire, UK), sampled with a CED Micro 1401 mk II (Cambridge Electronic Design, Cambridge, England) at a rate of 5 kHz and stored on a lab computer for offline analysis.

Transcranial magnetic stimulation (TMS) was applied over the optimal cortical representation for the right ADM using a slightly bent figure-of-8 coil (Medtronic MC-B70) connected to a Medtronic MagPro X100 + MagOption stimulator with a biphasic pulse configuration. Throughout the manuscript the current flow direction will be given as the direction of the induced current in the brain during the first quarter cycle of the pulse. While most studies use monophasic TMS pulses to assess corticospinal excitability we chose biphasic pulses to ensure that after effects of rTMS were measured in the same cortical circuits influenced by the intervention. The coil was held tangentially to the head with the handle pointing posterior and 45 degrees laterally.

Resting motor threshold (RMT) was determined as the minimum stimulator output at which at least 5 out of 10 consecutive TMS pulses induced MEPs of >50 μV in amplitude with the target muscle at rest. Active motor threshold (AMT) was measured under tonic contraction of the target muscle of approximately 20–30% of maximum EMG activity. The minimum stimulator output at which at least 5 out of 10 TMS pulses induced MEPs of >200 μV in amplitude was considered the AMT. The optimal coil position, RMT and AMT were determined for both current flow directions separately.

To assess changes in corticospinal excitability motor evoked potentials (MEP) were recorded from the relaxed ADM. At baseline the intensity of the magnetic pulse was adjusted to induce MEPs of about 1 mV peak-to-peak and kept constant for the measurement after intervention. Two blocks of 15 TMS pulses applied every 4 ± 0.4 s were recorded for either current direction at baseline. The order of current directions for the single pulse measurements was kept constant within one subject and counterbalanced between subjects.

Blocks of 15 MEPs for each current direction were measured again 1, 3, 5, 10, 15 and 30 min after the end of the intervention using the same intensities and order of current direction as before. RMT was assessed again following the measurement 15 min after the end of rTMS.

2.3. Repetitive transcranial magnetic stimulation (rTMS)

rTMS protocols were derived from a previously established subthreshold 5 Hz rTMS protocol of 1200 pulses in six blocks of 200 pulses each with an intertrain interval of 60 s using an intensity of 90% AMT (Sommer et al., 2006). In the present study 1200 pulses were applied either continuously or in blocks as described above with either anterior–posterior oriented or posterior–anterior oriented current direction in separate sessions, respectively.

For rTMS the slightly bent figure-of-8 coil (Medtronic MC-B70) was placed over the optimal representation of the ADM for the respective current direction as identified by single pulse TMS.

2.4. Statistical analysis

A repeated measures ANOVA with current direction for single pulse TMS (a–p, p–a), current direction for rTMS (a–p, p–a) and rTMS design (continuous vs. block) was calculated for the MEP amplitudes at baseline to exclude any systematic differences. MEP amplitudes were then normalized to the mean baseline amplitude of each individual session. A repeated measures ANOVA with current direction for single pulse TMS (a–p, p–a), current direction for rTMS (a–p, p–a), rTMS design (continuous vs. block) and time (1, 3, 5, 10, 15, 30 min after stimulation) as within subject factors was calculated. Based on significant result of the ANOVA paired two-tailed *t*-tests were calculated between individual time points and the respective baseline.

A repeated measures ANOVA was calculated for RMT with current direction for single pulse TMS (a–p, p–a), current direction for rTMS (a–p, p–a), rTMS design (continuous vs. block) and time (pre vs. post). For AMT a repeated measures ANOVA was calculated with current direction for single pulse TMS (a–p, p–a), current direction for rTMS (a–p, p–a), rTMS design (continuous vs. block) to exclude any differences in baseline values. A p-value <0.05 was considered significant for all statistical tests.

3. Results

3.1. MEP amplitudes

Only the 5 Hz rTMS protocol with posterior–anterior directed current flow in the brain in the block design led to significant facilitation, while the continuous protocol with posterior–anterior directed current flow tended towards inhibition instead (Fig. 1). Both protocols with an anterior–posterior directed current flow did not change corticospinal excitability significantly. The repeated measures ANOVA accordingly yielded a main effect of rTMS design ($df = 1$, $F = 5.256$, $p = 0.039$) and time ($df = 5$, $F = 4.226$, $p = 0.002$) and a two-way interaction of current direction for rTMS and rTMS design ($df = 1$, $F = 7.550$, $p = 0.017$). All other interactions were not statistically significant. Post-hoc *t*-tests showed significant differences for time points p3 and p15 ($p = 0.043$ and $p = 0.012$) in the continuous protocol with posterior–anterior directed current and for time points p5 and p15 ($p = 0.016$ and $p = 0.023$) in the block design with posterior–anterior directed current only (Fig. 2).

MEP amplitudes at baseline were not significantly different between different rTMS sessions or between the two test pulses (no significant main effects or interactions in ANOVA). The mean baseline values are summarized in Table 1.

3.2. Threshold measurements

For RMT the repeated measures ANOVA revealed a highly significant main effect of current direction for single pulse TMS ($df = 1$, $F = 187.839$, $p < 0.001$) and no other significant main effects or interactions. For AMT the repeated measures ANOVA revealed a significant main effects of current direction for single pulse TMS ($df = 1$, $F = 173.330$, $p < 0.001$) and current direction for rTMS ($df = 1$, $F = 9.620$, $p = 0.008$). The main effects of current direction

Please cite this article in press as: Rothkegel H et al. Breaks during 5 Hz rTMS are essential for facilitatory after effects. Clin Neurophysiol (2009), doi:10.1016/j.clinph.2009.11.016

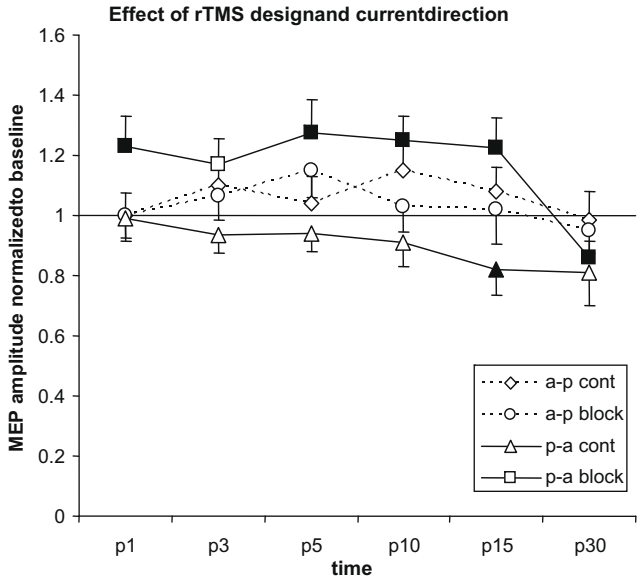


Fig. 1. Interaction of current direction for rTMS and rTMS design. MEP amplitudes measured after intervention using a–p and p–a test pulses are normalized to the respective mean baseline amplitudes and pooled, as there was no interaction term involving current direction for single pulse TMS, error bars indicate ± 1 SEM. Time is given in minutes after the end of the respective rTMS protocol (a–p = anterior–posterior current direction, p–a, posterior–anterior current direction, cont, continuous rTMS train, block, rTMS in blocks of 200 pulses each). Filled symbols represent time points with MEP ratios significantly different from 1 ($p < 0.05$).

for single pulses are due to higher thresholds for posterior–anterior directed pulses compared to anterior–posterior directed pulses (see Table 1).

4. Discussion

For conventional rTMS there is a general consensus, that low stimulation frequencies of around 1 Hz lead to inhibition while higher frequencies lead to facilitation (Fitzgerald et al., 2006; Hallett, 2007; Ziemann et al., 2008). Several studies have recently shown exceptions to this rule, which seems to be applicable only in a neutral resting condition of the motor system. Preconditioning might enhance neuroplastic effects of rTMS, when the direction of expected after effects is opposite for the preconditioning protocol and the rTMS intervention (Iyer et al., 2003; Lang et al., 2004; Siebner et al., 2004). On the contrary preconditioning with a protocol which induces neuroplastic effects of the same direction leads to an inversion of the rTMS induced after effects both for low and high frequency protocols (Lang et al., 2004; Siebner et al., 2004), which has been attributed to homeostatic mechanisms. Even changes in the level of excitability during application of rTMS by means of voluntary muscle contraction might invert the direction of externally induced neuroplasticity as has been shown for short trains of rTMS (Fujiwara and Rothwell, 2004) and transcranial direct current stimulation (Antal et al., 2007).

Furthermore rTMS protocols using short bursts of high frequency stimulation such as theta burst stimulation (TBS) or quadripulse TMS (QPS) show a more complex relationship between stimulation parameters and the direction of after effects. For TBS (Huang et al., 2005) not stimulation frequency but the presence of short breaks of a specific duration determines whether corticospinal excitability is facilitated or inhibited. While 40 s of continuous TBS leads to inhibition, breaking up this sequence every 2 s for 8 s switches inhibition to facilitation. For QPS (Hamada et al., 2008) the repetition rate in a short train of four monophasic pulses as well as the duration of stimulation determine the direction of the induced after effects in a non-linear way.

4.1. The role of breaks in conventional rTMS

Here we argue that the interval in excitatory high frequency stimulation of conventional rTMS plays a much bigger role in determining excitatory after effect than considered so far. The present study shows, that the continuous application of 1200 pulses of subthreshold 5 Hz rTMS does not induce facilitatory after effects as the standard condition with the same number of pulses split up in six blocks of 200 pulses each with intertrain intervals of 60 s. The continuous protocol rather tends toward inhibition which resembles the pattern seen after TBS. This indicates that even in conventional rTMS there is no clear frequency cut-off which separates inhibitory from facilitatory protocols. The importance of intervals as shown for TBS might also apply for conventional rTMS.

The mechanisms behind this functional importance of the breaks are not clear from the experiment. TMS is known to activate a combination of inhibitory and excitatory cortical pathways. As induction of inhibitory or facilitatory after effects follows a differ-

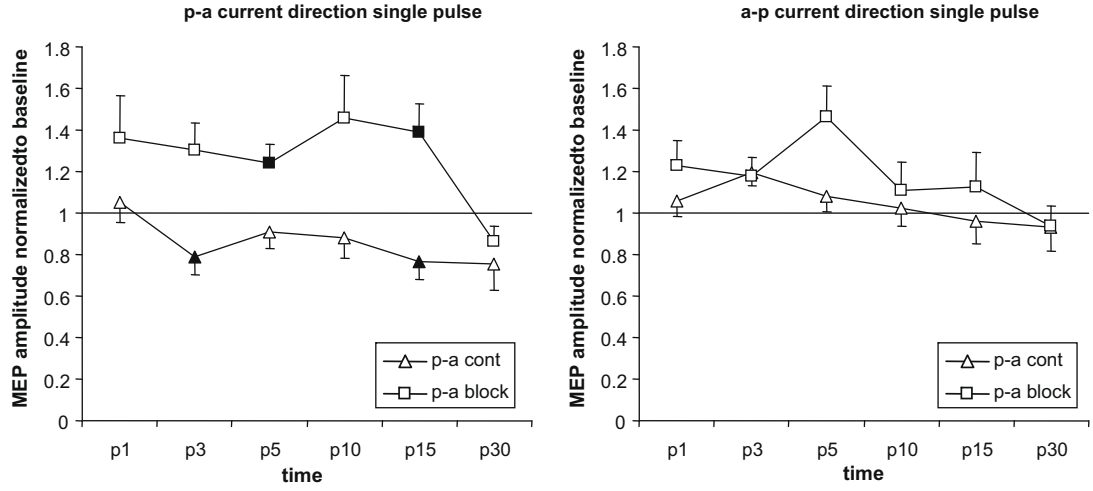


Fig. 2. Effect of rTMS design in protocols with posterior–anterior current direction during intervention for either current direction of single pulse TMS. MEP amplitudes measured after intervention are normalized to the respective mean baseline amplitudes, error bars indicate ± 1 SEM. Time is given in minutes after the end of rTMS. Filled symbols represent significant difference of a time point compared to baseline ($p < 0.05$).

Please cite this article in press as: Rothkegel H et al. Breaks during 5 Hz rTMS are essential for facilitatory after effects. Clin Neurophysiol (2009), doi:10.1016/j.clinph.2009.11.016

Table 1
Motor Threshold and baseline MEP amplitudes.

		p–a continuous		p–a block		a–p continuous		a–p block	
		Baseline	Post	Baseline	Post	Baseline	Post	baseline	post
Test pulse p–a	RMT [% MSO]	38.4 ± 4.8	38.4 ± 4.8	39.4 ± 6.4	39.3 ± 6.0	39.4 ± 6.8	38.9 ± 6.4	37.9 ± 5.6	38.1 ± 5.6
	AMT [% MSO]	30.8 ± 4.1		31.5 ± 4.4		30.6 ± 4.8		29.6 ± 4.5	
	SI1 mV [% MSO]	46.9 ± 7.4		47.8 ± 7.1		47.6 ± 9.0		46.7 ± 7.5	
	MEP-Ampl [mV]	1.13 ± 0.25		0.93 ± 0.21		1.09 ± 0.30		1.00 ± 0.23	
Test pulse a–p	RMT [% MSO]	32.6 ± 3.6	32.4 ± 4.1	32.9 ± 5.3	33.0 ± 5.1	32.7 ± 5.5	32.8 ± 5.7	32.4 ± 5.7	32.8 ± 5.2
	AMT [% MSO]	25.2 ± 3.8		25.8 ± 4.0		24.9 ± 4.0		24.9 ± 4.0	
	SI1 mV [% MSO]	40.8 ± 6.1		41.7 ± 6.2		40.9 ± 7.7		40.7 ± 6.6	
	MEP-Ampl [mV]	1.13 ± 0.28		1.06 ± 0.43		1.12 ± 0.29		1.06 ± 0.29	

The required stimulus intensity for posterior–anterior directed pulses (p–a) was significantly higher compared to stimulus intensity for anterior–posterior directed pulses (a–p). There was no statistically significant difference between sessions and no change in RMT after any of the rTMS protocols. All values are given as mean ± 1 SD. MSO, Maximum Stimulator Output.

ent time courses the presence of breaks might favour a facilitatory process which builds up faster while inhibitory processes are stronger but build up more slowly. From our data an interval of 60 s seems to be efficient in turning inhibition into excitation. But it remains to be determined if another timing pattern would lead to even stronger effects. In this context the duration of the rTMS train might be of interest. A recent study showed that 10 Hz rTMS at an intensity of 80% RMT with short trains of 1.5 s is capable of inducing facilitatory after effects while trains of 5 s lead to inhibition (Jung et al., 2008). The current direction for rTMS used in this study is not clear from the article.

An alternative explanation why uninterrupted high frequency stimulation inverts the direction of after effects into inhibition could be a homeostatic mechanism. As stated above several studies showed that the direction of after effects induced by rTMS depends on the previous state of the cortex or history of activation (Iyer et al., 2003; Lang et al., 2004; Siebner et al., 2004). Thus it is conceivable that prolonged trains of 5 Hz rTMS first increase cortical excitability which in turn causes the later part of the rTMS train to induce inhibition instead of facilitation. Breaks during the standard high frequency protocol after trains of limited duration might prevent that the level of excitability exceeds a threshold which would turn excitation into inhibition.

In addition intensity seems to play a role. A similar 5 Hz protocol as in the present study using blocks of 300 pulses each (biphasic, initially posterior–anterior current flow in the brain) only induced a significant facilitation at 90% RMT while there was no significant change in corticospinal excitability with the lower intensity of 90% AMT (Quartarone et al., 2005). In the present study the low intensity of 90% AMT was chosen to avoid any safety risk in the continuous condition and to ensure a cortical origin of neuroplastic changes. Previous studies have shown that TMS at intensities below AMT does not lead to detectable corticospinal volleys (Di Lazzaro et al., 1998) which makes a spinal mechanism of the reported excitability changes unlikely. However, as we did not measure spinal excitability in the present study we cannot fully exclude this possibility. Our protocol using shorter trains of stimulation seems to be more effective than the protocol used be Quartarone since in contrast we saw a clear excitatory effect at 90% AMT.

Furthermore we cannot exclude an effect of voluntary muscle contraction before rTMS due to the measurement of AMT, which was used to adjust stimulation intensity as in many previous studies. It has been shown for TBS, that voluntary muscle contraction might act as a priming condition favouring inhibitory after effects (Gentner et al., 2008).

4.2. Influence of current direction for rTMS

The present study confirmed previous data that only the p–a current direction leads to facilitatory after effects following the standard protocol in block design (Sommer et al., 2006). It has been shown that single pulses of p–a and a–p current direction preferentially activate different subsets of interneuron. While the initially a–p directed biphasic pulses tend to stimulate mainly those interneurons responsible for the I1-wave (Di Lazzaro et al., 2001), the I-wave pattern is much more complex for p–a current direction. However at low intensities p–a directed pulses seem to activate preferentially interneurons generating later I-waves such as I3. Thus a sequence of activation involving more synaptic connections after each TMS pulse might make the cortical network more susceptible to neuroplastic changes.

Another important factor might be the different physical intensities for p–a and a–p rTMS due to a higher motor threshold for p–a pulses. The pathways which are involved in the neuroplastic changes do not necessarily follow the same sensitivity to current direction as those structures generating the corticospinal volleys following TMS. The lack of after effects following a–p rTMS could simply result from lower stimulus intensity. The different physical intensities are a limitation of the present study so that we cannot draw firm conclusions regarding the question of current direction, which needs to be re-evaluated in future studies.

4.3. Influence of current direction for single pulse TMS

Interestingly the after effects of both the facilitatory (block design) and the inhibitory protocol were only significant for the p–a test pulse. This might implicate that subthreshold rTMS using a p–a current direction preferentially induces after effects in cortical circuits involved in the generation of later I-waves such as I3. This pattern differs from that found in studies on TBS: Facilitatory intermittent TBS preferentially affects later I-waves as well, while inhibitory continuous TBS mainly affects the I1-wave (Di Lazzaro et al., 2008) indicating a different mode of action for the inhibitory protocols.

Previous studies exploring the after effects of subthreshold high-frequency rTMS on short interval intracortical inhibition (SICI), which is predominantly an inhibition of later I-waves (Di Lazzaro et al., 1998), differ in the methods applied. However, the most consistent finding of these studies (Peinemann et al., 2000; Quartarone et al., 2005) is a decrease in SICI following subthreshold 5 Hz rTMS, which is paralleled by a decreased reduction of later I-waves compared to baseline (Di Lazzaro et al., 2002). If both facilitation and inhibition of MEP in our present experiment are mediated by changes of the same intracortical network an increase of SICI following the continuous protocol has to be postulated and will be subject of future experiments.

