Helsinki University of Technology
Department of Biomedical Engineering and Computational Science
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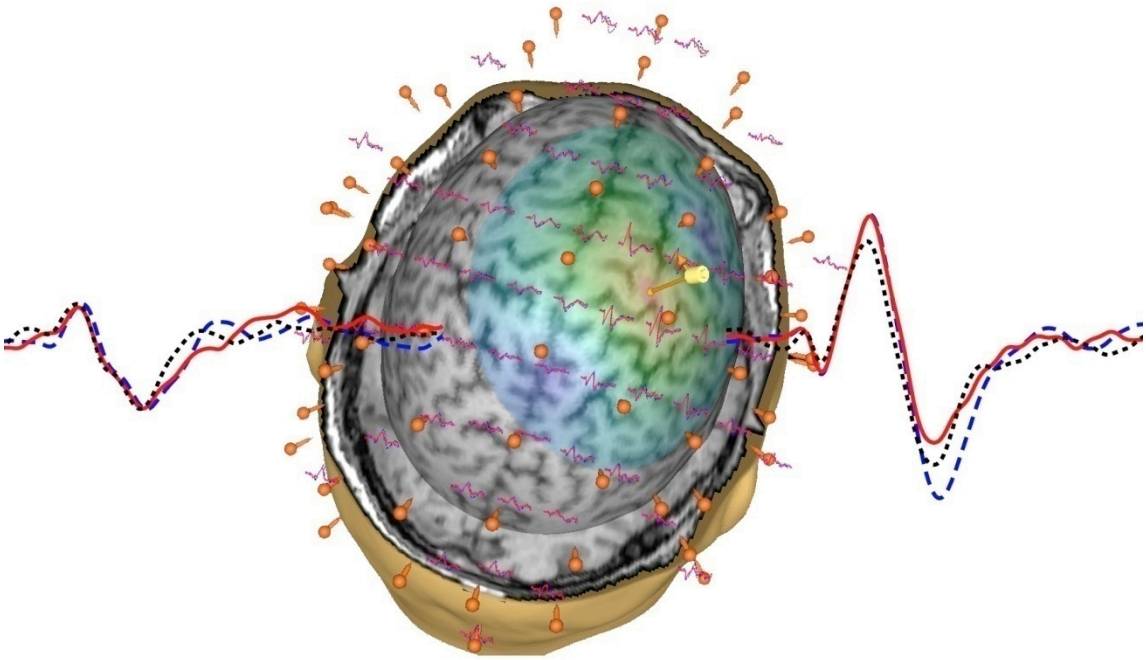
October, 2009 REPORT A13

PROBING CORTICAL EXCITABILITY WITH TRANSCRANIAL MAGNETIC STIMULATION

Dubravko Kičić



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Dubravko Kičić

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Helsinki University of Technology
Faculty of Information and Natural Sciences
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Department	Department of Biomedical Engineering and Computational Science
Field of research	Applied mathematics and neuroscience
Opponent(s)	Prof. John Rothwell
Supervisor	Prof. Risto Ilmoniemi
Instructor	Dr. Vadim V. Nikulin

Abstract

This thesis, consisting of seven original publications (I–VII), explored the technical and neurophysiological plausibility of combining neuro-navigated transcranial magnetic stimulation (nTMS) with neuroimaging techniques such as multichannel electroencephalography (EEG) and magnetoencephalography (MEG). This work has focused on the interaction between the current state of neuronal activity at the targeted cortical network and the effects of TMS. We took an integrative approach, including a correlation between cortical (EEG, MEG) vs. peripheral electromyographic (EMG) measurements. TMS-evoked EEG responses were used as probes for the current functional state of the cortex during the processing of sensory stimuli and the preparation/execution of different motor activities. Contrary to standard indirect approaches utilizing peripheral EMG measures, our study directly demonstrated graded excitability in contra- and ipsilateral hemispheres during the preparation/execution of unilateral movements. The obtained data suggest that the specific balance of interhemispheric excitability is tailored for the optimal performance of unilateral movement by preventing not only mirror movements through decreased excitability of ipsilateral hemisphere, but also via pre-emptive background tonic inhibition of this hemisphere. The utility of the TMS-EEG combination was further demonstrated by providing direct evidence for cortical involvement in short-latency afferent inhibition. We found a linear correlation between the attenuation of TMS-evoked EEG responses and the attenuation of muscle responses, thus revealing how changes in cortical neuronal activity are related to changes on the periphery. The clinical feasibility of the TMS-MEG combination was demonstrated by showing that delivering trains of TMS pulses to the motor cortex of Parkinson's patients successfully modulated the spontaneous beta-range oscillations measured with MEG over the rolandic cortical regions, suggesting probable alteration of the cortico-thalamo-basal ganglia networks. The present thesis demonstrates that the spatial accuracy of localizing primary motor representational areas with both MEG and nTMS is superior to electrical cortical stimulation via subdural grids. Furthermore, this work demonstrates very high reproducibility of TMS-evoked EEG deflections after repeated stimulation of both the primary motor and prefrontal cortices. This suggests new standards in preoperative clinical workup and a wide range of studies with test-retest design. Thus, this thesis provides a new methodological and technical framework for measuring the time-resolved functional connectivity and causality of activation in the observed neural networks of human cerebral cortex.