5. Conclusion

The present study shows the functional relevance of breaks during high-frequency rTMS. Somewhat unexpectedly a prolonged

Please cite this article in press as: Rothkegel H et al. Breaks during 5 Hz rTMS are essential for facilitatory after effects. Clin Neurophysiol (2009), doi:10.1016/j.clinph.2009.11.016

continuous train of rTMS tends towards inhibition while the classical block design leads to facilitation. These results points towards high frequency stimulation being a necessary but not sufficient condition to induce excitation. In addition the latter requires the involvement of stimulation intervals.

Acknowledgements

We wish to thank Prof. John Rothwell for helpful comments on the paper. H.R. and M.S. were supported by a Grant from the DFG (Deutsche Forschungsgemeinschaft) SO 429/2–2, W.P. by the Rose Foundation.

References

Antal A, Terney D, Poreisz C, Paulus W. Towards unravelling task-related modulations of neuroplastic changes induced in the human motor cortex. Eur J Neurosci 2007;26:2687–91.

Chen R, Classen J, Gerloff C, Celnik P, Wassermann EM, Hallett M, et al. Depression of motor cortex excitability by low-frequency transcranial magnetic stimulation. Neurology 1997;48:1398–403.

Di Lazzaro V, Oliviero A, Mazzone P, Insola A, Pilato F, Saturno E, et al. Comparison of descending volleys evoked by monophasic and biphasic magnetic stimulation of the motor cortex in conscious humans. Exp Brain Res 2001;141:121–7.

Di Lazzaro V, Oliviero A, Mazzone P, Pilato F, Saturno E, Dileone M, et al. Short-term reduction of intracortical inhibition in the human motor cortex induced by repetitive transcranial magnetic stimulation. Exp Brain Res 2002;147:108–13.

Di Lazzaro V, Pilato F, Dileone M, Profice P, Oliviero A, Mazzone P, et al. The physiological basis of the effects of intermittent theta burst stimulation of the human motor cortex. J Physiol 2008;586:3871–9.

Di Lazzaro V, Restuccia D, Oliviero A, Profice P, Ferrara L, Insola A, et al. Magnetic transcranial stimulation at intensities below active motor threshold activates intracortical inhibitory circuits. Exp Brain Res 1998;119:265–8.

Fitzgerald PB, Fountain S, Daskalakis ZJ. A comprehensive review of the effects of rTMS on motor cortical excitability and inhibition. Clin Neurophysiol 2006;117:2584–96.

Fujiwara T, Rothwell JC. The after effects of motor cortex rTMS depend on the state of contraction when rTMS is applied. Clin Neurophysiol 2004;115:1514–8.

Gentner R, Wankerl K, Reinsberger C, Zeller D, Classen J. Depression of human corticospinal excitability induced by magnetic theta-burst stimulation: evidence of rapid polarity-reversing metaplasticity. Cereb Cortex 2008;18:2046–53.

Gorsler A, Baumer T, Weiller C, Munchau A, Liepert J. Interhemispheric effects of high and low frequency rTMS in healthy humans. Clin Neurophysiol 2003;114:1800–7.

Hallett M. Transcranial magnetic stimulation: a primer. Neuron 2007;55:187–99.

Hamada M, Terao Y, Hanajima R, Shirota Y, Nakatani-Enomoto S, Furubayashi T, et al. Bidirectional long-term motor cortical plasticity and metaplasticity induced by quadripulse transcranial magnetic stimulation. J Physiol 2008;586:3927–47.

Huang YZ, Edwards MJ, Rounis E, Bhatia KP, Rothwell JC. Theta burst stimulation of the human motor cortex. Neuron 2005;45:201–6.

Iyer MB, Schleper N, Wassermann EM. Priming stimulation enhances the depressant effect of low-frequency repetitive transcranial magnetic stimulation. J Neurosci 2003;23:10867–72.

Jung SH, Shin JE, Jeong YS, Shin HI. Changes in motor cortical excitability induced by high-frequency repetitive transcranial magnetic stimulation of different stimulation durations. Clin Neurophysiol 2008;119:71–9.

Lang N, Siebner HR, Ernst D, Nitsche MA, Paulus W, Lemon RN, et al. Preconditioning with transcranial direct current stimulation sensitizes the motor cortex to rapid-rate transcranial magnetic stimulation and controls the direction of after-effects. Biol Psychiatry 2004;56:634–9.

Peinemann A, Lehner C, Mentschel C, Munchau A, Conrad B, Siebner HR. Subthreshold 5-Hz repetitive transcranial magnetic stimulation of the human primary motor cortex reduces intracortical paired-pulse inhibition. Neurosci Lett 2000;296:21–4.

Quartarone A, Bagnato S, Rizzo V, Morgante F, Sant'angelo A, Battaglia F, et al. Distinct changes in cortical and spinal excitability following high-frequency repetitive TMS to the human motor cortex. Exp Brain Res 2005;161:114–24.

Siebner HR, Lang N, Rizzo V, Nitsche MA, Paulus W, Lemon RN, et al. Preconditioning of low-frequency repetitive transcranial magnetic stimulation with transcranial direct current stimulation: evidence for homeostatic plasticity in the human motor cortex. J Neurosci 2004;24:3379–85.

Sommer M, Bachmann CG, Norden C, Rummel M, Speck S, Tings T, et al. Orientation-specific, non NMDA-receptor dependent facilitation in the human motor cortex elicited by fast biphasic repetitive transcranial magnetic stimulation. Soc Neurosci Abstr Program 2006, No. 705.12.

Wassermann EM. Risk and safety of repetitive transcranial magnetic stimulation: report and suggested guidelines from the International Workshop on the Safety of Repetitive Transcranial Magnetic Stimulation, June 5–7, 1996. Electroencephalogr Clin Neurophysiol 1998;108:1–16.

Ziemann U, Paulus W, Nitsche MA, Pascual-Leone A, Byblow WD, Berardelli A, et al. Consensus: motor cortex plasticity protocols. Brain Stimul 2008;1:164–82.

Please cite this article in press as: Rothkegel H et al. Breaks during 5 Hz rTMS are essential for facilitatory after effects. Clin Neurophysiol (2009), doi:10.1016/j.clinph.2009.11.016

2.4 Impact of pulse duration in single pulse TMS

The present design of TMS stimulator allows only pulses of a fixed duration due to the physical properties of a resonant circuit formed by the two main components of the machine, the capacitor bank and the stimulation coil. Based on measurements of motor threshold and calculations of neuronal membrane time constants a very brief pulse duration has been found to be more efficient with regard to energy consumption of the machine and coil heating (Barker et al., 1991).

The aim of the following study was to explore the impact of pulse duration in the range of commercially available monophasic systems on single pulse measures of cortical excitability. Two stimulators were connected in parallel in order to achieve an increase in pulse duration by a factor of 1.4. As expected the motor threshold expressed as percentage of maximum stimulator output (%MSO), which correlates to capacitor voltage, was lower using the longer pulse. There was no effect of pulse duration on the other parameters as long as intensities where adapted to the respective threshold. Pulse-to-pulse variation was decreased for the measurement of contralateral silent period using the longer pulse.

Thus changing pulse duration in the range tested here does not lead to stimulation of different neuronal populations. For subjects with high motor thresholds (e.g. neurodegenerative diseases or under pharmacological treatment) the longer pulse duration might be a valuable alternative. The present study implies that studies using different pulse duration or even different stimulator setup are comparable as long as measurements are adjusted to the individual threshold and the same type of coil is used.

Impact of pulse duration in single pulse TMS

H. Rothkege1[1], M. Sommer[1], W. Paulus[1], N. Lang[2]

[1]Department of Clinical Neurophysiology, University of Göttingen, Germany
[2]Department of Neurology, University of Kiel, Germany

Keywords: transcranial magnetic stimulation, motor evoked potential, cortical silent period

Abstract

Objective: The intensity of transcranial magnetic stimulation (TMS) is typically adjusted by changing the amplitude of the induced electrical field, while its duration is fixed. Here we examined the influence of two different pulse durations on several physiological parameters of primary motor cortex excitability obtained with single pulse TMS.

Methods: A Magstim Bistim² stimulator was used to produce TMS pulses of two distinct durations. For either pulse duration we measured in healthy volunteers resting and active motor thresholds, recruitment curves of motor evoked potentials in relaxed and contracting hand muscles as well as contralateral (cSP) and ipsilateral (iSP) cortical silent periods.

Results: Motor thresholds decreased by 20% using a 1.4 times longer TMS pulse compared to the standard pulse, while there was no significant effect on threshold adjusted measurements of cortical excitability. The longer pulse duration reduced pulse-to-pulse variability in cSP.

Conclusions: The strength of a TMS pulse can be adjusted both by amplitude or pulse duration. TMS pulse duration does not affect threshold-adjusted single pulse measures of motor cortex excitability.

Significance: Using longer TMS pulses might be an alternative in subjects with very high motor threshold. Pulse duration might not be relevant as long as TMS intensity is threshold-adapted. This is important when comparing studies performed with different stimulator types.

Introduction

Transcranial magnetic stimulation (TMS) is a non-invasive technique which allows stimulation of cortical neuronal networks in the awake behaving human subject. It has become a well established diagnostic tool for conduction studies of central motor pathways in neurology and neurosurgery. It is also a valuable research tool for assessment of cortical excitability in the motor and visual system as well as for modulation of cortical excitability in different cortical regions. Repetitive TMS is capable of inducing changes of cortical excitability outlasting the duration of stimulation thus making it a potential therapeutic option in a variety of neuropsychiatric disorders (Fregni and Pascual-Leone, 2007; Kobayashi and Pascual-Leone, 2003; Rossini and Rossi, 2007).

The technique of TMS is based on the principle of electromagnetic induction and uses a local rapidly changing magnetic field to induce an electrical field, which in turn leads to an electrical current in conductive tissue without attenuation by structure with high electrical impedance (e.g. the scull) or necessity of direct contact to electrodes. The basic stimulator design which is still used in all commercially available stimulators was first introduced in 1982 (Polson et al., 1982) for peripheral nerve stimulation and applied to transcranial cortical stimulation in 1985 (Barker et al., 1985). In order to achieve a sufficiently high rate of change of the magnetic field a high voltage from a capacitor bank is discharged via a magnetic coil. These components form an oscillator (RLC-circuit) with a resonant frequency f_0 mainly determined by the capacitance C of the stimulator and the inductance L of the coil according to the following equation (simplified for an undamped resonant circuit):

$$f_o = \frac{1}{2\pi\sqrt{LC}} \quad (1)$$

In conventional magnetic stimulators it is only possible to interrupt the effective stimulus duration at quarters of the full oscillation period leading to the so called monophasic pulse after the first quarter cycle, a halfsine pulse after the first two quarter cycles and the biphasic

pulse after a full period (Sommer et al., 2006). Due to the cosine shape of the induced electrical field all pulses of more than one quarter cycle will have a reversal of the direction of the electrical field after each odd quarter cycle. In contrast to electrical stimulation the pulse duration in magnetic stimulation (regarding a single phase) cannot easily be adjusted as this requires changing the resonant frequency and thus the stimulator hardware. The intensity of the TMS pulse is controlled by the capacitor voltage, which determines the initial steepness of the induced time-varying magnetic field and thereby the amplitude of the induced electrical field.

Using six different capacitor configurations in order to achieve monophasic TMS pulses of six distinct pulse durations Barker and colleagues demonstrated that a longer pulse requires more stored energy and leads to stronger coil heating compared to shorter pulses (Barker et al., 1991). However, the stimulation threshold in terms of capacitor voltage (which is proportional to the commonly used percentage of maximum stimulator output) is lower with a higher pulse duration. Comparing the stored energy required to evoke threshold motor responses at different stimulus intensities to analogue measurements with electronically defined time constants Barker and colleagues were able to estimate cortical membrane time constant in man to be in the order of 150µs.

Controlling the pulse duration of TMS might open the possibility to preferentially stimulate a specific neuronal population in a spatially overlapping cortical network. It has previously been shown that selection of a shorter pulse duration reduces stimulation of peripheral sensory nerves at skin level for a given intensity of motor cortex stimulation (Geddes, 1987).

So far the effect of pulse duration has only been investigated for motor threshold (Barker et al., 1991). The objective of the present study was to systematically investigate the effect of two distinct pulse durations offered by a commercially available TMS system on a set of single pulse parameters for corticospinal excitability.

Material and Methods

Subjects

12 healthy right-handed human subjects (6 women and 6 men, age range 19 to 43 years) participated in the experiment after giving informed consent. All subjects were non-smokers. Experimental procedures had the approval of the Ethics Committee of the University of Göttingen and were performed according to the ethical standards laid down in the Declaration of Helsinki.

Stimulator Setup

A commercially available Magstim Bistim² stimulator setup (The Magstim Company Limited, UK) was used to produce monophasic TMS-pulses of two distinct durations. This setup allows discharging two identical capacitor banks of the connected Magstim 200² stimulators simultaneously through the same coil. In this configuration the two capacitor banks are connected in parallel thus doubling the capacitance of the system. For a monophasic pulse the first phase of the induced electrical field is approximately a quarter cycle of a cosine wave followed by a relatively low electrical field in opposite direction induced by a slow decay of the magnetic field. Thus the first phase of the monophasic pulse can be considered as the "active" part so that calculations regarding pulse duration can be derived from the cosine shape. According to equation (1) doubling the capacitance of the system leads to a decrease of the resonance frequency of the system and thus an increase of the pulse duration by a factor of $\sqrt{2}$ (≈ 1.4) compared to a single stimulator. All parameters of corticospinal excitability were measured both in the simultaneous configuration and with a single stimulator discharging through the Bistim module in order to keep all other components of the system comparable. Figure 1 illustrates the time course of the magnetic field and the induced electrical field. The

magnetic field rise time was 82µs for the single stimulator and 114µs for the simultaneous mode.

Transcranial magnetic stimulation (TMS) was applied over the left primary motor cortex. The position of a figure-of-8 coil (70mm standard double coil 9925-00, appr. 16.35µH, The Magstim Company Limited, UK) connected to the Bistim² setup via a coil adapter (3110-00, The Magstim Company Limited, UK) was adjusted to yield maximum MEP amplitudes from the right first dorsal interosseus muscle (FDI, target muscle). MEPs from the right abductor digiti mini muscle (ADM, non-target muscle) were registered to test the focality of stimulation. The coil was held tangentially to the skull with the coil handle pointing posterolaterally at an angle of 45 degrees to the sagittal plane inducing a posterior-anterior directed current in the brain.

Surface EMG was recorded with Ag/AgCl cup electrodes in a belly-tendon montage from the FDI bilaterally and the ADM of the right hand. Analogue signals were band-pass filtered (2-3000 Hz) and amplified (Digitimer D360, Welwyn Garden City, Hertfordshire, UK), sampled at a rate of 5 kHz using a CED Micro 1401 mk II (Cambridge Electronic Design, Cambridge, England) and stored on a lab computer for offline analysis using customized Signal 2.16 software (Cambridge Electronic Design, Cambridge, England).

Parameters of corticospinal excitability

All of the following parameters were first measured with one of the pulse configuration and after a break of at least 10 minutes with the other one. The order of pulse configurations was pseudorandom and counterbalanced.

Resting motor threshold (RMT) was determined as the lowest stimulator output at which at least 5 out of 10 consecutive TMS pulses induced MEPs of >50µV in amplitude in the target muscle (right FDI) with all recorded muscles at rest. Values are given as a percentage of maximum stimulator output (MSO). For active motor threshold (AMT) subjects were asked to

keep a tonic contraction of the right FDI of approximately 20-30% of maximum EMG activity. The minimum stimulator output at which at least 5 out of 10 TMS pulses induced MEPs of >200μV in amplitude was considered the AMT.

MEP-amplitudes were measured peak to peak as recruitment curves in the relaxed muscle both for FDI and ADM of the right hand using intensities of 100-160% RMT. Stimulus intensities were adjusted to RMT to account for interindividual differences. The range of intensities was chosen because 160% RMT was the highest intensity that could be reached in all subjects tested. The intensity was increased in steps of 10% RMT with 10 MEPs recorded at each level. In addition to MEP amplitudes we measured MEP latency, duration of the first phase of the MEP and the area under the first phase of the MEP in the 160% condition only.

MEP amplitudes were also measured in the tonically contracting muscle at stimulus intensities of 120%, 140% and 160% AMT. Subjects were instructed to keep 20-30% of maximum voluntary force in the target and non-target muscles. Activation was controlled by mean rectified EMG activity. Again 10 MEPs were recorded at each intensity level. In these recordings also the contralateral (cSP) and ipsilateral (iSP) silent period (Ferbert et al., 1992) were assessed. The duration of the cSP was measured for each individual TMS pulse from the time of the stimulus to the point where the rectified EMG activity first reached the level of baseline activity determined in the 100ms preceding the TMS stimulus. The iSP was assessed in the tonically contracting left FDI muscle in the 160% AMT condition only. Data of all 10 recordings were rectified and averaged. Onset of the iSP was defined as the first point where the EMG activity fell below prestimulus EMG activity determined in the 100 ms preceding the TMS stimulus. The duration of the iSP was measured from the onset of the iSP to the point where the EMG-activity again reached the level of prestimulus EMG activity for more than 5 ms. The level of inhibition was measured as the ratio of the mean EMG activity during iSP divided be the prestimulus EMG activity.

In order to assess the pulse-to-pulse variability of MEP amplitudes and cSP the mean consecutive difference (MCD) was calculated and normalized to the respective mean values of amplitudes or cSP (Kiers et al., 1993).

Statistical Analysis

RMT and AMT values were compared using paired two-tailed t-tests. For MEP recruitment at rest a repeated measures separate ANOVAs with innersubject factors pulse duration (standard vs. simultaneous), muscle (target vs. non-target) and TMS intensity (7 levels) were calculated for MEP amplitudes and normalized MCD. Repeated measures ANOVAs with innersubject factors pulse duration and muscle were calculated for mean values of MEP latency, duration and area under curve of the first phase of the MEPs at 160% RMT.

For the MEPs measured under tonic contraction of both the target and non-target muscle repeated measures ANOVAs with inner subject factors pulse duration (standard vs. simultaneous), muscle (target vs. non-target) and TMS intensity (3 levels) were performed for mean amplitudes and cSP as well as for the respective normalized MCD.

Values for onset and duration of iSP as well as the ratio of EMG activity during iSP divided by the respective prestimulus activity were compared using paired two-tailed t-test.

All statistical tests were performed using SPSS 17.0. A p-Value < 0.05 was considered significant for all statistical tests.

Results

Motor Threshold

RMT and AMT values expressed as maximum stimulator output were approximately 20% lower using the longer pulse duration compared to the standard pulse (figure 2). Paired two-tailed t-tests accordingly showed a highly significant difference for pulse duration (RMT: $T = 13.403$, $p > 0.001$; AMT: $T = 15.117$, $p < 0.001$).

There was no statistically significant difference for the two pulses durations in the recruitment curves of the target and non-target hand muscle (FDI and ADM) at rest (figure 3). The repeated measures ANOVA showed a main effect of intensity ($F=31.633$, $df=6$, $p < 0.001$) as expected, but no other main effects or interactions. For the normalized MCD there was a main effect of intensity ($F=25.273$, $df=6$, $p < 0.001$) without any other significant main effects or interactions.

Mean MEP latencies at 160% RMT did not differ significantly between the two pulse durations or muscles (table 1). The first phase of the MEP was longer for the ADM compared to FDI (repeated measures ANOVA, main effect of muscle, $F=13,672$, $df=1$, $p=0.004$) without any effect of the pulse duration (table 1) while the area under the curve did not show any significant differences between muscles or pulse durations.

MEP amplitudes evoked under tonic contraction increased with increasing intensity as expected (figure 4). Repeated measures ANOVA showed a main effect of intensity ($F=45.536$, $df=2$, $p<0.001$) and no other significant main effects or interactions. The normalized MCD for MEP amplitudes decreased with intensity ($F=24.075$, $df=2$, $p < 0.001$). The ANOVA for normalized MCD also revealed an interaction of pulse duration and muscle ($F=8.565$, $df=1$, $p=0.014$) reflecting a slightly lower variability for the short pulse in the target muscle (FDI) and a reversed pattern for the non-target muscle (ADM). However, post-hoc t-tests did not confirm a significant difference for pulse duration at any level of intensity.

Contralateral silent period increased as expected with intensity (s. figure 5). At a stimulus intensity of 120% AMT six out of twelve subjects did not show a clearly discernable silent period in up to three out of 10 trials. These trials were not included in the calculation of mean values. ANOVA confirmed a main effect of intensity ($F=64.525$, $df=2$, $p<0.001$) without any other main effects or interactions. However the variability of cSP duration was significantly higher for the single pulse compared to the simultaneous pulse as revealed by a main effect of pulse duration (ANOVA for normalized MCD: $F=31.911$, $df=1$, $p<0.001$) and decreased with increasing intensity ($F=3.923$, $df=2$, $p=0.035$). Post-hoc t-test confirmed the effect of pulse duration for the ADM at 120% AMT only ($t=3.851$, $df=11$, $p=0.003$).

There were no statistically significant differences concerning ipsilateral silent period measured at 160% AMT (s. table 2). None of the subjects showed ipsilateral MEPs.

Discussion

The present study shows that an increase in stimulus duration by a factor of 1.4 compared to the standard configuration reduces both active and resting motor threshold expressed as %MSO by approximately 20%. Within the range of pulse durations tested here there is no difference in commonly used single pulse measures of corticospinal excitability as long as the intensity is adapted to the respective threshold. However, our data indicate that pulse duration might have an effect on variability of MEP amplitudes under tonic contraction and variability of silent period duration.

Effect of stimulus duration on motor threshold

The finding of a 20% decrease in motor threshold expressed as %MSO is in line with previous studies and calculations. Barker and colleagues (Barker et al., 1991) found that the

stored energy required to induce threshold responses was 1.27 times higher when the magnetic field rise time was prolonged by a factor of approximately 1.4 (standard capacitance vs. double capacitance). According to the equation for stored energy W on a capacitor $W = ½ C * V^2$ this translates to a 0.797 times lower voltage V when considering that the capacitance C is doubled for the longer pulse. In the present study we could confirm this stimulus duration – response relationship for measurement both in the resting and tonically contracting muscle.

Threshold-adapted measures of corticospinal excitability

This is the first study systematically comparing different parameters of corticospinal excitability for two different pulse durations of TMS. There were no statistically significant differences in these parameters for the two pulse durations as long as stimulus intensities are adapted to the respective threshold.

The chronaxie for a stimulus duration – response relationship has been estimated to be around 2.5 times the membrane time constant for magnetic stimulation when the shape of the electrical field is modelled by a triangle (instead of the first quarter of a cosine wave). Thus both pulse durations tested in the present study (82µs and 117µs) can be regarded as short compared to an estimated chronaxie of approximately 375µs). In order to selectively target different neuronal populations on the basis of different membrane time constants thus a wider range of pulse durations such as proposed for a near rectangular pulse (Peterchev et al., 2008) might be required.

Response Variability

MEPs after cortical stimulation typically vary considerably in amplitude and shape with a lower normalized MCD at higher stimulus intensities (Kiers et al., 1993). These fluctuations cannot be explained by methodological aspects such as changes in muscle relaxation, attentional modulation or slight displacement of the TMS coil alone. Based on studies using

the triple stimulation technique and brainstem stimulation Rösler and colleagues concluded that the main cause of this trial-to-trial variability are fluctuations in cortical and spinal excitability, which change the number of motor neuron that are close enough to their firing threshold to make them respond to the TMS pulse (Rosler et al., 2008). To our knowledge there are no published studies on the pulse-to-pulse variability of the contralateral silent period. It is conceivable, that the longer pulse duration increases the firing probability of neurons close to threshold leading to more stable effects. The preferential effect of pulse duration on cSP compared to the MEP amplitude would support the view that these parameters are mediated by distinct neuronal populations. In this context it is important to note that stimulus intensity for cSP measurements were adapted to AMT which might differ from the cSP threshold.

Implications for future studies

Even though energy transfer from the stimulator to neural membranes is less efficient with longer pulses and coil heating is increased, the possibility to expand the range of stimulator strength is certainly useful in a number of settings. It has been shown that motor thresholds increase with age even in healthy humans (Rossini et al., 1992), and a further increase has been described in diseases affecting the corticospinal tract such as multiple sclerosis, stroke, brain or spinal cord injury (Kobayashi and Pascual-Leone, 2003). Motor threshold has also been shown to be increased after intake of certain drugs, especially Na^+-channel antagonists (Paulus et al., 2008; Ziemann, 2003). In some of these cases a stronger stimulus than 100% MSO with the standard pulse duration might be required and could be realized using a longer pulse duration. However, increasing the pulse duration does not seem to change the saturation level of MEP amplitudes. That means using longer pulses would not increase MEP amplitudes if they already saturate at a low level because of increase temporal dispersion in the corticospinal conduction e.g. in MS.

The lack of differences in threshold adapted measures of corticospinal excitability allows the combination of different pulse durations within the same study if higher stimulus strength is required in some subjects. This also means that studies using different stimulator systems which might differ slightly in pulse duration are comparable as long as pulse configurations (current direction, phase configuration) are identical and stimulus intensities are adapted to the individual motor threshold. The use of longer TMS pulses might reduce variability in cSP measurements, while it does not affect the mean duration, which has been shown to differ between different pulse configurations (Orth and Rothwell, 2004).

It is important to note that even changing the TMS coil might change the pulse duration for a given stimulator as the resonant frequency also depends on the inductivity L (s. equation 1). However changing the coil leads to more complex changes than just pulse duration, as it will also change the geometry of the magnetic field resulting in different functional effects of TMS (Lang et al., 2006).

Conclusion

Increasing the pulse duration for TMS within the limits of commercially available systems produces stronger pulses which might be needed in subjects with higher threshold due to age, medication or disease. It might also be useful to reduce trial-to-trial variability. The results further suggest that pulse duration does not have an effect on several measures of corticospinal excitability as long as it is adapted to the motor threshold.

Acknowledgements: The authors wish to thank Dr. Lars Matthäus for help with the measurement of the pulse configuration. H.R. and M.S. were supported by a grant from the DFG (Deutsche Forschungsgemeinschaft) SO 429/2-2, W.P. by the Rose Foundation. A part of this work has been presented as poster at the 52nd annual meeting of the German Society of Clinical Neurophysiology, Magdeburg, April 10-12, 2008.

References:

Barker AT, Garnham CW, Freeston IL. Magnetic nerve stimulation: the effect of waveform on efficiency, determination of neural membrane time constants and the measurement of stimulator output. Electroencephalogr Clin Neurophysiol Suppl 1991; 43: 227-37.

Barker AT, Jalinous R, Freeston IL. Non-invasive magnetic stimulation of human motor cortex. Lancet 1985; 1: 1106-7.

Ferbert A, Priori A, Rothwell JC, Day BL, Colebatch JG, Marsden CD. Interhemispheric inhibition of the human motor cortex. J Physiol 1992; 453: 525-46.

Fregni F, Pascual-Leone A. Technology insight: noninvasive brain stimulation in neurology-perspectives on the therapeutic potential of rTMS and tDCS. Nat Clin Pract Neurol 2007; 3: 383-93.

Geddes LA. Optimal stimulus duration for extracranial cortical stimulation. Neurosurgery 1987; 20: 94-9.

Kiers L, Cros D, Chiappa KH, Fang J. Variability of motor potentials evoked by transcranial magnetic stimulation. Electroencephalogr Clin Neurophysiol 1993; 89: 415-23.

Kobayashi M, Pascual-Leone A. Transcranial magnetic stimulation in neurology. Lancet Neurol 2003; 2: 145-56.

Lang N, Harms J, Weyh T, Lemon RN, Paulus W, Rothwell JC, et al. Stimulus intensity and coil characteristics influence the efficacy of rTMS to suppress cortical excitability. Clin Neurophysiol 2006; 117: 2292-301.

Orth M, Rothwell JC. The cortical silent period: intrinsic variability and relation to the waveform of the transcranial magnetic stimulation pulse. Clin Neurophysiol 2004; 115: 1076-82.

Paulus W, Classen J, Cohen LG, Large CH, Di Lazzaro V, Nitsche M, et al. State of the art: Pharmacologic effects on cortical excitability measures tested by transcranial magnetic stimulation. Brain Stimulation 2008; 1: 151-163.

Peterchev AV, Jalinous R, Lisanby SH. A transcranial magnetic stimulator inducing near-rectangular pulses with controllable pulse width (cTMS). IEEE Trans Biomed Eng 2008; 55: 257-66.

Polson MJ, Barker AT, Freeston IL. Stimulation of nerve trunks with time-varying magnetic fields. Med Biol Eng Comput 1982; 20: 243-4.

Rosler KM, Roth DM, Magistris MR. Trial-to-trial size variability of motor-evoked potentials. A study using the triple stimulation technique. Exp Brain Res 2008; 187: 51-9.

Rossini PM, Desiato MT, Caramia MD. Age-related changes of motor evoked potentials in healthy humans: non-invasive evaluation of central and peripheral motor tracts excitability and conductivity. Brain Res 1992; 593: 14-9.

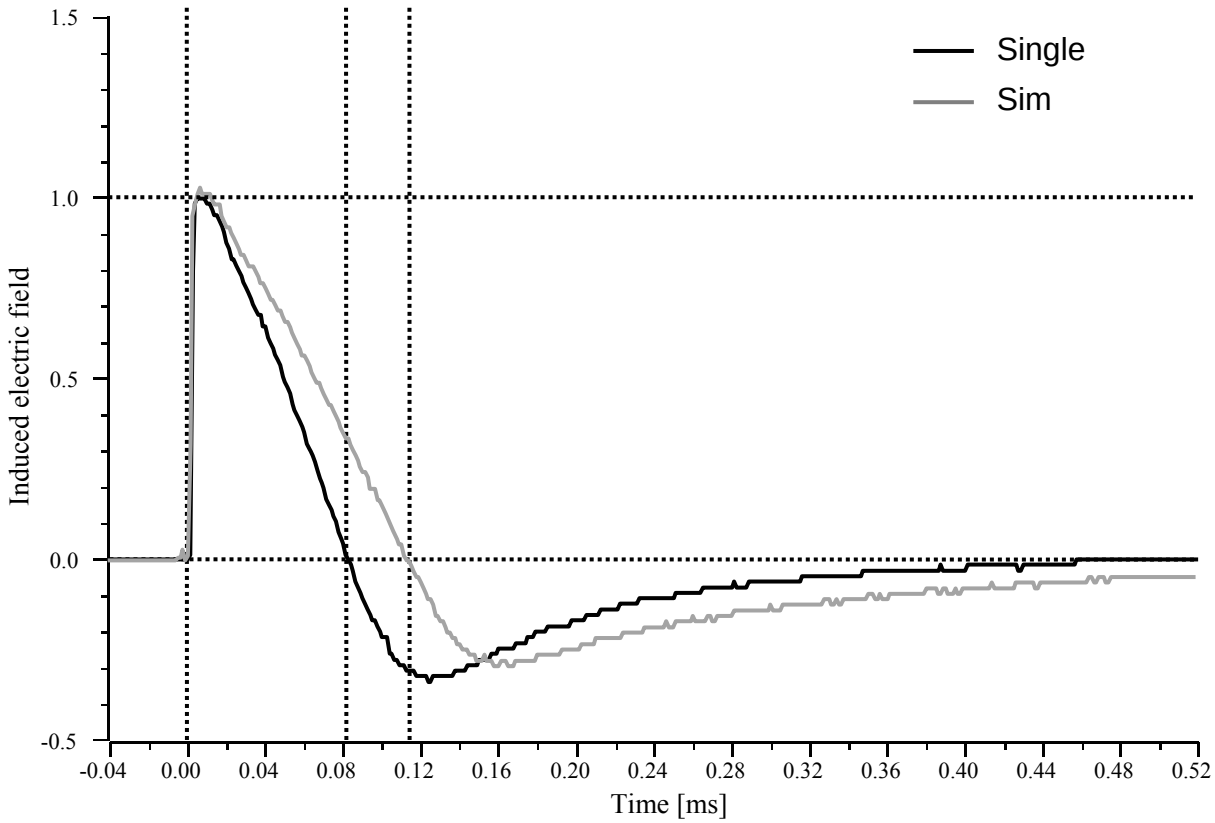
Rossini PM, Rossi S. Transcranial magnetic stimulation: diagnostic, therapeutic, and research potential. Neurology 2007; 68: 484-8.

Sommer M, Alfaro A, Rummel M, Speck S, Lang N, Tings T, et al. Half sine, monophasic and biphasic transcranial magnetic stimulation of the human motor cortex. Clin Neurophysiol 2006; 117: 838-44.

Ziemann U. Pharmacology of TMS. Suppl Clin Neurophysiol 2003; 56: 226-31.

Figure 1: Pulse configuration. Time course of the induced electric field in a wire probe (measured data) at 75% maximum stimulator output (a) and the estimated magnetic field calculated as integral of the original data (b) for the pulse of a single stimulator and the simultaneous mode respectively. All values are given in arbitrary units. The amplitude of the induced electrical field is identical for both stimulator configurations which corresponds to an identical initial rate of change for the magnetic field. The duration of the first phase of the electrical field (= magnetic field rise time) is 0.082ms for the single stimulator and 0.114ms for the simultaneous mode respectively.

a)



b)

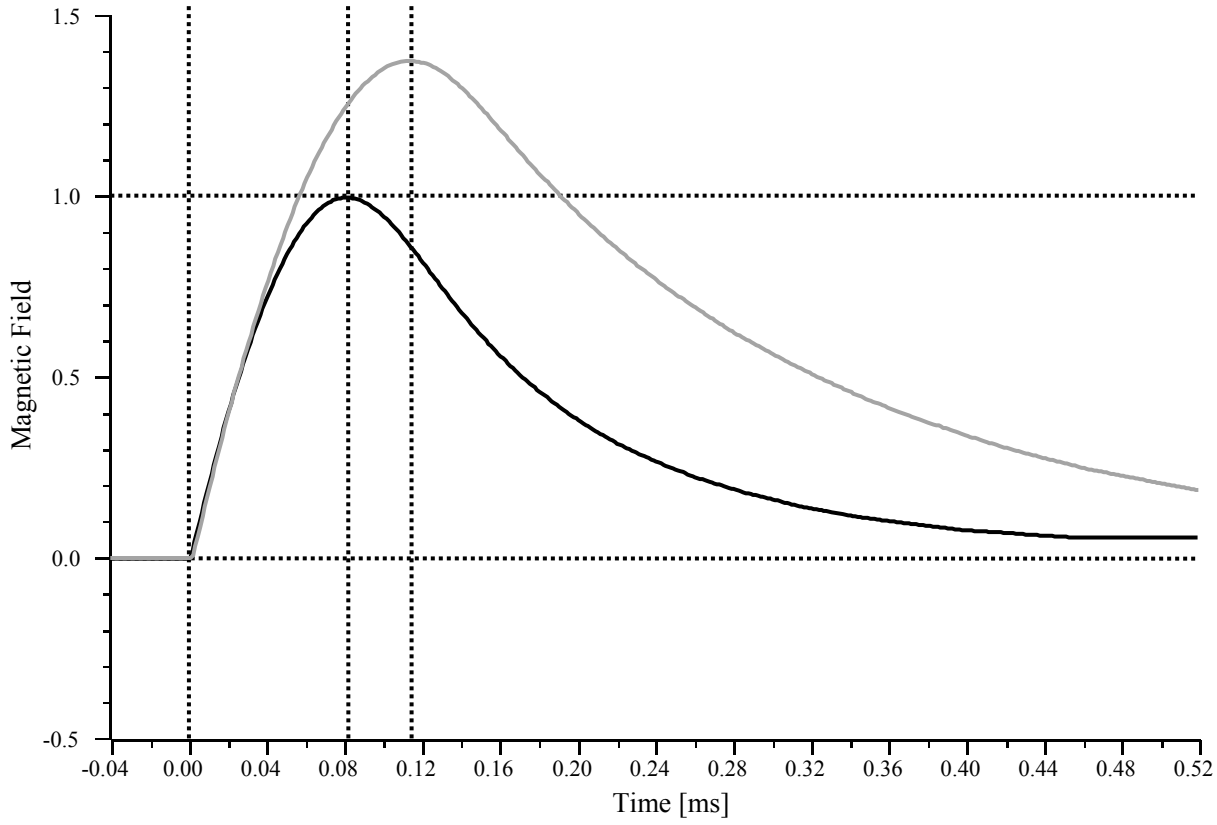

1.5
1.0
0.5
0.0
-0.5
Magnetic Field
-0.04
0.00
0.04
0.08
0.12
0.16
0.20
0.24
0.28
0.32
0.36
0.40
0.44
0.48
0.52
Time [ms]

Figure 2: Motor Threshold. Resting (RMT) and active motor threshold (AMT) for the longer (simultaneous) pulse are significantly lower in terms of maximum stimulator output (i.e. capacitor voltage) compared to the standard (single) pulse. Boxes represent all values between 25th and 75th percentile with a horizontal bar at the position of the median. Upper and lower Whiskers represent the maximum and minimum values respectively. *** marks highly significant differences ($p < 0.001$, paired two-tailed t-tests).