Keywords transcranial magnetic stimulation, electroencephalography, motor cortex	
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Academic dissertation

Probing cortical excitability with transcranial magnetic stimulation

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Preface and acknowledgements

Professor Jari Karhu, a colleague and a dear friend of mine, told me several years ago: ”doctors of science are just common people who are stubborn enough to carry out the work that virtually no one would do!” Jari, thanks for putting this bug into my ears, it kept me going on all this time! However, the present thesis is not solely my personal achievement. Rather, it is the result of successful collaboration between a number of people and organizations.

I started my scientific career in the BioMag Laboratory of the Helsinki University Central Hospital. For extended period of time, this lab has been a pleasurable and stimulating place to work, where I carried out all the experiments and measurements for this thesis. I'm indebted to the director, Dr. Jyrki Mäkelä, for being truly supportive both as a supervisor and coauthor. The fact that he was always available when we needed a subject to test our wild scientific ideas, surely makes his brain one of the most TMS'd and MEG'd brains in the Helsinki brain research community! My special thanks also go to Dr. Juha Montonen, present technical director of the BioMag Lab, for being a perpetual force pushing us forward. I shall always remember his excellent running of the BioMag laboratory during the toughest phases of its existence. From BioMag, I have to mention also Dr. Juha Heiskala, Dr. Leena Lauronen, Dr. Heidi Wikström, Chris Bailey, Karen Johanne Pallesen, Lauri Lipiäinen, Kalle Kotilahti, Tommi Noponen, Dr. Milena Korostenskaja, Suvi Heikkilä, Ville Mäntynen, and Simo Monto.

With all of my heart, I would like to express my gratitude to Jussi Nurminen for being an unprecedented computer geek and a Matlab guru to me - Jussi has opened many secret doors in my perception of the world of ones and zeros.

When, in the winter of 2000, Dr. Vadim V. Nikulin told me that he has some interesting preliminary TMS-EEG data, I immediately replied: show me! ”Is that really what you want”, Dr. Nikulin was persistent. ”What else could I want?” was my answer, as well as my ticket to the most important part of my scientific career. Under the guidance of Dr. Nikulin, I was able to both crystallize my methodological approach for studying the functional aspects of the human brain, as well as to successfully integrate our results into a framework of known neurophysiological data. I'm honored and deeply thankful to Dr. Nikulin for adding a scientific essence to my (most of the times) scattered thoughts, and for being a true friend and spiritus movens of my work.

I have been extremely fortunate in being given the opportunity to collaborate with top professionals from several companies with the mission of transferring scientific expertise into solutions for both the clinics and laboratories. In world of TMS, Dr. Jarmo Ruohonen, technology director at Nexstim Oy, was the first person to instill in me in the fire of applied neuroscience. Thanks to collaborators from Nexstim - Henri Hannula, Marko Ollikainen, Tuomas Neuvonen, and Perttu Sipilä - I have learned that only a team effort between dedicated scientists excited about their work can successfully bring about development in a field.

Working as a consultant for Elekta Neuromag Oy provided me with both a very deep

insight into the processing of magnetic multichannel signals and a robust experience of working with a top-notch expert MEG group. I'm especially grateful to Dr. Jukka Nenonen, method development manager in Elekta Neuromag, for being the most responsive collaborator I have ever met; to Dr. Samu Taulu for informal discussions on mathematical aspects of the analysis of MEG data; to Dr. Veikko Jousmäki for our endless discussions about the technical solutions undelying MEG physics and Apple computers; and to Mrs. Candice Weir and Mr. Juha Virola for their support.

Professor Risto Ilmoniemi, the head of Department of Biomedical Engineering and Computational Science supervised my postgraduate studies and research from the very beginning, and his contribution to this thesis goes far beyond its scientific borders. I'm indebted to him for treating me always primarily as a human being and a friend, and then as a student. My take-for-life message from Risto is definitely: "do not blindly believe scientific results, even if they are your own!" With his unique approach to teaching and scientific conduct, Risto surely provided me with qualities that will forever remain as my own elements of style. I'm also grateful to Prof. (emer.) Toivo Katila for supervising my graduate studies and for immersing me into the world of multidisciplinary assessment of neuronal phenomena.

It was honor and my immense pleasure to work and collaborate with Prof. Risto Näätänen, former head of the Helsinki Brain Research Center, the Center of Excellence of Academy of Finland. Without him and his support for our science, much of the work presented in this thesis would not have been possible. He was really instrumental in development the hypothesis-driven science in many young researchers, including me. I'm deeply thankful to Prof. Näätänen for his contribution in making my work a successful one.

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The list of names of my Finnish and international collaborators would be too long to be presented here. However, several people I would like to emphasize as contributors which significantly shaped and contributed certain phases of my scientific development. I would especially like to mention Prof. Claudia Tesche, Dr. Elina Pihko, Prof. Pekka Meriläinen, Dr. Klaus Linkenkaer-Hansen, Prof. Yoshio Okada, Prof. Yoshikazu Ugawa, Nobuyuki Igarashi-san, Prof. Pedro Valdes-Sosa, Prof. Mireille Besson, Mireille Bonnard, Prof. Selma Supek, Prof. Riitta Hari, Dr. Anna Shestakova, Dr. Ilkka Nissillä, Dr. Ville Mäkinen, Prof. Hannu Tiitinen, and Dr. Patrick May.

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I'm deeply grateful to my mother Ana and my brothers Miroslav and Nevenko for their continuous support.