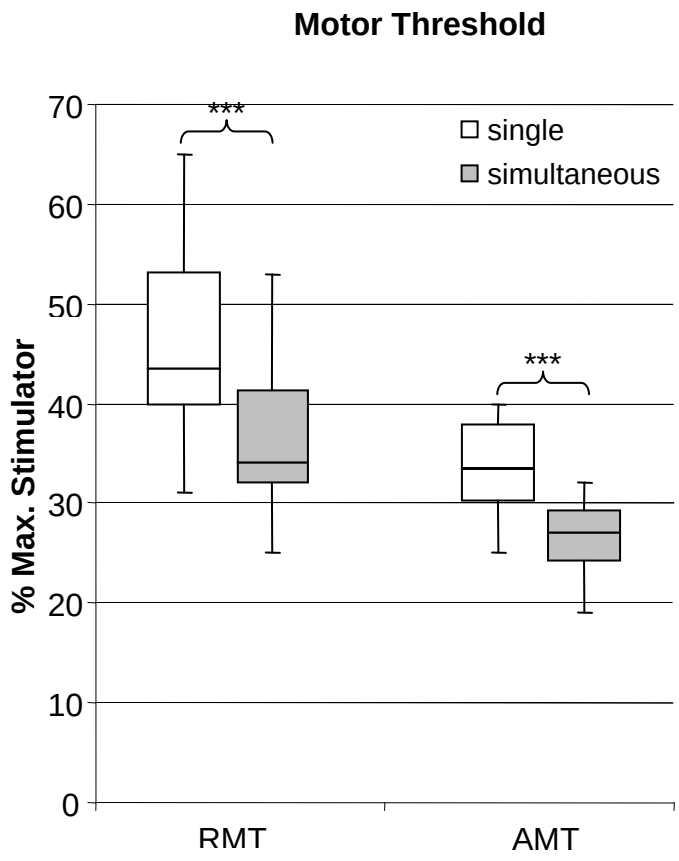
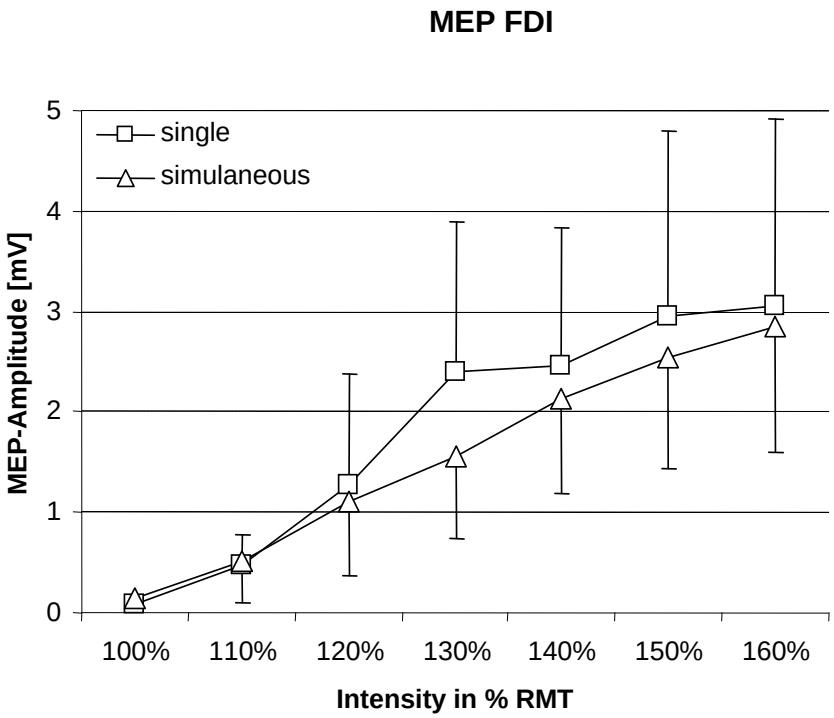


Figure 3: Input-Output curves for MEP amplitudes [mV] at rest for the target muscle (FDI) and non-target muscle (ADM) of the right hand.). There were no statistically significant effects of pulse duration. All values are presented as mean ± standard deviation.



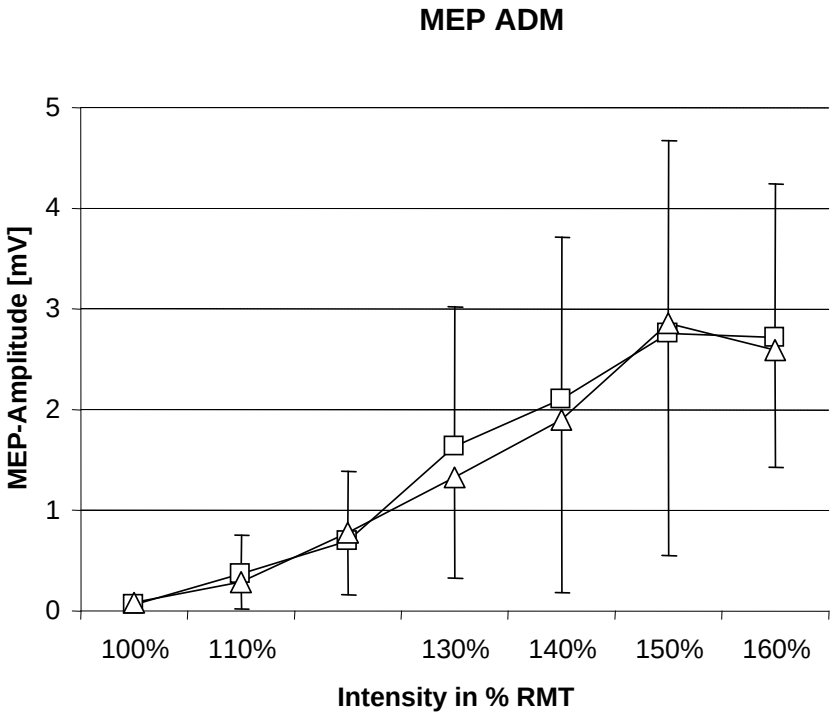
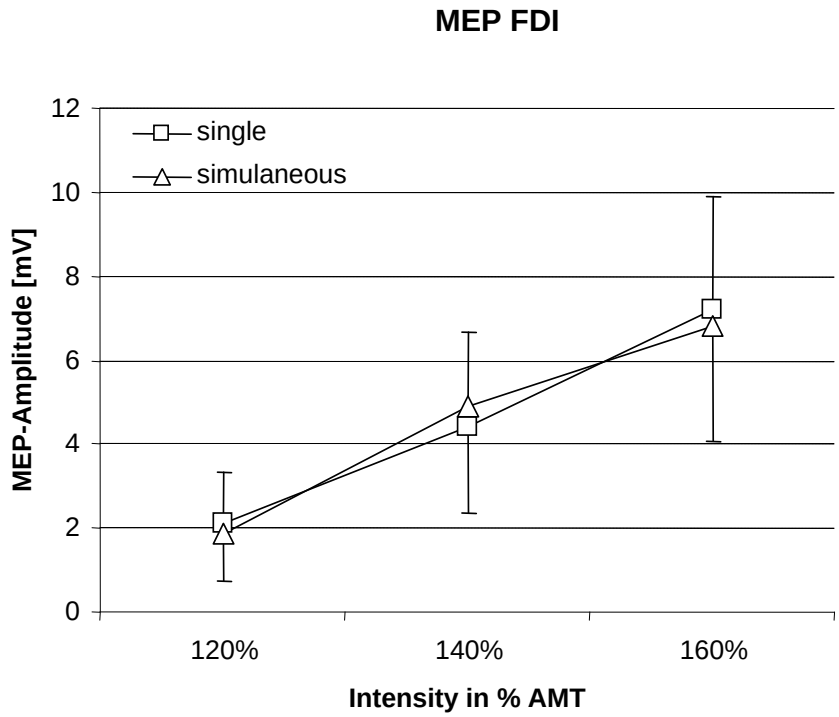


Figure 4: MEP amplitudes under tonic contraction. Mean amplitudes of the target muscle (FDI) and non-target muscle (ADM) of the right hand in mV. All values are presented as mean ± standard deviation.



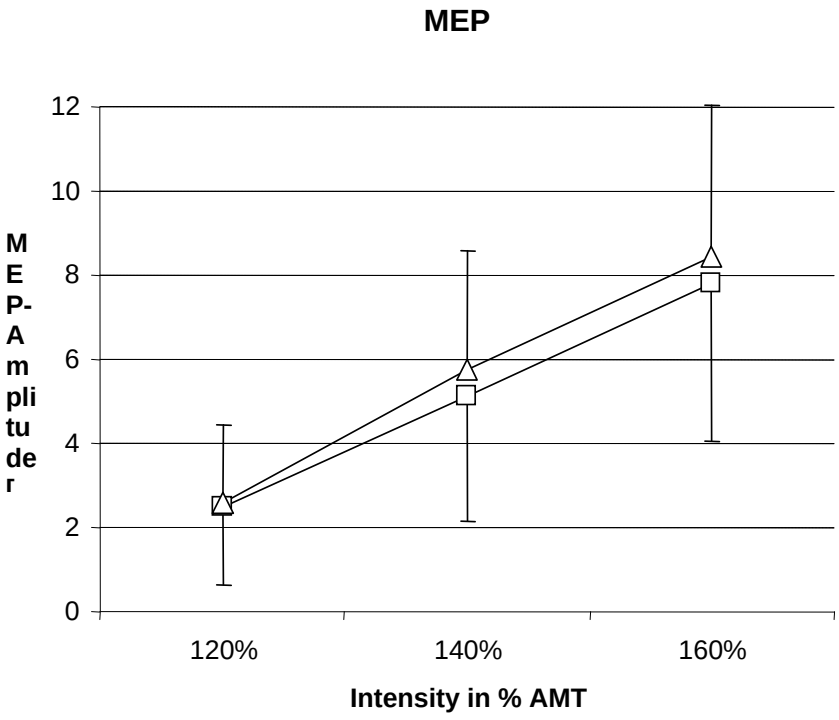


Figure 5: Contralateral silent period. (a) Mean duration of the contralateral silent period in the target muscle (FDI) and non-target muscle (ADM). (b) Normalized MCD for the cSP duration. All values are presented as mean ± standard deviation. Filled symbols in (b) represent a significant difference between the two pulse durations ($p < 0.05$, paired two-tailed t-test).

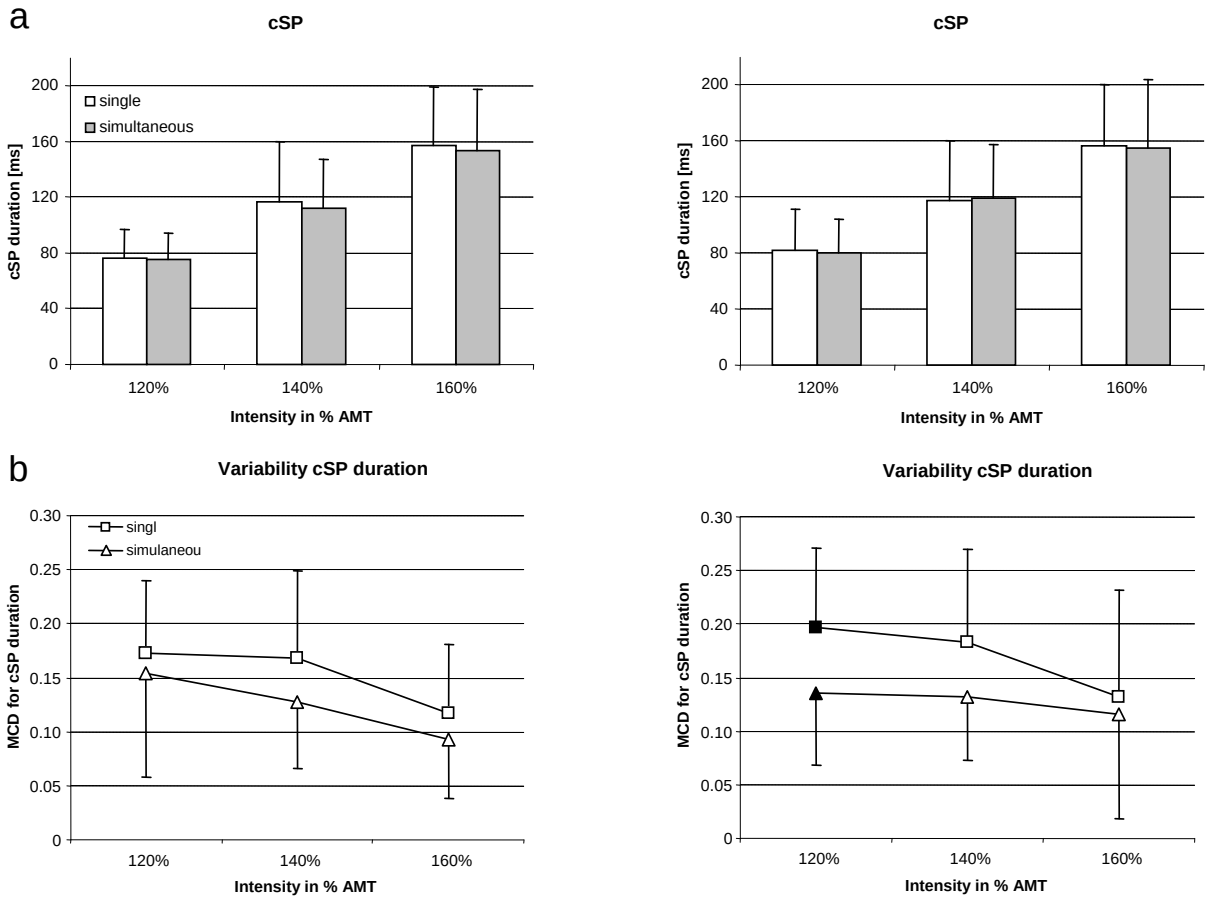


Table 1: Characteristics of MEPs measured at 160% RMT. Latency, duration and area of the first phase of the MEP recorded from the target muscle (FDI) and non-target muscle (ADM) of the right hand. All values are presented as mean across subjects ± standard deviation.

	single		simultaneous	
	FDI	ADM	FDI	ADM
latency	22.2 ± 1.4	21.8 ± 1.0	22.3 ± 1.4	21.9 ± 1.1
duration first phase	7.5 ± 1.2	8.5 ± 1.6	7.5 ± 1.1	8.5 ± 1.4
area first phase	7.2 ± 4.9	7.0 ± 4.7	6.5 ± 3.3	6.5 ± 3.4

Table 2: Ipsilateral silent period. Latency, duration and ratio of the mean rectified EMG activity during iSP divided by the level of prestimulus activity for the FDI of the left hand. There were no statistically significant differences for the two pulse durations. All values are presented as mean values ± standard deviation.

	single	sim	p-value
iSP latency	33.1 ± 5.3	31.4 ± 5.1	0.461
iSP duration	28.1 ± 7.9	30.6 ± 5.8	0.331
EMG Ratio iSP	68.5% ± 12.0%	66.0% ± 15.8%	0.494

2.5 The effect of rTMS over left and right dorsolateral premotor cortex on movement timing of either hand

A precise timing ability is crucial for dextrous hand motor function, which is impaired in a number of movement disorders. Functional interactions in a cerebello-thalamo-cortical network (Pollok et al., 2005) and a specific function of the left dorsolateral premotor cortex (dPMC) (Pollok et al., 2006) have been proposed as the basis for a unimanual auditorily paced synchronisation task. The aim of the following study was to further clarify the role and functional hemispheric asymmetry of the dPMC for movement timing. Inhibitory rTMS and pairs of TMS pulses were used as a transient functional lesion to elucidate the functional relevance and time window of dPMC action in healthy human subjects.

Inhibition of the left dPMC resulted in an increase in asynchrony and variability for either hand, while there was no significant effect of right dPMC stimulation. The time window for disturbance of the contralateral right hand was at 160ms prior to the auditory signal, while synchronization of the left hand was disturbed at 200ms prior to the pacing signal. This implies that the relevant connections between left dPMC and right primary motor cortex for movement timing are most likely mediated by an indirect pathway.

These results highlight the role of the left dPMC in movement timing which might be and interesting target in movement disorders.

European Journal of Neuroscience, Vol. 27, pp. 757–764, 2008 doi:10.1111/j.1460-9568.2008.06044.x

The effect of rTMS over left and right dorsolateral premotor cortex on movement timing of either hand

Bettina Pollok,[1] Holger Rothkegel,[2] Alfons Schnitzler,[1,3] Walter Paulus[2] and Nicolas Lang[2,4]
[1]Department of Neurology, Heinrich-Heine University, Duesseldorf, Germany
[2]Department of Clinical Neurophysiology, Georg-August University Goettingen, Germany
[3]Wolfson Centre for Clinical and Cognitive Neuroscience, School of Psychology, University of Wales, Bangor, UK
[4]Department of Neurology, Christian-Albrechts University Kiel, Germany

Keywords: dPMC, interaction, synchronization, tapping

Abstract

It has been suggested that the left dorsolateral premotor cortex (dPMC) controls timing abilities of either hand. To further clarify its functional significance for movement timing, low-frequency repetitive transcranial magnetic stimulation (rTMS) was applied over the left and right dPMC, respectively, while subjects performed an auditorily paced finger-tapping task with each hand. rTMS over the left dPMC decreased tapping accuracy of both hands, whereas no behavioural effects occurred following right dPMC stimulation. To elucidate the time window in which left dPMC TMS disturbs synchronization abilities, pairs of TMS pulses were applied over the left dPMC and the left anterior parietal cortex serving as control condition. TMS pulses were applied randomly at 40 ms, 80 ms, 120 ms, 160 ms, 200 ms and 240 ms before pacer onset, as taps precede the pacing signal for about 20–60 ms. Again, the analysis revealed that TMS over the left dPMC disturbed synchronization abilities of either hand; however, this effect was shown at different times suggesting that the left dPMC affects the right M1 via at least one additional relay station. The present data support the hypothesis that the left dPMC is crucial for accurate timing of either hand. Additionally, they reveal a piece of evidence that the left dPMC affects the left hand not via a direct left dPMC–right M1 connection.

Introduction

A fundamental prerequisite for exact timing abilities is the temporally precise interaction between spatially distributed brain areas comprising cortical as well as subcortical structures (for an overview, see Wing, 2002). A recent study suggests that a unimanual synchronization task, which requires subjects to press a button in synchrony with a regular auditory pacing signal, is associated with functional interaction in a cerebello-thalamo-cortical network (Pollok *et al*., 2005). Furthermore, a specific significance of the left dorsolateral premotor cortex (dPMC) for movement timing has been evidenced, showing functional interaction between the left dPMC and bilateral primary sensorimotor cortices (S1/M1). In contrast, the right dPMC was shown to be functionally connected with ipsilateral S1/M1 only (Pollok *et al*., 2006). These data suggest that the left dPMC modulates neural activity in bilateral S1/M1, at least, in tasks that require precise timing abilities. The specific functional relevance of this interaction pattern for synchronization tasks, however, has yet to be solved.

One possibility to elucidate the functional significance of a certain brain area for a specific task is to transiently affect its function by transcranial magnetic stimulation (TMS; for reviews, see Pascual-Leone *et al*., 2000; Sack & Linden, 2003; O'Shea & Walsh, 2007). Thus, TMS provides the possibility to transiently and non-invasively modulate neural activity in focal brain regions. TMS can be applied as single- or paired-pulses, or repetitively at different frequencies. Whereas single- and paired-pulses depolarize cortical neurons for the stimulation period, repetitive TMS (rTMS) at intensities below motor threshold modifies the excitability of focal brain areas, which outlasts the stimulation period (reviewed in Anninos *et al*., 2006; Pascual-Leone *et al*., 2000; Kobayashi & Pascual-Leone, 2003). Under most conditions, low-frequency rTMS at 1 Hz results in reduced cortical excitability (for reviews, see George *et al*., 2003; Kobayashi & Pascual-Leone, 2003). Consequently, low-frequency rTMS reveals the possibility to affect circuitries relevant for the execution of a specific task and, therefore, allows establishing the causal role of a given cortical region by directly investigating the relation between behaviour and brain function. In addition, single- or paired-pulse TMS allows to trace the time course of this contribution to behaviour (for an overview, see Pascual-Leone *et al*., 2000).

Data from our previous study (Pollok *et al*., 2006) imply that the left dPMC controls both hands in tasks that require precise timing abilities. Additionally, these data suggest that the left dPMC controls both hands via direct left dPMC–M1 connection. Thus, we hypothesized: (i) that rTMS over the left dPMC affects timing abilities of both hands. Because temporal processing, at least timing of sequential movements, has been primarily related to the left hemisphere, we further hypothesized: (ii) that rTMS over the right dPMC should have no behavioural effects. Additionally, our previous data suggest that the left dPMC affects the left hand performance via a direct left dPMC–right M1 connection. We therefore hypothesized that: (iii) performance of both hands should be affected by TMS in comparable time windows. Because data from a pilot study revealed that single-pulse

Correspondence: Dr B. Pollok, as above.
E-mail: bettina.pollok@uni-duesseldorf.de

Received 17 September 2007, revised 15 November 2007, accepted 11 December 2007

TMS is not effective in disturbing synchronization abilities, we applied paired-pulse TMS in order to boost the stimulation effects.

Materials and methods

Subjects and paradigm

Twelve healthy right-handed subjects participated in each experiment. They gave their written informed consent prior to the study and were naïve with regard to its exact purpose. All applied TMS parameters are in accordance with general safety guidelines (Wassermann, 1998). The study was approved by the ethics committee of the medical faculty of the Georg-August University, Göttingen, and is in accordance with the declaration of Helsinki.

Subjects performed a unimanual synchronization task. To this end, they pressed the space bar of a computer keyboard with respect to a regular auditory pacing signal with the left and the right index finger, respectively. The onset of space bar presses was determined using eprime (http://www.pstnet.com). The pacing signal was presented binaurally with a constant interstimulus interval of 800 ms and with a duration of 10 ms. Handedness was assessed using the Edinburgh inventory (Oldfield, 1971). Subjects were comfortably seated in a reclining chair. During rTMS they were instructed to relax and to keep their eyes open. During the tapping task, subjects closed their eyes to avoid visual feedback. A short training period of about 10 finger-taps preceded both experiments, respectively.

Behavioural data were analysed with respect to two measures: (i) the asynchrony, which is defined as the temporal distance between tap onset and pacer onset; and (ii) the inter-tap variability. Usually, subjects show a negative asynchrony, indicating that the tap leads over the pacing signal for about 20–60 ms.

TMS procedure

TMS was administered using a Magstim standard figure-of-eight coil with an outer winding diameter of 70 mm connected to a Magstim Rapid^2 stimulator (Magstim Company, Dyfed, Wales, UK) and placed tangentially to the scalp. The handle pointed backwards and laterally at 45° away from the midline, inducing an initial posterior–anterior current flow in the brain. The magnetic stimulus had a biphasic waveform with a pulse width of about 300 μs.

All stimulation areas of interest were localized with reference to the primary motor cortex. To this end, surface electromyography (EMG) of the first dorsal interosseus (FDI) muscle of the contralateral hand was recorded. We first localized the optimal cortical representation of the FDI by eliciting motor-evoked potentials (MEP; for an overview, see Kobayashi & Pascual-Leone, 2003). By moving the coil in 0.5-cm steps anterior, posterior, medial and lateral to this area, the exact localization of the point that invoked the maximum motor response of the FDI muscle was determined as the motor hot spot and marked with a skin pen on the scalp. EMG was only recorded during localization of the M1 hand area and determination of motor thresholds.

PMC was localized 2.5 cm anterior to the motor hot spot. This procedure is in accordance with previous studies (e.g. Schluter *et al.*, 1998, 1999; Munchau *et al.*, 2002; Schlaghecken *et al.*, 2003; Mochizuki *et al.*, 2004, 2005), and agrees well with data from functional imaging studies indicating that the dPMC is located about 20 mm anterior to the M1 hand area (Fink *et al.*, 1997; Picard & Strick, 2001). In addition, in the second experiment we localized the anterior part of the posterior parietal cortex (APC) 2.5 cm posterior to the motor hot spot most likely corresponding to the primary somatosensory cortex.

Experiment 1

Twelve healthy right-handed volunteers (six males) participated in the study. The mean age was 29.2 ± 2.1 years (mean ± SEM), and the overall age ranged between 21 and 43 years. We applied rTMS over the left and right dPMC in separate runs at 1 Hz for 20 min, resulting in 1200 TMS pulses, respectively. Stimulation intensity was set to 90% of the individual active motor threshold (AMT). AMT is defined as the intensity needed to evoke MEPs in the tonically contracted FDI muscle of about 200 μV in five of 10 consecutive trials. This intensity has been shown to induce a decrease of cortico-spinal excitability, which outlasts the stimulation for several minutes when applied as rTMS over the dPMC (Gerschlager *et al.*, 2001). Subjects performed the finger-tapping task with each hand in consecutive runs before and immediately after rTMS. In each experimental condition subjects performed 50 auditorily cued finger-taps. Thus, each run lasted for about 40 s. To avoid carry-over effects of the magnetic stimulation, the second rTMS session was performed 48 h after the first one. All experimental conditions were pseudorandomized and counterbalanced across subjects.

Experiment 2

The second experiment was performed to determine the time window in which the left dPMC affects synchronization abilities of the left and right hand, respectively. To this end, pairs of TMS (ppTMS) pulses with an interstimulus interval of 20 ms were applied while subjects performed the finger-tapping task with both hands, each. Again 12 subjects (seven males) participated in the experiment. Nine of them were investigated in the first study. The mean age was 29.4 (± 1.7; range: 23–43) years.

Because data from the first experiment showed that merely left dPMC stimulation affects synchronization accuracy, TMS was applied to the left hemisphere, only. In addition to dPMC stimulation, in a separate run ppTMS were applied over the APC serving as control condition. In each subject stimulation over the APC and the dPMC was applied sequentially in consecutive runs on 1 day. Experimental runs were pseudorandomized and counterbalanced across subjects. Stimulation intensities were set to 90% of the individual resting motor threshold (RMT). RMT is defined as the intensity needed to evoke MEPs in the relaxed FDI muscle of about 50 μV in five of 10 consecutive trials. This intensity was chosen because it has been shown that single-pulse stimulation with 90% RMT over the dPMC inhibits MEPs of ipsilateral hand muscles (Mochizuki *et al.*, 2004). Thus, we expected stimulation at 90% RMT to activate inhibitory pathways between the dPMC and the contralateral primary motor cortex. Because in two pilot data a selective effect of single TMS pulses did not occur, we administered paired-pulses to increase the effect of stimulation without increasing the stimulation intensity (Mochizuki *et al.*, 2005). We chose this procedure because: (i) it is likely that higher stimulation intensities lead to spread towards the adjacent motor cortex; and (ii) higher stimulation intensities are associated with stronger tactile sensations, which may induce unspecific behavioural effects.

Due to the fact that subjects usually show a negative asynchrony (i.e. the taps precede the pacing signal for about 20–60 ms), TMS pulses were applied 40 ms, 80 ms, 120 ms, 160 ms, 200 ms or 240 ms before the onset of every third pacing signal. The temporal distance between pacing signal and TMS pulses was randomized (Fig. 1).

Hereby, TMS pulses were predictable, but the temporal distance between pacer and TMS was unpredictable. This procedure was

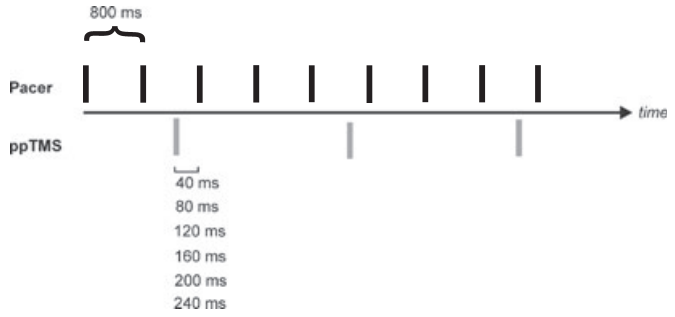


FIG. 1. Summary of the experimental procedure used in the second experiment. The pacing signal was presented with a constant interstimulus interval of 800 ms. With respect to the onset of each third pacing signal, pairs of transcranial magnetic stimulation (ppTMS) were applied with varying intervals (i.e. 40 ms, 80 ms, 120 ms, 160 ms, 200 ms or 240 ms). The temporal distance between ppTMS onset and onset of the pacing signal was randomized across subjects and across trials.

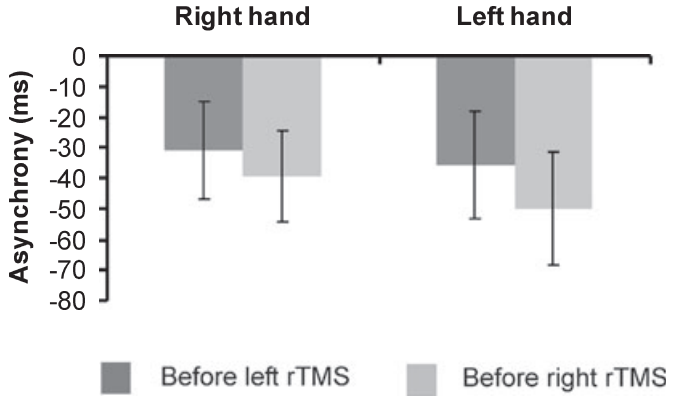


FIG. 2. Mean negative asynchrony values immediately before left and right repetitive transcranial magnetic stimulation (rTMS). Error bars indicate standard error of mean.

chosen because pilot data demonstrate that the unpredictable presentation of TMS pulses disturbed synchronization abilities in a temporally and spatially unspecific way (i.e. synchronization accuracy was declined in all time windows and following stimulation over the dPMC as well as over the APC).

Each experimental run started with a baseline measurement, in which the coil was held over the respective stimulation site without applying TMS pulses. Following 20 taps without stimulation, ppTMS was applied. For each stimulation interval subjects performed 20 taps. Additionally, between stimulation 240 taps without ppTMS were performed. All in all, each experimental run consisted of 380 taps.

TMS pulses produce additional tactile and auditory cues, which may facilitate simple reaction times (Terao *et al*., 1997). To make subjects familiar with the procedure, a first training condition was conducted in which subjects performed the finger-tapping task with the right hand while TMS pulses were applied about 10 cm behind each subject's head. Hereby, effects of the auditory cue were expected, which tally with that during real TMS stimulation. This test condition was performed in each subject before TMS was applied over the cortex.

Results

Experiment 1

Analysis of the handedness inventory revealed quotients that ranged between 95 and 100, indicating that all subjects were strictly right-handed. The mean AMT was 52.0 ± 3.1% max. stimulator output for stimulation of the right hemisphere and 46.0 ± 1.6% max. stimulator output for stimulation of the left hemisphere. Although this difference was not significant ($t_{11} = 1.9$; $P = 0.08$), stimulation of the right hemisphere required higher stimulation intensities as compared with the left side.

Analysis of asynchrony values revealed a negative asynchrony (i.e. taps preceding the pacing signal) in all experimental conditions. In a first step, we compared synchronization abilities preceding each rTMS session. Analysis using a two-way analysis of variance (ANOVA) with factors session (before left rTMS vs before right rTMS) and hand (left vs right) revealed a slight but not significant increase of the negative asynchrony preceding right dPMC stimulation ($P > 0.2$). The mean values are depicted in Fig. 2.

Because we were interested in effects due to rTMS, we analysed data with respect to the baseline values immediately preceding each rTMS session.

Statistical analysis using a three-way ANOVA with factors hand (left vs right), stimulation (rTMS vs pre-rTMS) and location (left dPMC vs right dPMC) revealed a significant main effect of stimulation ($F_{1,11} = 9.9$; $P = 0.01$), and a significant interaction between stimulation and location ($F_{1,11} = 10.2$; $P = 0.01$). *Post hoc* analysis using Scheffé test revealed that rTMS over the left dPMC significantly increased the negative asynchrony of each hand, whereas rTMS over the right dPMC did not result in significant behavioural changes (Fig. 3).

Inter-tap variability was analysed by calculating relative changes following rTMS. To this end, pre-rTMS values were set to 100%, and the individual changes in each subject following rTMS were calculated with respect to these baseline values. Analysis revealed a significant increase of variability following left dPMC stimulation ($F_{1,11} = 5.5$; $P = 0.04$). No significant effects were observed following right dPMC stimulation ($P > 0.3$). Figure 4 summarizes relative changes of inter-tap variability of both hands during all experimental conditions.

Finally, we investigated the mean inter-tap interval in all conditions to estimate whether rTMS affects the subjects' tapping speed. Analysis using a three-way ANOVA with factors hand (left vs right), stimulation (rTMS vs pre-rTMS) and location (left dPMC vs right dPMC) revealed neither significant main effects nor interaction ($P > 0.1$).

Experiment 2

Values of the Oldfield inventory ranged between 95 and 100. The mean RMT was 52.4 ± 1.7% max. stimulator output. To estimate whether the dPMC affects synchronization abilities in a specific time window, paired *t*-tests were performed. To this end, we compared synchronization accuracy during ppTMS with those during the respective baseline condition. Right-hand asynchrony was increased by ppTMS over the dPMC at about 160 ms before onset of the pacing signal, corresponding to 50 ms before onset of the finger-tap ($t_{11,\ \text{one-tailed}} = 1.8$; $P = 0.05$). Contrary, left-hand asynchrony was increased when ppTMS was applied at about 200 ms prior to the pacing signal (i.e. 90 ms prior to the tap; $t_{11,\ \text{one-tailed}} = 2.0$; $P = 0.04$). Figure 5 summarizes the effects of ppTMS on synchronization abilities.

Discussion

Recent data imply that in sensorimotor synchronization tasks the left dPMC controls movement timing of either hand (Pollok *et al*., 2006).

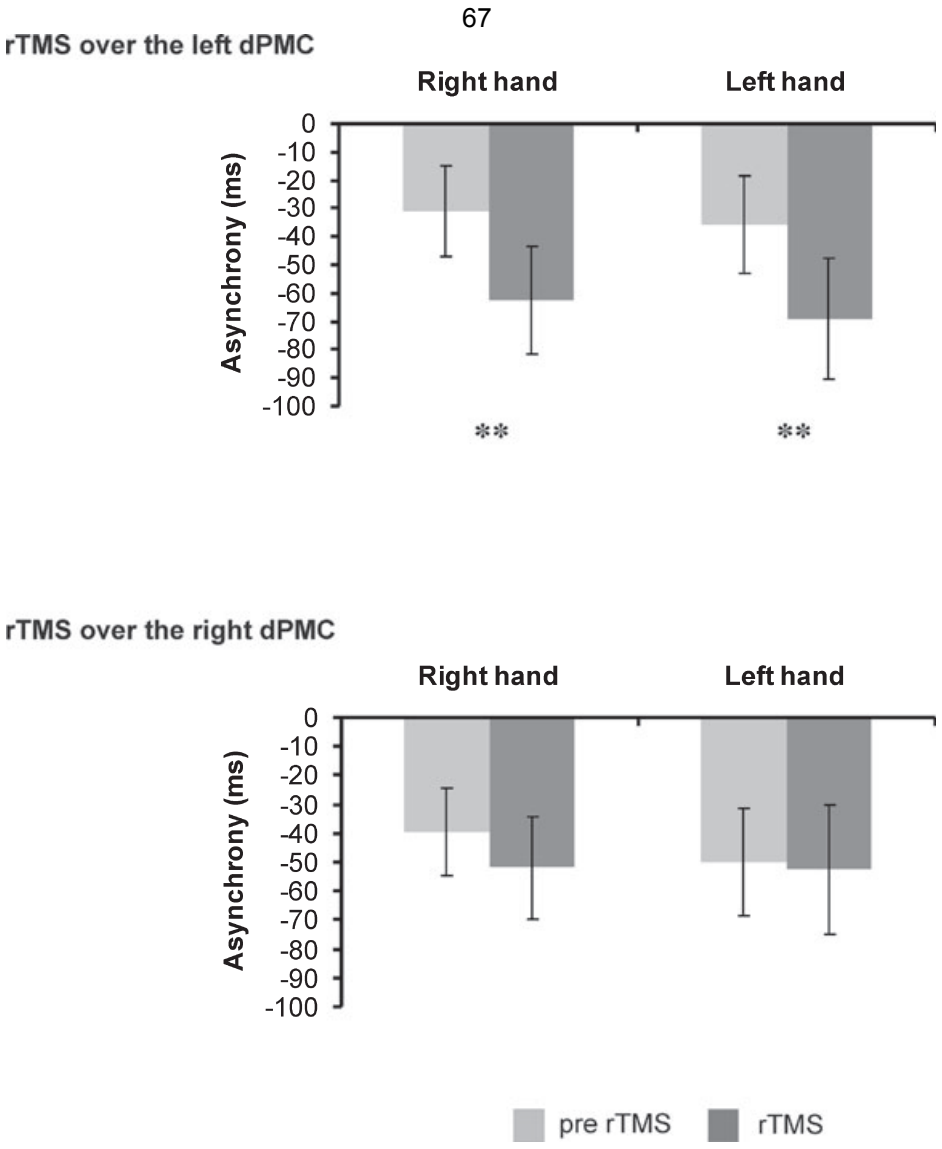


FIG. 3. Effects of repetitive transcranial magnetic stimulation (rTMS) over the left and right dorsolateral premotor cortex (dPMC). Asynchrony values following stimulation were compared with baseline values immediately before rTMS. Please note that rTMS over the left dPMC increased asynchrony values of both hands, while no effect was observed following right dPMC stimulation. $^{**}P \leq 0.01$.