I love my wife Jagoda and my daughters Lucija and Tonka for being the light of creation in my life.

Helsinki, October 2009

Dubravko Kičić

Contents

List of Publications

Publications included in this thesis are:

I Nikulin V.V., Kičić D., Kähkönen S., and Ilmoniemi R.J. (2003). Modulation of electroencephalographic responses to transcranial magnetic stimulation: evidence for changes in cortical excitability related to movement. Eur J Neurosci 18(5), 1206-12.

II Kičić D., Lioumis P., Ilmoniemi R.J., and Nikulin V.V. (2008). Bilateral changes in excitability of sensorimotor cortices during unilateral movement: combined electroencephalographic and transcranial magnetic stimulation study. Neuroscience 152(4), 1119-29.

III Bikmullina R., *Kičić D., Carlson S., and Nikulin V.V. (2009). Electrophysiological correlates of short-latency afferent inhibition: combined EEG and TMS study. Exp Brain Res 194(4), 517-26.

IV Lioumis P., Kičić D., Savolainen P., Mäkelä J.P., and Kähkönen S. (2009). Reproducibility of TMS-Evoked EEG responses. Hum Brain Mapp 30(4), 1387-96.

V Raij T., Karhu J., Kičić D., Lioumis P., Julkunen P., Lin F.H., Ahveninen J., Ilmoniemi R.J., Mäkelä J.P., Hämäläinen M., Rosen B.R., and Belliveau J.W. (2008). Parallel input makes the brain run faster. Neuroimage 40(4), 1792-7.

VI Kičić D., Bikmullina R., Lioumis P., Nurminen J., Kaakkola S., Mäkelä J.P., and Pekkonen E. (2007). Effects of 10 Hz rTMS on spontaneous brain oscillations in non-demented Parkinson's patients: Preliminary results of combined MEG-rTMS study. International Congress Series 1300, 1717-20.

VII Vitikainen A.M., Lioumis P., Paetau R., Salli E., Komssi S., Metsähonkala L., Paetau A., Kičić D., Blomstedt G., Valanne L., Mäkelä J.P., and Gaily E. (2008). Combined use of non-invasive techniques for improved functional localization for a selected group of epilepsy surgery candidates. Neuroimage 45(2), 342-8.

* Dubravko Kičić and Rozaliya Bikmullina equally contributed to the study.

Author's contribution

The theoretical and experimental basis for this thesis was developed in Publication I together with the first author. Publication III was used to test the accuracy and to justify the experimental approach, which was used in Publications II–VI. Publications II, IV, and V expanded this basic theory into a more general framework and tested it in a variety of neurophysiological systems and experimental settings.

Publication I: The author performed extensive optimization measurements in order to test and establish experimental setup and to obtain reliable TMS-EEG recordings without artifact. He conducted TMS-EEG measurements, data acquisition, pre-processing, a significant amount of data analysis, and actively participated in interpreting the results as well as writing the article.

Publication II: The author designed the experimental paradigm, planned and tested the experimental setup, performed all measurements, data analysis and writing of the article. He is the principal author of the article.

Publication III: The author adapted the experimental paradigm into the TMS-EEG environment and performed optimization of the measurements and analysis methods. He supervised and monitored data analysis at all stages and participated equally with the first author in interpreting the results and writing of the manuscript. His contribution was equal to that of the first author of the article.

Publication IV: The author pointed out the methodological necessity for this study, designed the experimental paradigm and performed test measurements. He monitored the data analysis at all stages, significantly contributed to data interpretation, and wrote significant parts of the article.

Publication V: The author technically adapted the study paradigm into the experimental TMS-EEG environment and performed optimization measurements. He designed the data analysis approach and performed the initial stages of data analysis. He actively contributed in writing the article.

Publication VI: The author proposed the methodology for this clinical study. He designed, tested and evaluated the paradigm prior to actual patient measurements. He performed all MEG and rTMS measurements, data acquisition, pre-processing and data analysis, and wrote the article. He is the principal author of the article.

Publication VII: The author contributed to the TMS part of the study. He performed a technical setup of the paradigm together with the second author, performed optimization measurements, and suggested implementation strategies with results from other imaging modalities used in the study.

List of Abbreviations

CSF	Cerebro-Spinal Fluid
D2	Index Finger
DLPFC	Dorsolateral Prefrontal Cortex
EEG	Electroencephalography
EMG	Electromyography
ECD	Equivalent Current Dipole
ECS	Electrical Cortical Stimulation
EOG	Electro-oculogram
ER	Evoked Response
ERF	Event-Related Field
ERP	Event-Related Potential
fMRI	Functional MRI
GABA	γ-Aminobutyric acid
GABA-A	γ-Aminobutyric acid type A
GABA-B	γ-Aminobutyric acid type B
LTP	Long-Term Potentiation
LTD	Long-Term Depression
M1	Primary Motor Cortex
MEG	Magnetoencephalography
MEP	Motor-Evoked Potential
MRI	Magnetic Resonance Imaging
NIRS	Near-Infrared Spectroscpy
NREM	Non-Rapid Eye Movement
nTMS	Navigated TMS
PAS	Paired Associative Stimulation
PD	Parkinson's Disease
PET	Positron Emission Tomography
REM	Rapid Eye Movement
ROI	Region Of Interest
rTMS	Repetitive TMS
SAI	Short-latency Afferent Inhibition
SEF	Somatosensory Evoked Field
SP	Spectral Power
TBS	Theta-Burst Stimulation
TMS	Transcranial Magnetic Stimulation
TMS-EEG	TMS combined with EEG
UPDRS	Unified Parkinson's Disease Rating Scale

1 Aims of the study

The specific aims of Publications I–VII were as follows.