Additionally, these data suggest that dPMC might affect the ipsilateral hand via a direct left dPMC–right M1 connection. The present study aimed at elucidating the significance of this interaction pattern for movement timing. Our data suggest that explicit timing of both hands is premised on unimpaired left dPMC function. However, the time course of functional interaction between the left dPMC and bilateral M1 reveals a piece of evidence for the assumption that dPMC controls the ipsilateral hand not via a direct left dPMC–right M1 connection.

Behavioural effects of rTMS

In all conditions we found the tap preceding the pacing signal. This so-called negative asynchrony is a well-established phenomenon demonstrated in a variety of behavioural studies (reviewed in Repp, 2005). We found the negative asynchrony of both hands to be increased following rTMS of the left but not the right dPMC. Because stimulation intensity of rTMS over the right hemisphere was stronger as compared with left dPMC stimulation, this result cannot be explained by ineffective stimulation of the right hemisphere.

Interestingly, a previous study did not show an effect of rTMS over left PMC for such synchronization tasks (Doumas *et al.*, 2005). In this study, rTMS was applied over the left hemisphere with stimulation intensities of 90% RMT, whereas in the present study stimulation intensity was set to 90% AMT. Although this discrepancy is not yet clear, one might speculate that the behavioural effects of rTMS might vary with stimulation intensity. However, results from our second experiment weaken this assumption, as ppTMS at 90% RMT results in an increase of the negative asynchrony as well.

We further investigated whether rTMS affects the subjects' ability to perform a movement with a certain frequency. Analysis revealed that despite rTMS, subjects performed their finger-taps with the requested mean interval of 800 ms. However, inter-tap variability of both hands increased following left rTMS. Thus, rTMS over the left dPMC affects the subjects' ability to keep in time with a specific event but not to perform a movement at a certain frequency. These results

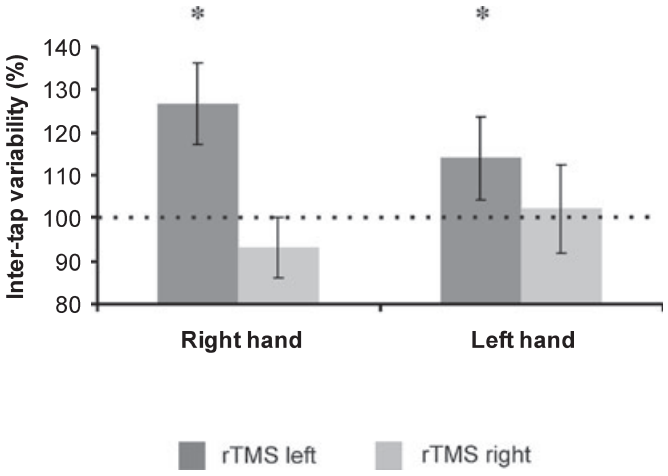


FIG. 4. Effects of repetitive transcranial magnetic stimulation (rTMS) on relative changes of inter-tap variability. The dotted line indicates prestimulation values, which were set to 100%. Relative changes with respect to baseline values are demonstrated. Error bars depict standard error of mean. Only rTMS over the left dPMC increased behavioural variability. *$P \leq 0.05$.

pose the question for the putative functional significance of the left dPMC for movement timing.

Functional significance of the left dPMC for motor control

It is well established that in right-handed subjects the left hemisphere is crucial for motor control, particularly for sequence production (for overviews, see Harrington & Haaland, 1992; Haaland & Harrington, 1996; Serrien *et al*., 2006).

In several studies, TMS stimulation over the dPMC has been used to investigate its effects on reaction times (Schluter *et al*., 1998; Schlaghecken *et al*., 2003; Mochizuki *et al*., 2005). These studies show that left dPMC stimulation delayed reaction times of the contralateral right hand. The effects on ipsilateral left-hand performance, however, were contradictory. Whereas Schluter *et al*. (1998) found the left-hand reaction times to be delayed, other studies did not replicate these findings (Schlaghecken *et al*., 2003; Mochizuki *et al*., 2005). All in all, these data indicate that left dPMC stimulation affects reaction times in complex choice reaction tasks that require response selection, but not in simple reaction tasks, an interpretation corroborated by a recent TMS study (Koch *et al*., 2006), showing that the left

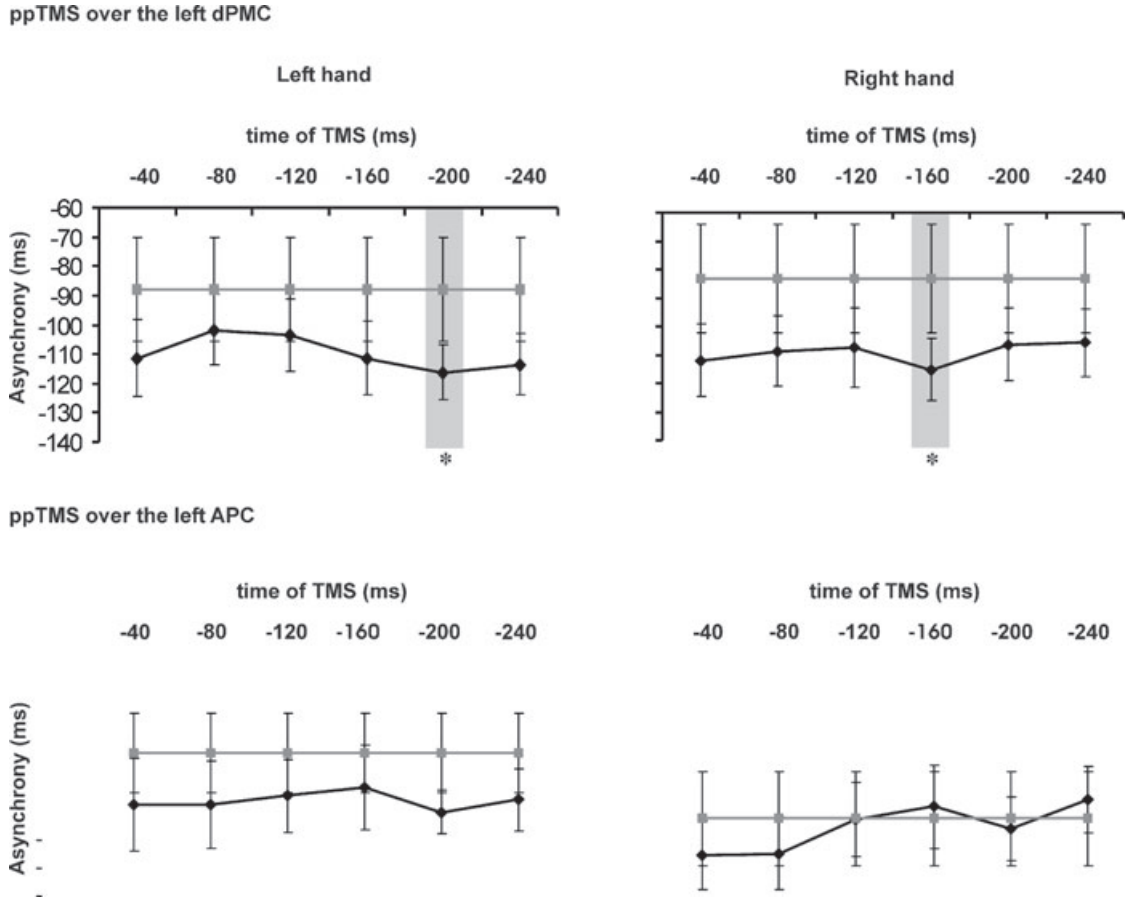


FIG. 5. Effects of pairs of transcranial magnetic stimulation (ppTMS) over the left dorsolateral premotor cortex (dPMC) and the left anterior parietal cortex (APC) administered at different time windows on asynchrony values. The grey line indicates the mean negative asynchrony during the baseline condition preceding each stimulation. Whereas no significant effects of APC stimulation were evident, TMS over the dPMC affects both hands in different time windows (grey bars). Error bars indicate standard error of mean. Please note that TMS pulses were applied with respect to the pacing signal. Therefore, left-hand performance was disturbed when the dPMC was stimulated at about 90 ms prior to tap onset, whereas the right hand was affected following TMS at about 50 ms before tap onset. *$P \leq 0.05$.

dPMC may facilitate movements of the ipsilateral hand. Although these data suggest that the left dPMC controls both hands, this function does not seem to be exclusively related to the left hemisphere as right dPMC stimulation delayed complex reactions of the contralateral left hand (Schluter *et al*., 1998). In contrast, the data of the present study support the hypothesis that at least in tasks that require a movement with respect to a certain external event the left hemisphere, in particular the left dPMC, is crucial. Although a left hemispheric dominance for precise timing abilities is well established (for review, see Serrien *et al*., 2006), it is the cerebellum that has been related to such event timing in the subsecond range (for reviews, see Ivry *et al*., 2002; Ivry & Spencer, 2004). Because it is well established that the cerebellum is closely connected to the cerebral cortex via a cerebello-thalamo-cortical loop (for review, see Horne & Butler, 1995), one might speculate that the cerebellum indicates the point in time when a specific event occurs, whereas the left dPMC is crucial for the implementation of a movement at this time. The hypothesis of a functionally relevant functional connectivity between the cerebellum and dPMC is supported by a recent TMS study (Del Olmo *et al*., 2007). But, these results do not necessarily mean that left dPMC is directly related to movement timing. Alternatively, it has been evidenced that dPMC has a specific meaning for movement preparation (Churchland & Shenoy, 2007). In this study, PMC microstimulation in macaque monkeys increased reaction times when applied around the go-cue, whereas stimulation of the primary motor cortex did not result in reaction time changes. Compiling these results and those from the present study, the effect observed might be due to a disturbance of preparatory activity within the dPMC.

However, this would not necessarily explain why the negative asynchrony increased following rTMS. Presently, we do not have a conclusive answer on this question. One highly speculative hypothesis might be that a possible function of dPMC is not only to initiate but also to inhibit a movement. Thus, rTMS might have disturbed this putative inhibition, resulting in an increase of the negative asynchrony. It should be stressed that this interpretation is highly speculative and needs to be investigated directly.

Effects of sites remote from the dPMC

Electroencephalographic recordings suggest a rapid spread of activation following TMS (Ilmoniemi *et al*., 1997). Combination of TMS with positron emission tomography and functional magnetic resonance imaging indicates that cerebral blood flow changes in regions that are anatomically connected to the target region (Paus *et al*., 1997; Bestmann *et al*., 2004). Further evidence for the hypothesis that low-frequency rTMS results in activation changes in connected brain areas comes from several TMS studies (Wassermann *et al*., 1998; Gerschlager *et al*., 2001; Munchau *et al*., 2002; Baumer *et al*., 2006). Thus, one might argue that present data are due to: (i) a widespread stimulation rather than to local stimulation of the dPMC; or to (ii) changes of cortico-cortical or cortico-spinal connections. The relatively low stimulation intensity of 90% AMT weakens the former interpretation (Ilmoniemi *et al*., 1997; Nahas *et al*., 2001; Baumer *et al*., 2003). It is well known that low-frequency rTMS over the primary motor cortex affects the excitability of motor cortical neurons (Lang *et al*., 2006) without changes of basic motor behaviour, as determined by maximum tapping speed (Chen *et al*., 1997) or muscle force and movement acceleration of the thumb (Muellbacher *et al*., 2000), although in one study a slowing of fastest tapping was observed (Jancke *et al*., 2004), which, however, was not required in the present study. Therefore, it is unlikely that the observed disturbance of synchronization abilities results from activation changes within M1. Particularly, because in the studies of Chen *et al*. (1997), and of Muellbacher *et al*., (2000), TMS over M1 was applied with suprathreshold stimulation. Compiling these data, the effect observed in the present study is most likely due to alterations of the functional interplay between the left dPMC and the primary motor cortices.

Time course of stimulation effects

To further elucidate the pattern of functional interaction between the left dPMC and bilateral M1, ppTMS was applied over the left dPMC and over the left APC. APC stimulation did not affect synchronization abilities. This result is in line with a previous study showing that conditioning rTMS over the APC did not change the excitability of the ipsilateral motor cortex (Gerschlager *et al*., 2001). Thus, the effect of dPMC stimulation was again shown to be spatially specific.

Although there is growing evidence that the left dPMC exerts dominance over the right hemisphere, the underlying mechanism remains unclear. The data from our previous EMG study (Pollok *et al*., 2006) imply a direct functional interaction between the left dPMC and bilateral S1/M1. Anatomically, transcallosal connections between the left dPMC and the right M1 have been evidenced in animal studies (Rouiller *et al*., 1994; Marconi *et al*., 2003). From the first experiment, we can not rule out the possibility that: (i) the left dPMC affects the contralateral hand indirectly via subcortical structures, possibly the thalamus; or (ii) via other cortical areas like the contralateral dPMC or the ipsilateral M1. Alternatively: (iii) direct cortico-spinal projections originating in dPMC might contribute to the effect observed. The latter opportunity is unlikely, as it has been shown that the electrical stimulation threshold of cortico-spinal projection of the PMC is higher than those originating in M1 (Cerri *et al*., 2003).

Previous studies tracking the time course of interhemispheric facilitation and inhibition suggest a direct pathway between the left dPMC and the right M1 (Mochizuki *et al*., 2004; Baumer *et al*., 2006). However, it has yet to be solved whether this connection is behaviourally relevant. Data from the present study suggest that ppTMS over the left dPMC affects synchronization abilities of both hands in different time windows. Whereas performance of the ipsilateral left hand is disturbed by ppTMS at 200 ms before onset of the pacing signal (i.e. about 90 ms prior to the tap), synchronization of the right hand is altered when TMS is applied at 160 ms prior to the pacing signal (i.e. 50 ms before tap onset). Thus, this effect is possibly not due to direct connections between the left dPMC and the right M1. Rather, it is more likely that the dPMC affects the left hand via an indirect pathway running via the left M1 or the right dPMC or via a subcortical locus.

Interestingly, the negative asynchrony during baseline was increased in the second as compared with the first experiment. This result indicates that the subjects' performance was disturbed not only by TMS pulses but also by the procedure itself without any stimulation. Thus, one might speculate that the small effects observed in the second experiment might be due to a simple ceiling effect. All in all, we realize that the effect observed in the second experiment is weak, but we would like to stress that the present data should only be seen as first evidence against the idea that the left hand is controlled via a direct left dPMC–right M1 connection.

Conclusion

The present data suggest a spatially and temporally specific effect of the left dPMC on event timing. Our data support the hypothesis that

the left dPMC modulates neural activity in bilateral primary motor cortices, which is behaviourally relevant. Moreover, the present data reveal first evidence against the assumption that the effect of the left dPMC on right-hand performance is due to a direct connection between left dPMC and right M1.

Acknowledgements

Bettina Pollok was supported by the Deutsche Forschungsgemeinschaft (PO 806/2-2) and by two grants from the medical department of the Heinrich Heine University (977275; 9772328). AS was supported by the VolkswagenStiftung (I/80191).

Abbreviations

AMT, active motor threshold; APC, anterior part of the posterior parietal cortex; dPMC, dorsolateral premotor cortex; EMG, electromyography; FDI, first dorsal interosseus; MEP, motor-evoked potentials; ppTMS, pairs of TMS; RMT, resting motor threshold; rTMS, repetitive transcranial magnetic stimulation; TMS, transcranial magnetic stimulation.

References

Anninos, P., Kotini, A., Tamiolakis, D. & Tsagas, N. (2006) Transcranial magnetic stimulation. A case report and review of the literature. *Acta Neurol. Belg.*, **106**, 26–30.

Baumer, T., Bock, F., Koch, G., Lange, R., Rothwell, J.C., Siebner, H.R. & Munchau, A. (2006) Magnetic stimulation of human premotor or motor cortex produces interhemispheric facilitation through distinct pathways. *J. Physiol.*, **572**, 857–868.

Baumer, T., Rothwell, J. & Munchau, A. (2003) Functional connectivity of the human premotor and motor cortex explored with TMS. *Clin. Neurophysiol. Suppl.*, **56**, 160–169.

Bestmann, S., Baudewig, J., Siebner, H.R., Rothwell, J.C. & Frahm, J. (2004) Functional MRI of the immediate impact of transcranial magnetic stimulation on cortical and subcortical motor circuits. *Eur. J. Neurosci.*, **19**, 1950–1962.

Cerri, G., Shimazu, H., Maier, M.A. & Lemon, R.N. (2003) Facilitation from ventral premotor cortex of primary motor cortex outputs to macaque hand muscles. *J. Neurophysiol.*, **90**, 832–842.

Chen, R., Classen, J., Gerloff, C., Celnik, P., Wassermann, E.M., Hallett, M. & Cohen, L.G. (1997) Depression of motor cortex excitability by low-frequency transcranial magnetic stimulation. *Neurology*, **48**, 1398–1403.

Churchland, M.M. & Shenoy, K.V. (2007) Delay of movement caused by disruption of cortical preparatory activity. *J. Neurophysiol.*, **97**, 348–359.

Del Olmo, M.F., Cheeran, B., Koch, G. & Rothwell, J.C. (2007) Role of the cerebellum in externally paced rhythmic finger movements. *J. Neurophysiol.*, **98**, 145–152.