I To identify electroencephalographic (EEG) correlates of increased cortical premovement excitability using cortical response to transcranial magnetic stimulation (TMS) as a probe.

II To demonstrate the inhibitory role of the ipsilateral hemisphere in the performance of unilateral movement.

III To demonstrate the cortical origins of short-latency afferent inhibition (SAI) with direct EEG recordings.

IV To evaluate repeatable probing of cortical excitability with transcranial magnetic stimulation combined with concurrent EEG (TMS-EEG).

V To evaluate the effects of serial and parallel cortical processing in a behavioural task.

VI To validate the use of magnetoencephalography (MEG) for mapping the direct effects of rapid-rate TMS (rTMS) on specific cortical circuits in patients with Parkinson's disease (PD).

VII To evaluate the combined use of MEG and navigated TMS (nTMS) as non-invasive protocols for localization of the epileptogenic and sensorimotor cortical regions in patients with epilepsy.

2 Introduction

Transcranial magnetic stimulation is a non-invasive technique for stimulating the human brain by means of rapidly changing magnetic fields (Barker et al. 1985). The stimulating effect is achieved by induction of weak, brief intracortical currents, which depolarize the cell membranes of both cortical excitatory pyramidal cells and inhibitory interneurons. If the depolarization exceeds a threshold level, the nerve cell will discharge and, as the propagated action potential greatly outlives the electrical pulse, the effect of one TMS pulse can last tens of milliseconds. This TMS-evoked activity can be measured with a variety of electrophysiological methods and a number of parameters can be studied in the activated network. The neural impact of TMS stimulus is not determined only by the properties of that stimulus, but also by the initial state of the activated brain region, which is usually referred to as neuronal excitability (Abbruzzese and Trompetto 2002; Amassian et al. 1989).

In general terms, the neuronal excitability can be understood as the responsiveness of the neuronal population to the incoming signals. Current neuronal states of the cortex might be shaped by the sensory inputs as well as by the activity of other neuronal structures projecting into the given area. Cognitive neuroscience has predominantly focused on the cerebral cortex, which is also easily reachable by TMS. Because of the wealth of information regarding TMS impacts on the motor system, particularly due to measurable compound motor evoked potentials (MEPs) from peripheral muscles, motor cortex excitability has become the most common topic in TMS studies. However, a proper study of motor cortex excitability with TMS should clearly differentiate between the indices of the overall excitability of the corticospinal system (corticospinal excitability), and those specifically reflecting the excitability of the motor cortex (cortical excitability) and spinal cord.

MEPs caused by TMS were used routinely in research and clinical evaluation – abnormalities in the latency of amplitude of MEPs, or in the duration of the electromyographically (EMG) observed silent period were often taken as indicators of cortical pathology (Meyer 2002; Morita et al. 2008; Liepert et al. 2009). The problem with EMG in general, and with MEPs in particular, is that they are affected by a combination of cortical, subcortical, and spinal-cord mechanisms, which usually coincide in time, making their separation very difficult. If drawn exclusively on MEP recordings, conclusions about cortical pathologies, or in general about cortical involvement of the primary motor cortex (M1) in a given process might be uncertain. The present thesis provides an alternative approach, utilizing a combination of magnetic resonance image (MRI) guided TMS with both MEG and concurrent EEG to distinguish cortical involvement in a range of experimental paradigms.

The motivation for the studies in this thesis came from multichannel EEG mapping of cortical responses to TMS (Ilmoniemi et al. 1997; Komssi et al. 2002) and multi-modal stimulation experiments (Nikouline et al. 1999; Schürmann et al. 2001; Tiitinen et al. 1999) conducted in the BioMag Laboratory (HUSLAB, Hospital District of Helsinki and Uusimaa). Those studies showed on one side that TMS-evoked EEG responses can be reliably mapped over the whole scalp, and on the other side

that the TMS-EEG technique is suitable for detection of subtle changes in cortical excitability. Questions concerning intersensory facilitation and cross-modality suppression raised in a study by Nikouline et al. (1999) encouraged the idea to further investigate functionally-specific modulation of TMS-evoked EEG responses. In particular, local interaction between TMS-induced activity and the neural activation caused by peripheral somatosensory stimulation, as well as an indicated relationship between evoked responses (ER) and spontaneous EEG (Schürmann et al. 2001), encouraged the idea to develop a methodological framework to further study changes in the cortical excitability of healthy subjects and patients. For this purpose, we utilized the unique characteristic of TMS to interfere with ongoing neural processes of the living human brain.

Using a multitude of brain mapping techniques, we established and empirically tested a novel framework for probing the subtle, functionally specific, and importantly, transient changes in cortical excitability.

All Publications (I–VII) in this thesis used TMS as a probe of cortical excitability.