Doumas, M., Praamstra, P. & Wing, A.M. (2005) Low frequency rTMS effects on sensorimotor synchronization. *Exp. Brain Res.*, **167**, 238–245.

Fink, G.R., Frackowiak, R.S., Pietrzyk, U. & Passingham, R.E. (1997) Multiple nonprimary motor areas in the human cortex. *J. Neurophysiol.*, **77**, 2164–2174.

George, M.S., Nahas, Z., Lisanby, S.H., Schlaepfer, T., Kozel, F.A. & Greenberg, B.D. (2003) Transcranial magnetic stimulation. *Neurosurg. Clin. N. Am.*, **14**, 283–301.

Gerschlager, W., Siebner, H.R. & Rothwell, J.C. (2001) Decreased corticospinal excitability after subthreshold 1 Hz rTMS over lateral premotor cortex. *Neurology*, **57**, 449–455.

Haaland, K.Y. & Harrington, D.L. (1996) Hemispheric asymmetry of movement. *Curr. Opin. Neurobiol.*, **6**, 796–800.

Harrington, D.L. & Haaland, K.Y. (1992) Motor sequencing with left hemisphere damage. Are some cognitive deficits specific to limb apraxia? *Brain*, **115**, 857–874.

Horne, M.K. & Butler, E.G. (1995) The role of the cerebello-thalamo-cortical pathway in skilled movement. *Prog. Neurobiol.*, **46**, 199–213.

Ilmoniemi, R.J., Virtanen, J., Ruohonen, J., Karhu, J., Aronen, H.J., Naatanen, R. & Katila, T. (1997) Neuronal responses to magnetic stimulation reveal cortical reactivity and connectivity. *Neuroreport*, **8**, 3537–3540.

Ivry, R.B. & Spencer, R.M. (2004) The neural representation of time. *Curr. Opin. Neurobiol.*, **14**, 225–232.

Ivry, R.B., Spencer, R.M., Zelaznik, H.N. & Diedrichsen, J. (2002) The cerebellum and event timing. *Ann. N. Y. Acad. Sci.*, **978**, 302–317.

Jancke, L., Steinmetz, H., Benilow, S. & Ziemann, U. (2004) Slowing fastest finger movements of the dominant hand with low-frequency rTMS of the hand area of the primary motor cortex. *Exp. Brain Res.*, **155**, 196–203.

Kobayashi, M. & Pascual-Leone, A. (2003) Transcranial magnetic stimulation in neurology. *Lancet Neurol.*, **2**, 145–156.

Koch, G., Franca, M., Del Olmo, M.F., Cheeran, B., Milton, R., Alvarez Sauco, M. & Rothwell, J.C. (2006) Time course of functional connectivity between dorsal premotor and contralateral motor cortex during movement selection. *J. Neurosci.*, **26**, 7452–7459.

Lang, N., Harms, J., Weyh, T., Lemon, R.N., Paulus, W., Rothwell, J.C. & Siebner, H.R. (2006) Stimulus intensity and coil characteristics influence the efficacy of rTMS to suppress cortical excitability. *Clin. Neurophysiol.*, **117**, 2292–2301.

Marconi, B., Genovesio, A., Giannetti, S., Molinari, M. & Caminiti, R. (2003) Callosal connections of dorso-lateral premotor cortex. *Eur. J. Neurosci.*, **18**, 775–788.

Mochizuki, H., Franca, M., Huang, Y.Z. & Rothwell, J.C. (2005) The role of dorsal premotor area in reaction task: comparing the 'virtual lesion' effect of paired pulse or theta burst transcranial magnetic stimulation. *Exp. Brain Res.*, **167**, 414–421.

Mochizuki, H., Huang, Y.Z. & Rothwell, J.C. (2004) Interhemispheric interaction between human dorsal premotor and contralateral primary motor cortex. *J. Physiol.*, **561**, 331–338.

Muellbacher, W., Ziemann, U., Boroojerdi, B. & Hallett, M. (2000) Effects of low-frequency transcranial magnetic stimulation on motor excitability and basic motor behavior. *Clin. Neurophysiol.*, **111**, 1002–1007.

Munchau, A., Bloem, B.R., Irlbacher, K., Trimble, M.R. & Rothwell, J.C. (2002) Functional connectivity of human premotor and motor cortex explored with repetitive transcranial magnetic stimulation. *J. Neurosci.*, **22**, 554–561.

Nahas, Z., Lomarev, M., Roberts, D.R., Shastri, A., Lorberbaum, J.P., Teneback, C., McConnel, K., Vincent, D.J., Li, X., George, M.S. & Bohning, D.E. (2001) Unilateral left prefrontal transcranial magnetic stimulation (TMS) produces intensity-dependent bilateral effects as measured by interleaved BOLD fMRI. *Biol. Psychiatry*, **50**, 712–720.

O'Shea, J. & Walsh, V. (2007) Transcranial magnetic stimulation. *Curr. Biol.*, **17**, R196–R199.

Oldfield, R.C. (1971) The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia*, **9**, 97–113.

Pascual-Leone, A., Walsh, V. & Rothwell, J. (2000) Transcranial magnetic stimulation in cognitive neuroscience – virtual lesion, chronometry, and functional connectivity. *Curr. Opin. Neurobiol.*, **10**, 232–237.

Paus, T., Jech, R., Thompson, C.J., Comeau, R., Peters, T. & Evans, A.C. (1997) Transcranial magnetic stimulation during positron emission tomography: a new method for studying connectivity of the human cerebral cortex. *J. Neurosci.*, **17**, 3178–3184.

Picard, N. & Strick, P.L. (2001) Imaging the premotor areas. *Curr. Opin. Neurobiol.*, **11**, 663–672.

Pollok, B., Gross, J., Müller, K., Aschersleben, G. & Schnitzler, A. (2005) The cerebral oscillatory network associated with auditorily paced finger movements. *Neuroimage*, **24**, 646–655.

Pollok, B., Gross, J. & Schnitzler, A. (2006) Asymmetry of interhemispheric interaction in left-handed subjects. *Exp. Brain Res.*, **175**, 268–275.

Repp, B.H. (2005) Sensorimotor synchronization: a review of the tapping literature. *Psychon. Bull. Rev.*, **12**, 969–992.

Rouiller, E.M., Babalian, A., Kazennikov, O. & Moret, V., Yu, X.-H. & Wiesendanger, M. (1994) Transcallosal connections of the distal forelimb representations of the primary and supplementary motor cortical areas in macaque monkeys. *Exp. Brain Res.*, **102**, 227–243.

Sack, A.T. & Linden, D.E. (2003) Combining transcranial magnetic stimulation and functional imaging in cognitive brain research: possibilities and limitations. *Brain Res. Brain Res. Rev.*, **43**, 41–56.

Schlaghecken, F., Munchau, A., Bloem, B.R., Rothwell, J. & Eimer, M. (2003) Slow frequency repetitive transcranial magnetic stimulation affects reaction times, but not priming effects, in a masked prime task. *Clin. Neurophysiol.*, **114**, 1272–1277.

Schluter, N.D., Rushworth, M.F., Mills, K.R. & Passingham, R.E. (1999) Signal-, set-, and movement-related activity in the human premotor cortex. *Neuropsychologia*, **37**, 233–243.

Schluter, N.D., Rushworth, M.F., Passingham, R.E. & Mills, K.R. (1998) Temporary interference in human lateral premotor cortex suggests dominance for the selection of movements. A study using transcranial magnetic stimulation. *Brain*, **121**, 785–799.

Serrien, D.J., Ivry, R.B. & Swinnen, S.P. (2006) Dynamics of hemispheric specialization and integration in the context of motor control. *Nat. Rev. Neurosci.*, **7**, 160–166.

Terao, Y., Ugawa, Y., Suzuki, M., Sakai, K., Hanajima, R., Gemba-Shimizu, K. & Kanazawa, I. (1997) Shortening of simple reaction time by peripheral electrical and submotor-threshold magnetic cortical stimulation. *Exp. Brain Res.*, **115**, 541–545.

Wassermann, E.M. (1998) Risk and safety of repetitive transcranial magnetic stimulation: report and suggested guidelines from the International Workshop on the Safety of Repetitive Transcranial Magnetic Stimulation, June 5–7, 1996. *Electroencephalogr. Clin. Neurophysiol.*, **108**, 1–16.

Wassermann, E.M., Wedegaertner, F.R., Ziemann, U., George, M.S. & Chen, R. (1998) Crossed reduction of human motor cortex excitability by 1-Hz transcranial magnetic stimulation. *Neurosci. Lett.*, **250**, 141–144.

Wing, A.M. (2002) Voluntary timing and brain function: an information processing approach. *Brain Cogn.*, **48**, 7–30.

Chapter 3 – Discussion

A causal treatment of movement disorders such as PD is not possible at present. Pharmacological treatment strategies do not have a sufficient effect on all symptoms of the disease. Some drugs lose their effect in the course of the disease and are associated with adverse effects. Therefore additional non-pharmacological treatment options would be desirable. The studies included in this thesis elucidate different factors that are important for development and application of non-invasive brain stimulation protocols and contribute to the understanding of the mechanisms underlying rTMS induced after effects in healthy human subjects in order to facilitate the rational design of suitable stimulation protocols in movement disorders.

Several questions arise from the first study which was designed to compare short term effects of conventional rTMS with the newly introduced TBS protocols. The baseline assessment of motor performance in several tasks derived from standard clinical tests showed strong training effects in the PD patients ON medication. This has to be considered even in other patient studies that use repeated clinical evaluation. On the other hand these changes might not reflect pure motor learning by repeated performance of the respective tasks. An additional unspecific effect of repeated rTMS sessions cannot be excluded, even if no significant short term effect of a single rTMS session was present in this study, not even after TBS. Two recent studies (Khedr et al., 2006; Lomarev et al., 2006) indicate that repeated sessions of rTMS might be needed to achieve clinically significant improvement of motor function. This resembles the approach for rTMS in the treatment of depression (O'Reardon et al., 2007), where repeated sessions have become the most common strategy.

One of the most important points for the application of rTMS in PD might be whether the intervention should be combined with the patient's previous medication, an adapted medication or a transient interruption of drug intake. While the size of possible effects could be bigger in the OFF condition an impairment of neuroplastic processes strongly argues against a dopamine depleted state. Several studies have shown impaired practice-dependent (Meintzschel and Ziemann, 2006) or externally induced neuroplasticity (Nitsche et al., 2006) under dopamine antagonists in healthy controls or in the dopamine-depleted OFF state

in PD (Ueki et al., 2006). Accordingly training effects in study I were only present in the ON group and not in a dopamine-depleted state. In healthy subjects activation of dopamine receptors enhances inhibitory effects of rTMS (study II) or cathodal tDCS (Nitsche et al., 2006), while it turns unspecific excitability enhancement of anodal tDCS into inhibition (Kuo et al., 2008). In contrast levodopa stabilizes synapse-specific plasticity facilitation following PAS (Kuo et al., 2008). However, the use of dopaminergic drugs to enhance externally induced neuroplasticity in PD patients is limited to a degree as doses exceeding the patient's previous medication might lead to undesirable dyskinesias. For the application of rTMS in other disorders it is interesting to note that dopaminergic potentiation of rTMS induced after effect might not be effective in other cortical areas or pathological conditions such as the auditory cortex in tinnitus patients (Kleinjung et al., 2009). Beyond dopaminergic medication a frequently used drug for the treatment of PD is amantadine which acts as a NMDA receptor antagonist. The exact mechanism by which amantadine improves motor function and reduces levodopa induced dyskinesias is still a matter of debate (Paquette et al., 2008). Nevertheless, if we assume that rTMS acts via the induction of neuroplastic changes, the use of NMDA receptor antagonists might prevent these effects. Practice-dependent representational plasticity has been shown to be impaired by amantadine and memantine while there were no effects on motor learning (Ziemann et al., 2006).

In addition to neuroplastic effects in the motor cortex itself even a change in dopamine release in the striatum has to be considered in studies using rTMS in PD. Strafella and colleagues showed that rTMS over the prefrontal (Strafella et al., 2001) and primary motor cortex (Strafella et al., 2003) in healthy subjects as well as in PD patients (Strafella et al., 2005) leads to a striatal dopamine release. This effect was specific for the putamen ipsilateral to the stimulated hemisphere with a smaller amount of dopamine release from a spatially enlarged area in the more affected hemisphere in PD patients. However, an unspecific bilateral dopamine release was also found after sham rTMS (Strafella et al., 2006). These studies might imply that the main mechanism for rTMS induced improvements in motor function in PD patient is an increased dopamine release from remaining nigrostriatal projections rather than a neuroplastic effect in the primary motor

cortex. Furthermore the sham study (Strafella et al., 2006) highlights the importance of control experiments using sham stimulation.

Another important aspect for the design of studies using non-invasive brain stimulation in PD is a possible dissociation of motor function and parameters of pathologically altered excitability of the primary motor cortex. A recent study demonstrated that rTMS over the premotor cortex normalized SP without clinical effect, while DBS improved motor function without significant effect on SP (Baumer et al., 2009). L-DOPA significantly improved motor function and restored a shortened SP.

Despite these restrictions the motor cortex might still be an interesting target for symptom relief in PD. Based on experiments in an animal model it has been suggested recently that the effect of DBS on motor performance is mainly mediated by corticosubthalamic projections originating in layer V of the primary motor cortex (Gradinaru et al., 2009). However, in order to mimic the effects of DBS a constant and selective stimulation of these projections would be required which might be a mechanism underlying the effects of chronic epidural motor cortex stimulation (Priori and Lefaucheur, 2007). Alternatively it might be possible to activate these projections by neuroplastic changes induced in more specific neuronal populations. For this purpose the underlying mechanism of rTMS induced after effects need to be better understood.

In study III it could be shown that a high frequency of TMS pulses is not sufficient to induce facilitatory after effects, but a pattern of stimulation trains and breaks is required. Further studies are needed to clarify the optimal relationship between stimulation intensity, stimulation train duration and breaks. Furthermore it is not clear from this study which processes are active during the breaks. A first approach might be to combine the rTMS protocol with voluntary muscle contraction during the breaks in order to further increase excitability or utilize surround inhibition. Even changes in the configuration and duration of single TMS pulses as well as the direction of the induced electrical field have to be considered, as the subsets of neurons targeted by the stimulation differ depending on theses factors although spatially overlapping. In study III this is evident from the effects of current direction for both the rTMS protocol and single pulses for the assessment of cortical excitability. Study IV focuses on the impact of pulse duration for single TMS pulses and shows that this factor is most relevant for the

relationship between the maximum amplitude of the induced electrical field and motor threshold. For threshold adjusted measures no significant difference was found in the range of pulse durations that can be realized with commercially available systems. This is important for the comparison of studies performed with different TMS setups.

Precise motor timing requires the coordinated activation of a complex network of different cortical and subcortical areas. Therefore improvement of motor symptoms might be achieved by interactions with different targets in this network. An interesting target might be the left dPMC, which has been shown to be involved in an auditorily paced motor synchronisation task (Pollok et al., 2005) among other functions. Using a virtual lesion approach the functional relevance of the left dPMC for either hand could be demonstrated (study V). It would be interesting to expand these experiments to patient studies. It is know that movement initiation and self-paced finger tapping at maximum speed is impaired in PD. External cues can be utilized to overcome these problems. In addition, the premotor cortex can be easily targeted by rTMS and is highly connected to bilateral primary motor cortices.

In conclusion the studies included in this thesis stress the importance of clarifying the mechanisms underlying non-invasive brain stimulation. The application as a therapeutic tool in movement disorders seems promising but at present premature. Changes in cortical excitability caused by the primary pathology or adaptive processes lead to an altered susceptibility to externally induced neuroplastic changes. Therefore stimulation parameters cannot simply be copied from studies in healthy subjects but need to be adapted to the underlying pathophysiology. As the combination of Parkinsonian symptoms and the respective neurophysiological alterations might differ between patients, it might even be necessary to adjust several stimulation parameters individually.

References

Albin RL, Young AB, Penney JB. The functional anatomy of basal ganglia disorders. Trends Neurosci 1989; 12: 366-75.

Alexander GE, Crutcher MD. Functional architecture of basal ganglia circuits: neural substrates of parallel processing. Trends Neurosci 1990; 13: 266-71.

Barker AT, Garnham CW, Freeston IL. Magnetic nerve stimulation: the effect of waveform on efficiency, determination of neural membrane time constants and the measurement of stimulator output. Electroencephalogr Clin Neurophysiol Suppl 1991; 43: 227-37.

Barker AT, Jalinous R, Freeston IL. Non-invasive magnetic stimulation of human motor cortex. Lancet 1985; 1: 1106-7.

Baumer T, Hidding U, Hamel W, Buhmann C, Moll CK, Gerloff C, et al. Effects of DBS, premotor rTMS, and levodopa on motor function and silent period in advanced Parkinson's disease. Mov Disord 2009; 24: 672-6.

Bliss TV, Lomo T. Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. J Physiol 1973; 232: 331-56.

Boylan LS, Pullman SL, Lisanby SH, Spicknall KE, Sackeim HA. Repetitive transcranial magnetic stimulation to SMA worsens complex movements in Parkinson's disease. Clin Neurophysiol 2001; 112: 259-64.

Braak H, Rub U, Gai WP, Del Tredici K. Idiopathic Parkinson's disease: possible routes by which vulnerable neuronal types may be subject to neuroinvasion by an unknown pathogen. J Neural Transm 2003; 110: 517-36.

Buhmann C, Glauche V, Sturenburg HJ, Oechsner M, Weiller C, Buchel C. Pharmacologically modulated fMRI--cortical responsiveness to levodopa in drug-naive hemiparkinsonian patients. Brain 2003; 126: 451-61.

Cantello R, Gianelli M, Bettucci D, Civardi C, De Angelis MS, Mutani R. Parkinson's disease rigidity: magnetic motor evoked potentials in a small hand muscle. Neurology 1991; 41: 1449-56.

Chen R, Classen J, Gerloff C, Celnik P, Wassermann EM, Hallett M, et al. Depression of motor cortex excitability by low-frequency transcranial magnetic stimulation. Neurology 1997; 48: 1398-403.

DeFelipe J. Brain plasticity and mental processes: Cajal again. Nat Rev Neurosci 2006; 7: 811-7.

DeLong MR. Primate models of movement disorders of basal ganglia origin. Trends Neurosci 1990; 13: 281-5.

Di Lazzaro V, Oliviero A, Profice P, Saturno E, Pilato F, Insola A, et al. Comparison of descending volleys evoked by transcranial magnetic and electric stimulation in conscious humans. Electroencephalogr Clin Neurophysiol 1998; 109: 397-401.