Anatomical structures seen in MRI were utilized for the selection of cortical TMS targets in Publications III–VII. We named this technology navigated TMS (nTMS). Additional accuracy in TMS targeting was gained from activation sites determined by MEG inverse solutions (Publications V and VII). Publication I identifies the EEG correlates of increased cortical excitability related to the preparation and execution of movement, while Publication II represents its methodological extension to the role of the ipsilateral hemisphere in the control of unilateral movements. Publication III presents the first demonstration of a correlation between the EEG and MEP manifestations of the short-latency afferent inhibition phenomenon. The very important issue of the reproducibility of TMS-evoked EEG responses was evaluated in Publication IV. Of special interest in that study is the introduction of cortical excitability probing of non-motor areas, which was further developed in Publication V. Based on our interest in the use of rTMS for treatment of neurological diseases, Publication VI is pioneering an offline combination of rTMS and MEG for monitoring rTMS effects on spontaneous cortical oscillations in Parkinsonian patients. Publication VII evaluates the use of nTMS as an additional tool in preoperative motor mapping in patients with epilepsy.

2.1 Cerebral cortex

The cerebral cortex is a greatly convoluted sheet of neural cells on the outer surface of the brain, just under the skull and the cerebrospinal fluid (CSF). It is about 3 mm thick, consisting of small folds called sulci, large grooves called fissures, and bulges between them called gyri (Fig. 2.1). Approximately two-thirds of the cortical surface is located in the sulci and fissures. The cortex is grossly divided into three functionally separate groups: sensory, motor and association cortices. On the same spatial scale, the cortex in each hemisphere is anatomically divided into the four lobes: the frontal, temporal, parietal and occipital lobe (Fig. 2.1). The central sulcus separates the frontal lobe from the parietal lobe, and the lateral (or Sylvian)

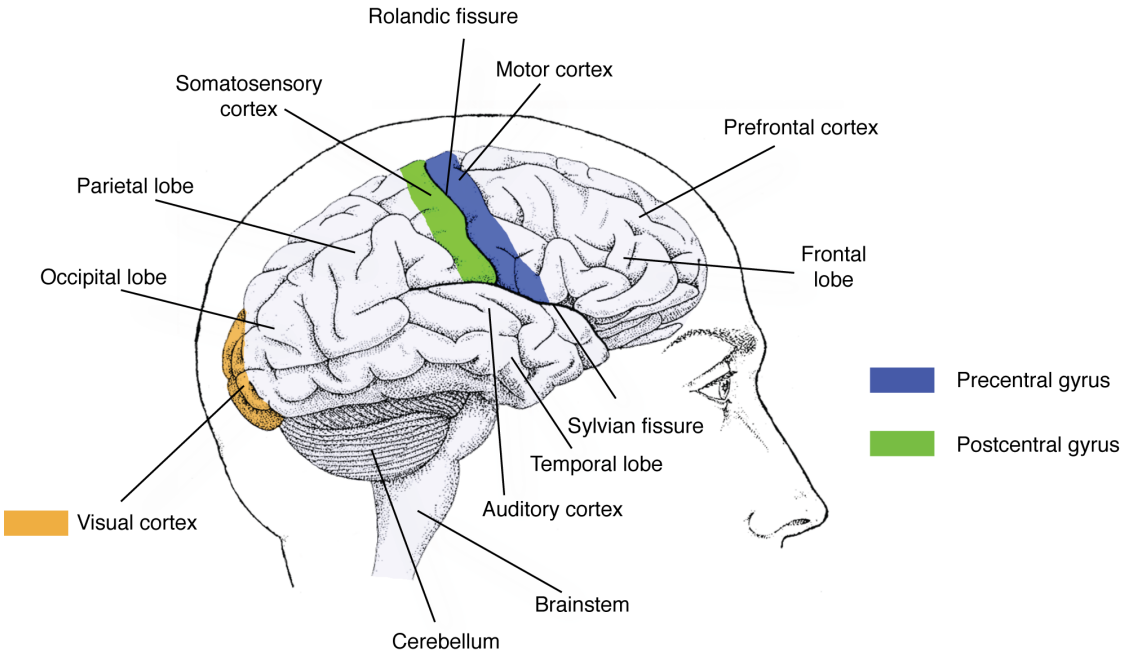

Figure 2.1: Gross functional and anatomical divisions indicated on the right lateral view of the human brain. Some of the important structural landmarks and special areas of the cerebral cortex are highlighted.

fissure separates the temporal lobe from the overlying frontal and temporal lobes (Fig. 2.1). The motor cortex controls the movement and is located in the precentral sulcus of the frontal cortex, just opposite to the somatosensory cortex. The visual cortex occupies most of the occipital lobe but stretches into the temporal lobe. The auditory cortex lies in the temporal sulcus, while the somatosensory cortex is situated in the postcentral gyrus and receives an input from the sensory systems via the thalamus. The associative cortex includes parts of the parietal and the frontal cortex and plays an important role in performing higher cognitive functions, such as memory and learning. Roughly, cortical neurons can be subdivided into the interneurons and the pyramidal cells. The interneurons project locally, while the pyramidal cells might also project globally into remote cortical structures. The cortical neurons are massively interconnected. A single pyramidal neuron has been estimated to receive around 60 000 synaptic inputs and may directly contact around 5000 other neurons. A volume of 1 mm^3 contains the axons corresponding in length to approximately 1–4 km.

The cortex of the cerebral hemispheres is a six-layered mixture of cell bodies and local fibres that varies in size and configuration from one cortical region to the other (Fig. 2.2). In general, the upper four cortical layers receive input projections from other cortical areas, the brainstem, and the subcortical nuclei (*e.g.,* basal ganglia and thalamus), whereas the lower two layers comprise the output projection layers. Layer 5 (called internal pyramidal layer, or ganglion cell layer, Fig. 2.2) is particularly prominent in the motor cortex, where it contains large pyramidal Betz cells that give rise to a portion of the descending pyramidal motor tract. Layer 1

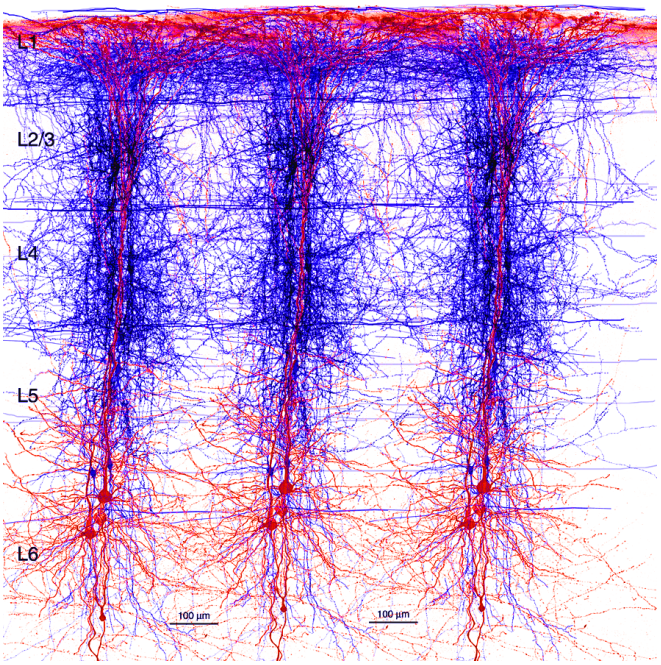

Figure 2.2: The six layers of the cerebral cortex. Note the large layer V and VI pyramidal neurons (in red), with their apical shafts ascending to layer I. The inhibitory fibres (in blue) wrap around these apical shafts in order to control the level of excitability in the cortex. Figure adapted with permission from The Blue Brain Project (http://bluebrain.epfl.ch/).

has a sparse abundance of neurons, being thus less important from the aspect of TMS-evoked activity. Even though layer 4 is very thin in the motor cortex (Gatter et al. 1978), it is rich in inhibitory fibres (blue traces in Fig. 2.2), which control the level of excitability in the cortex.

2.2 Neural basis of TMS, EEG, and MEG

Bioelectric activity studied with MEG or EEG is described in terms of a primary or source current:

$$\mathbf{J}^p(\mathbf{r}) = \mathbf{J}(\mathbf{r}) - \mathbf{J}^v(\mathbf{r}), \tag{2.1}$$

which results from activation of cortical cells. The primary current alters distribution of charges in the surrounding tissue, thus generating an electric field $\mathbf{E}(\mathbf{r})$, which in turn drives the ohmic volume current:

$$\mathbf{J}^v(\mathbf{r}) = \sigma(\mathbf{r})\mathbf{E}(\mathbf{r}), \tag{2.2}$$

which is determined by the conductivity of the tissue σ. Total current distribution at point $\mathbf{r}$ is $\mathbf{J}(\mathbf{r})$, representing the sum of the primary and the volume currents. EEG measures the voltage distribution on the scalp that arises from the altered charge distribution. Synchronous activation of neurones in a number of cortical columns is required to generate dipole moments in the order of 10 nAm. Such dipole moments

are associated with magnetic fields large enough to be detected by the MEG sensors and correspond to typical event-related potentials (ERP) and event-related fields (ERF) (Chapman et al. 1984).

MEG is particularly sensitive to superficial and tangentially oriented sources. On the other hand, EEG measures both tangential and radial sources, where the major source of primary neuronal currents originates at the apical dendrites that are oriented perpendicularly to the surface of the cortex (Proverbio and Zani 2003).

A stimulation effect of TMS in the cortex is due to the induced electric field, which affects the transmembrane potential of the neuronal cell by opening its voltage-sensitive ion channels. Since the cell membrane behaves as a leaky capacitor, faster and stronger changes in the electromagnetic environment are more effective for excitation (Panizza et al. 1992; Nagarajan et al. 1993). The gradient of a TMS-induced electric field along a distal axon has been considered as the primary mechanism of activation (Basser et al. 1992), though perpendicular electric field components have also been shown to change the membrane potentials of neurones (Ruohonen et al. 1996). Bends and other non-uniformities of the neural structure have been determined as locations of increased excitability for magnetic stimulation (Maccabee et al. 1993; Ilmoniemi et al. 1997).

2.3 Theory of TMS is the converse of MEG

The volume current $\mathbf{J}^v$ is passive and results from the macroscopic electric field on charge carriers in the conducting medium. Everything else is represented as the primary current $\mathbf{J}^p$ (Ilmoniemi et al. 1999):

$$\mathbf{J}(\mathbf{r}) = \mathbf{J}^p(\mathbf{r}) + \sigma(\mathbf{r})\mathbf{E}(\mathbf{r}) = \mathbf{J}^p(\mathbf{r}) - \sigma(\mathbf{r})\nabla V(\mathbf{r}). \tag{2.3}$$

Neural activity gives rise to primary current mainly inside or within the vicinity of a cell, whereas the volume current flows passively everywhere in the medium (Hämäläinen et al. 1993). By finding the primary current, we can locate the source of brain activity, as described by a current dipole.