Dick JP, Cowan JM, Day BL, Berardelli A, Kachi T, Rothwell JC, et al. The corticomotoneurone connection is normal in Parkinson's disease. Nature 1984; 310: 407-9.

Diener H, Putzki N, editors. Leitlinie für Diagnostik und Therapie in der Neurologie. Stuttgart: Thieme, 2008.

Ellaway PH, Davey NJ, Maskill DW, Dick JP. The relation between bradykinesia and excitability of the motor cortex assessed using transcranial magnetic stimulation in normal and parkinsonian subjects. Electroencephalogr Clin Neurophysiol 1995; 97: 169-78.

Feldman DE. Synaptic mechanisms for plasticity in neocortex. Annu Rev Neurosci 2009; 32: 33-55.

Ferbert A, Priori A, Rothwell JC, Day BL, Colebatch JG, Marsden CD. Interhemispheric inhibition of the human motor cortex. J Physiol 1992; 453: 525-46.

Fitzgerald PB, Fountain S, Daskalakis ZJ. A comprehensive review of the effects of rTMS on motor cortical excitability and inhibition. Clin Neurophysiol 2006; 117: 2584-96.

Ghabra MB, Hallett M, Wassermann EM. Simultaneous repetitive transcranial magnetic stimulation does not speed fine movement in PD. Neurology 1999; 52: 768-70.

Gradinaru V, Mogri M, Thompson KR, Henderson JM, Deisseroth K. Optical deconstruction of parkinsonian neural circuitry. Science 2009; 324: 354-9.

Hebb DO. The organization of behavior. A neuropsychological theory. New York: Wiley, 1949.

Helmich RC, Siebner HR, Bakker M, Munchau A, Bloem BR. Repetitive transcranial magnetic stimulation to improve mood and motor function in Parkinson's disease. J Neurol Sci 2006; 248: 84-96.

Huang YZ, Edwards MJ, Rounis E, Bhatia KP, Rothwell JC. Theta burst stimulation of the human motor cortex. Neuron 2005; 45: 201-6.

Iyer MB, Schleper N, Wassermann EM. Priming stimulation enhances the depressant effect of low-frequency repetitive transcranial magnetic stimulation. J Neurosci 2003; 23: 10867-72.

Kaneko K, Kawai S, Fuchigami Y, Morita H, Ofuji A. The effect of current direction induced by transcranial magnetic stimulation on the corticospinal excitability in human brain. Electroencephalogr Clin Neurophysiol 1996; 101: 478-82.

Khedr EM, Farweez HM, Islam H. Therapeutic effect of repetitive transcranial magnetic stimulation on motor function in Parkinson's disease patients. Eur J Neurol 2003; 10: 567-72.

Khedr EM, Rothwell JC, Shawky OA, Ahmed MA, Hamdy A. Effect of daily repetitive transcranial magnetic stimulation on motor performance in Parkinson's disease. Mov Disord 2006; 21: 1311-6.

Kleinjung T, Steffens T, Landgrebe M, Vielsmeier V, Frank E, Hajak G, et al. Levodopa does not enhance the effect of low-frequency repetitive transcranial magnetic stimulation in tinnitus treatment. Otolaryngol Head Neck Surg 2009; 140: 92-5.

Kobayashi M, Pascual-Leone A. Transcranial magnetic stimulation in neurology. Lancet Neurol 2003; 2: 145-56.

Kujirai T, Caramia MD, Rothwell JC, Day BL, Thompson PD, Ferbert A, et al. Corticocortical inhibition in human motor cortex. J Physiol 1993; 471: 501-19.

Kuo MF, Paulus W, Nitsche MA. Boosting focally-induced brain plasticity by dopamine. Cereb Cortex 2008; 18: 648-51.

Lang N, Siebner HR, Ernst D, Nitsche MA, Paulus W, Lemon RN, et al. Preconditioning with transcranial direct current stimulation sensitizes the

motor cortex to rapid-rate transcranial magnetic stimulation and controls the direction of after-effects. Biol Psychiatry 2004; 56: 634-9.

Lefaucheur JP. Motor cortex dysfunction revealed by cortical excitability studies in Parkinson's disease: influence of antiparkinsonian treatment and cortical stimulation. Clin Neurophysiol 2005; 116: 244-53.

Lefaucheur JP, Drouot X, Von Raison F, Menard-Lefaucheur I, Cesaro P, Nguyen JP. Improvement of motor performance and modulation of cortical excitability by repetitive transcranial magnetic stimulation of the motor cortex in Parkinson's disease. Clin Neurophysiol 2004; 115: 2530-41.

Lomarev MP, Kanchana S, Bara-Jimenez W, Iyer M, Wassermann EM, Hallett M. Placebo-controlled study of rTMS for the treatment of Parkinson's disease. Mov Disord 2006; 21: 325-331.

Meintzschel F, Ziemann U. Modification of practice-dependent plasticity in human motor cortex by neuromodulators. Cereb Cortex 2006; 16: 1106-15.

Merton PA, Morton HB. Stimulation of the cerebral cortex in the intact human subject. Nature 1980; 285: 227.

Nitsche MA, Lampe C, Antal A, Liebetanz D, Lang N, Tergau F, et al. Dopaminergic modulation of long-lasting direct current-induced cortical excitability changes in the human motor cortex. Eur J Neurosci 2006; 23: 1651-7.

Nitsche MA, Paulus W. Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. J Physiol 2000; 527 Pt 3: 633-9.

O'Reardon JP, Solvason HB, Janicak PG, Sampson S, Isenberg KE, Nahas Z, et al. Efficacy and safety of transcranial magnetic stimulation in the acute treatment of major depression: a multisite randomized controlled trial. Biol Psychiatry 2007; 62: 1208-16.

Paquette MA, Brudney EG, Putterman DB, Meshul CK, Johnson SW, Berger SP. Sigma ligands, but not N-methyl-D-aspartate antagonists, reduce levodopa-induced dyskinesias. Neuroreport 2008; 19: 111-5.

Pascual-Leone A, Valls-Sole J, Wassermann EM, Hallett M. Responses to rapid-rate transcranial magnetic stimulation of the human motor cortex. Brain 1994; 117 (Pt 4): 847-58.

Paulus W. Toward establishing a therapeutic window for rTMS by theta burst stimulation. Neuron 2005; 45: 181-3.

Peinemann A, Reimer B, Loer C, Quartarone A, Munchau A, Conrad B, et al. Long-lasting increase in corticospinal excitability after 1800 pulses of subthreshold 5 Hz repetitive TMS to the primary motor cortex. Clin Neurophysiol 2004; 115: 1519-26.

Pollok B, Gross J, Muller K, Aschersleben G, Schnitzler A. The cerebral oscillatory network associated with auditorily paced finger movements. Neuroimage 2005; 24: 646-55.

Pollok B, Gross J, Schnitzler A. Asymmetry of interhemispheric interaction in left-handed subjects. Exp Brain Res 2006; 175: 268-75.

Priori A, Berardelli A, Inghilleri M, Accornero N, Manfredi M. Motor cortical inhibition and the dopaminergic system. Pharmacological changes in the silent period after transcranial brain stimulation in normal subjects, patients with Parkinson's disease and drug-induced parkinsonism. Brain 1994; 117 (Pt 2): 317-23.

Priori A, Lefaucheur JP. Chronic epidural motor cortical stimulation for movement disorders. Lancet Neurol 2007; 6: 279-86.

Quartarone A, Bagnato S, Rizzo V, Morgante F, Sant'angelo A, Battaglia F, et al. Distinct changes in cortical and spinal excitability following high-frequency repetitive TMS to the human motor cortex. Exp Brain Res 2005; 161: 114-24.

Ridding MC, Inzelberg R, Rothwell JC. Changes in excitability of motor cortical circuitry in patients with Parkinson's disease. Ann Neurol 1995; 37: 181-8.

Roick H, von Giesen HJ, Benecke R. On the origin of the postexcitatory inhibition seen after transcranial magnetic brain stimulation in awake human subjects. Exp Brain Res 1993; 94: 489-98.

Rossini PM, Rossi S. Transcranial magnetic stimulation: diagnostic, therapeutic, and research potential. Neurology 2007; 68: 484-8.

Sabatini U, Boulanouar K, Fabre N, Martin F, Carel C, Colonnese C, et al. Cortical motor reorganization in akinetic patients with Parkinson's disease: a functional MRI study. Brain 2000; 123 (Pt 2): 394-403.

Siebner HR, Lang N, Rizzo V, Nitsche MA, Paulus W, Lemon RN, et al. Preconditioning of low-frequency repetitive transcranial magnetic stimulation with transcranial direct current stimulation: evidence for homeostatic plasticity in the human motor cortex. J Neurosci 2004; 24: 3379-85.

Siebner HR, Mentschel C, Auer C, Conrad B. Repetitive transcranial magnetic stimulation has a beneficial effect on bradykinesia in Parkinson's disease. Neuroreport 1999; 10: 589-94.

Siebner HR, Rossmeier C, Mentschel C, Peinemann A, Conrad B. Short-term motor improvement after sub-threshold 5-Hz repetitive transcranial magnetic stimulation of the primary motor hand area in Parkinson's disease. J Neurol Sci 2000; 178: 91-4.

Sommer M, Kamm T, Tergau F, Ulm G, Paulus W. Repetitive paired-pulse transcranial magnetic stimulation affects corticospinal excitability and finger tapping in Parkinson's disease. Clin Neurophysiol 2002; 113: 944-50.

Stefan K, Kunesch E, Cohen LG, Benecke R, Classen J. Induction of plasticity in the human motor cortex by paired associative stimulation. Brain 2000; 123 Pt 3: 572-84.

Strafella AP, Ko JH, Grant J, Fraraccio M, Monchi O. Corticostriatal functional interactions in Parkinson's disease: a rTMS/[11C]raclopride PET study. Eur J Neurosci 2005; 22: 2946-52.

Strafella AP, Ko JH, Monchi O. Therapeutic application of transcranial magnetic stimulation in Parkinson's disease: The contribution of expectation. Neuroimage 2006.

Strafella AP, Paus T, Barrett J, Dagher A. Repetitive transcranial magnetic stimulation of the human prefrontal cortex induces dopamine release in the caudate nucleus. J Neurosci 2001; 21: RC157.

Strafella AP, Paus T, Fraraccio M, Dagher A. Striatal dopamine release induced by repetitive transcranial magnetic stimulation of the human motor cortex. Brain 2003; 126: 2609-15.

Teitti S, Maatta S, Saisanen L, Kononen M, Vanninen R, Hannula H, et al. Non-primary motor areas in the human frontal lobe are connected directly to hand muscles. Neuroimage 2008; 40: 1243-50.

Tergau F, Wassermann EM, Paulus W, Ziemann U. Lack of clinical improvement in patients with Parkinson's disease after low and high frequency repetitive transcranial magnetic stimulation. Electroencephalogr Clin Neurophysiol Suppl 1999; 51: 281-8.

Terney D, Chaieb L, Moliadze V, Antal A, Paulus W. Increasing human brain excitability by transcranial high-frequency random noise stimulation. J Neurosci 2008; 28: 14147-55.

Thickbroom GW. Transcranial magnetic stimulation and synaptic plasticity: experimental framework and human models. Exp Brain Res 2007; 180: 583-93.

Tokimura H, Di Lazzaro V, Tokimura Y, Oliviero A, Profice P, Insola A, et al. Short latency inhibition of human hand motor cortex by somatosensory input from the hand. J Physiol 2000; 523 Pt 2: 503-13.

Ueki Y, Mima T, Kotb MA, Sawada H, Saiki H, Ikeda A, et al. Altered plasticity of the human motor cortex in Parkinson's disease. Ann Neurol 2006; 59: 60-71.

Ueno S, Tashiro T, Harada K. Localized stimulation of neural tissues in the brain by means of a paired configuration of time-varying magnetic fields. J Appl Phys 1988; 64: 5862-5864.

Valls-Sole J, Pascual-Leone A, Brasil-Neto JP, Cammarota A, McShane L, Hallett M. Abnormal facilitation of the response to transcranial magnetic stimulation in patients with Parkinson's disease. Neurology 1994; 44: 735-41.

Wassermann EM. Risk and safety of repetitive transcranial magnetic stimulation: report and suggested guidelines from the International Workshop on the Safety of Repetitive Transcranial Magnetic Stimulation, June 5-7, 1996. Electroencephalogr Clin Neurophysiol 1998; 108: 1-16.

Wassermann EM, Lisanby SH. Therapeutic application of repetitive transcranial magnetic stimulation: a review. Clin Neurophysiol 2001; 112: 1367-77.

Wassermann EM, Wedegaertner FR, Ziemann U, George MS, Chen R. Crossed reduction of human motor cortex excitability by 1-Hz transcranial magnetic stimulation. Neurosci Lett 1998; 250: 141-4.

Watts RL. The role of dopamine agonists in early Parkinson's disease. Neurology 1997; 49: S34-48.

Wolters A, Sandbrink F, Schlottmann A, Kunesch E, Stefan K, Cohen LG, et al. A temporally asymmetric Hebbian rule governing plasticity in the human motor cortex. J Neurophysiol 2003; 89: 2339-45.

Wolters A, Schmidt A, Schramm A, Zeller D, Naumann M, Kunesch E, et al. Timing-dependent plasticity in human primary somatosensory cortex. J Physiol 2005; 565: 1039-52.

Ziemann U. Pharmacology of TMS. Suppl Clin Neurophysiol 2003; 56: 226-31.

Ziemann U, Meintzschel F, Korchounov A, Ilic TV. Pharmacological modulation of plasticity in the human motor cortex. Neurorehabil Neural Repair 2006; 20: 243-51.

Acknowledgements

First and foremost, I would like to express my deepest gratitude to Prof. Walter Paulus for giving me the opportunity to work in his department. His constant and tremendous support, academic supervision, innovative ideas and patience provided the basis for the work included in my thesis which would not have been possible without him. I also wish to thank the members of my thesis committee Prof. Stefan Treue and Prof. Thomas Rammsayer for their valuable advice and support.

I am very grateful to Prof. Claudia Trenkwalder for her tremendous support during the study on rTMS effect in Parkinson's Disease which was done at the Paracelsus Elena Klinik in Kassel. I am deeply grateful to Dr. Martin Sommer for his guidance, support and many fruitful discussions. I would like to express my sincere gratitude to Dr. Nicolas Lang for sharing his expertise in the field of neuroplasticity, his support and cooperation. I would also like to thank Dr. Bettina Pollok for introducing me to the exciting topic of movement timing.

In addition I would like to thank all members of the Department of Clinical Neurophysiology who have supported me. I especially would like to acknowledge Dr. Andrea Antal, Nicole Neef, Min-Fang Kuo and Tommaso Fadini for many inspiring discussions. A very special thanks goes to Marion Kurze who has helped me in a variety of situations.

Finally, I would like to express my deepest gratitude to the members of my family who have always supported me and have been a source of inspiration.

Curriculum Vitae

Given Name	Holger
Family Name	Rothkegel
Date of Birth:	27th April 1977
Place of Birth:	Bonn, Germany
Citizenship:	German

Education:

12/2005-present	PhD program Systems Neuroscience, Göttingen Graduate School for Neurosciences and Molecular Biosciences (GGNB), Georg-August-Universtity Göttingen, Germany
1997-2004	Medical Education, University of Bonn, Germany State examination November 4, 2004, full license to practise as a physician
2004	Internship (PJ) in internal medicine at Danderyd University Hospital, Stockholm, Sweden
2004	Internship (PJ) in surgery at the University Central Hospital of Turku, Finland
2003-2004	Internship (PJ) paediatrics, St.-Marien Teaching Hospital of the University of Bonn, Germany
2002	Two terms as exchange student at Karolinska Institutet and Huddinge University Hospital, Stockholm, Sweden
1996	Graduation from High School („Abitur“), Collegium Josephinum Bonn

Work Experience:

2005-present	Research Assistant and Resident at the Department of Clinical Neurophysiology, University Hospital Göttingen since May 15, 2005
2003-2004	Student job as assisting physician during internship (PJ) at St.-Marien Teaching Hospital of the University of Bonn, Germany

2002	Clinical Assistant (“läkarassistent”) at the Department of Internal Medicine, Sollefteå Hospital, Sweden
1999-2001	Student tutor for seminars in physiology for medical students, University of Bonn, Germany
1999-2000	Student tutor for the courses of microscopic anatomy/histology and macroscopic anatomy for medical students, University of Bonn, Germany
1997-2003	Student job as additional nurse at the Division of Paediatric Haematology/ Oncology, University Children’s Hospital Bonn, Germany

Publications:

Rothkegel H, Sommer M, Paulus W, Lang N. Impact of pulse duration in single pulse TMS. [submitted]

Rothkegel H, Sommer M, Paulus W. Breaks during 5Hz rTMS are essential for facilitatory after effects. Clin Neurophysiol 2009 [Epub ahead of print, available online 16 Dec 2009].

Fadini T, Matthäus L, Rothkegel H, Sommer M, Tergau F, Schweikard A, Paulus W, Nitsche MA. Reply to "Motor evoked potential latency, motor threshold and electric field measurements as indices of transcranial magnetic stimulation depth". Clin Neurophysiol 2009.

von Lewinski F, Hofer S, Kaus J, Merboldt KD, Rothkegel H, Schweizer R, Liebetanz D, Frahm J, Paulus W. Efficacy of EMG-triggered electrical arm stimulation in chronic hemiparetic stroke patients. Restor Neurol Neurosci. 2009;27(3):189-97.

Fadini T, Matthäus L, Rothkegel H, Sommer M, Tergau F, Schweikard A, Paulus W, Nitsche MA. H-coil: Induced electric field properties and input/output curves on healthy volunteers, comparison with a standard figure-of-eight coil. Clin Neurophysiol. 2009 Jun;120(6):1174-82.

Rothkegel H, Sommer M, Rammsayer T, Trenkwalder C, Paulus W. Training Effects Outweigh Effects of Single-Session Conventional rTMS and Theta Burst Stimulation in PD Patients. Neurorehabil Neural Repair 2009; 23: 373-81.

Pollok B, Rothkegel H, Schnitzler A, Paulus W, Lang N. The effect of rTMS over left and right dorsolateral premotor cortex on

movement timing of either hand. Eur J Neurosci 2008; 27: 757-64.

Lang N, Speck S, Harms J, Rothkegel H, Paulus W, Sommer M. Dopaminergic potentiation of rTMS-induced motor cortex inhibition. Biol Psychiatry 2008; 63: 231-3.

Göttingen, 31st December 2009