The (equivalent) current dipole $\mathbf{Q}$ is a theoretical, infinitely small current element. It is a convenient building block for constructing mathematically equivalent models of electrical activity patterns in the brain. Current dipole is an approximation of the localized primary current and is a widely used concept in neuromagnetism. Let us consider the concentration of $\mathbf{J}^p(\mathbf{r})$ to a single point $\mathbf{r}_Q$:

$$\mathbf{J}^p(\mathbf{r}) = \mathbf{Q}\delta(\mathbf{r} - \mathbf{r}_Q), \tag{2.4}$$

where $\delta(\mathbf{r})$ is the Dirac delta function (Arfken and Weber 1995). In EEG and MEG applications, a current dipole is used as an equivalent source for the unidirectional primary current that may extend over several square centimetres of cortex.

The theory of lead fields is very important for spatial analysis of EEG and MEG signals. It yields a measure of the sensitivity of sensors for electromagnetic field

quantities, depending on lead configuration, source location and conductivity distribution. The existence of the lead field is a direct consequence of the linearity (principle of superposition) of electromagnetic fields (Hämäläinen and Ilmoniemi 1994). This principle predicts that a measurement of an electromagnetic scalar entity - be it the electric potential or the components of the magnetic field - must be proportional to the magnitude of each of the components of the current source - in this case the primary currents. This can be written as:

$$B = \mathbf{L}(\mathbf{r}') \cdot \mathbf{J}^p(\mathbf{r}'\ ), \tag{2.5}$$

where $\mathbf{L}(\mathbf{r}$') is termed the lead vector and B is the amplitude of the sensor. Note that the sensor can be either a magnetometer or an electrode pair and that the lead field is specific for each sensor. The flux in a magnetometer coil

$$\Phi = \int_{\text{coil i}} \mathbf{B} \cdot \mathrm{d}\mathbf{A} \tag{2.6}$$

depends linearly on the primary current distribution. Therefore, we can define a sensitivity function $\mathbf{L}_i(\mathbf{r}')$ called lead field for each sensor i. Integrating 2.5 over a volume containing sources yields:

$$B_i = \int \mathbf{L}_i(\mathbf{r}') \cdot \mathbf{J}^p(\mathbf{r}')\ dv'\ , \tag{2.7}$$

where $B_i = \Phi_i / A_i$, (A_i is the coil area) is the magnetic field in the detection coil of magnetometer i. The lead field depends on the coil geometry and its location and orientation with respect to the head as well as on the tissue conductivity distribution $\sigma = \sigma(\mathbf{r})$ (Ilmoniemi et al. 1999).

The lead field of a TMS coil is the same as the lead field of a magnetometer coil of the same size, shape, location, and orientation. This allows us to summarize: if $\mathbf{L}_i(\mathbf{r}')$ is the lead field of coil i and current $I_i = I_i(t)$ is fed into the coil, the total electric field, induced directly and caused by charges at conductivity boundaries, is

$$\mathbf{E}(\mathbf{r}') = -A_i \frac{\mathrm{d}I_i}{\mathrm{d}t}\ \mathbf{L}_i(\mathbf{r}'). \tag{2.8}$$

The complicated part here is the precise calculation of $\mathbf{L}_i(\mathbf{r}')$, which not only depends on the stimulator coil, its location, but also on the minute local conductivity distribution of the head.

3 TMS as a tool for probing cortical excitability

3.1 Methodological aspects: review of literature

TMS is unique in that it offers a non-invasive, painless method for stimulating the brain. The stimulating effect depends on several important factors, including the geometry of the stimulating coil (circular, figure-of-eight, cone-shaped), the waveform of the current pulse driven through the coil (monophasic or biphasic), or the cytoarchitectonic structure of the stimulated area. With commonly used stimulation parameters and focal figure-of-eight coils (Ueno et al. 1988), the superficial cortical structures are activated within the cone-shaped volume of few cube centimetres, extending approximately 2–3 centimetres in depth from the surface of the human skull (Bohning et al. 1997).

Pulses of sufficient intensity can evoke a sequence of descending cortico-spinal volleys (Day et al. 1989). They can be measured with peripheral EMG in the form of MEPs to provide information on the anatomical and functional organization of the motor system, useful for precise mapping of motor cortex representations (Kammer et al. 2005; Bestmann et al. 2008; Julkunen et al. 2009). After it was shown that abnormal central motor conduction could be associated with neuronal deficit (Barker et al. 1987), TMS methodology was widely introduced to patient studies, demonstrating excitability alterations in various diseases, including Parkinson's disease (Pascual-Leone et al. 1994b; Lefaucheur 2005; Fisher et al. 2008), dystonia (Edwards et al. 2003; Sohn and Hallett 2004; Bütefisch et al. 2005; Quartarone et al. 2005), Huntington's disease (Meyer et al. 1992; Lorenzano et al. 2006), Tourette's syndrome (Ziemann et al. 1997; Berardelli et al. 2003; Gilbert et al. 2004), and essential tremor (Romeo et al. 1998; Modugno et al. 2002).

Delivering two consecutive TMS pulses to the motor cortex (paired-pulse TMS, Kujirai et al. 1993) with independently adjusted stimulus intensities and a short inter-stimulus interval (1–200 ms) allows modulation of M1 excitability to be investigated by local circuits, as well as the study of inhibition and facilitation within the motor pathway (Di Lazzaro et al. 2000; Kujirai et al. 1993; Manganotti et al. 2002; Shimizu et al. 1999; Tamburin et al. 2004; Valls-Solé et al. 1992). If a conditioning stimulus is given to the brain areas other than M1, an area-to-area facilitation and inhibition can be estimated by observing changes in the size of conditioned MEPs relative to test MEPs alone (double-pulse TMS: Bajbouj et al. 2004; Daskalakis et al. 2002; Di Lazzaro et al. 1999; Kujirai et al. 1993; Meyer et al. 1998; Ridding et al. 2000). In general, modulating inputs from conditioning pulses elicit inhibitory or facilitatory effects on the motor cortex trough intracortical (Kujirai et al. 1993; Orth et al. 2003; Ziemann et al. 1996; Chen et al. 1998a), intrahemispheric (Bajbouj et al. 2004; Hanajima et al. 1996; Strafella et al. 2000; Pierantozzi et al. 2002; Buhmann et al. 2004), or interhemispheric connections (Ferbert et al. 1992; Boroojerdi et al. 1999; Cracco et al. 1989; Di Lazzaro et al. 1999; Wilkins et al. 1984; Hanajima et al. 2001; Chen et al. 2003).

A new approach to TMS paradigms was introduced by showing that a subject's

performance in a character identification task was transiently impaired when single TMS pulses were administered to the occipital cortex at specific latency after onset of the visual stimulus (Amassian et al. 1989). Disruption of the ongoing cortical processing was named the lesion paradigm, and is broadly used in cognitive neuroscience with the objective of interfering in the neural activity associated with cognitive processes. TMS applied in healthy subjects during a cognitive process most commonly leads to disruptions in task performance (Cowey 2005; Walsh and Pascual-Leone 2003). Nevertheless, there is a growing number of reports indicating that TMS can also facilitate behaviour if single TMS pulses are applied shortly before the onset of a cognitive process (*e.g.,* Töpper et al. 1998; Grosbras and Paus 2003). Some of these 'paradoxical' facilitatory effects of TMS can be accounted for by a disinhibition of an unstimulated brain area whose function is normally suppressed by the TMS target region (Walsh and Pascual-Leone 2003). Such functional release suggests that TMS-induced neuronal activity can spread beyond the directly stimulated area to anatomically connected sites (Fox et al. 1997; Ilmoniemi et al. 1997; Komssi et al. 2002; Paus et al. 1997; Paus et al. 2001; Strafella et al. 2001).

An important development in TMS technology was the introduction of rapid-rate TMS (mode of stimulation with frequency higher than 1 Hz; Cadwell Laboratories Inc., Kennewick, USA, 1988), showing that rTMS of language areas in the dominant hemisphere can arrest the speech production (Pascual-Leone et al. 1991; Jennum et al. 1994; Stewart et al. 2001). This sparked immediate interest among clinical researchers because TMS avoids systemic side effects and stimulates the brain with a spatial and temporal specificity that currently cannot be achieved pharmacologically or via electroconvulsive therapy. At the same time, rTMS makes it possible to test behavioural effects of brain stimulation in healthy volunteers. To be of lasting benefit beyond the period of stimulation, enduring changes in the functioning of the target pathways would need to be invoked. The duration of the after effects can last for 30–60 minutes, depending on parameters such as the number of pulses applied, the rate of application, and the intensity of each stimulus. One of the possible mechanisms for such rTMS effects can be long-term potentiation (LTP). However, the above-mentioned plasticity effects are often weak, highly variable between individual subjects, and rarely last longer than 30 minutes. Because rTMS of the cortex has the potential to induce epileptic activity even in healthy subjects, safety instructions have to be followed (Wassermann 1998).

To enhance LTP effects, new protocols, such as theta burst stimulation (TBS), have been introduced (Huang et al. 2005). In TBS, the 50-Hz bursts are repeated at a frequency of 5 Hz (theta range) and the protocol holds promise as a powerful LTP inducer. TBS has been applied to the primary motor cortex, tending to result in improved motor recovery following stroke (Talelli et al. 2007), as well as to brain regions outside the M1, with evidence of lasting inhibition demonstrated in the frontal eye field (Nyffeler et al. 2006), and the occipital cortex (Franca et al. 2006). With advanced technical solutions, new protocols and paradigms are continuously being tested and explored: paired associative stimulation (PAS) produces long-term plasticity effects, measured by an increase of MEPs in the target muscle for more than 30 minutes (Stefan et al. 2000; Meunier et al. 2007), triple-pulse TMS (Komissarow et al. 2004; Sacco et al. 2009) exerts a facilitatory effects on MEP

amplitude, quadripulse TMS (Hamada et al. 2007b) induces long-lasting locally restricted facilitation of motor cortex excitability for up to 75 minutes (Hamada et al. 2007a), which is considered to be a cortical event (Hamada et al. 2008).

In recent years TMS has been combined with EEG (Ilmoniemi et al. 1997; Esser et al. 2006; Massimini et al. 2005; Komssi et al. 2002; Kähkönen and Wilenius 2007), positron emission tomography (PET: Fox et al. 1997; Paus et al. 1997; Strafella et al. 2003; Ko et al. 2008), functional magnetic resonance imaging (fMRI: Bohning et al. 1997; Bestmann et al. 2006; Kemna and Gembris 2003; Siebner et al. 2003; Denslow et al. 2005), near-infrared spectroscopy (NIRS: Nissilä et al. 2002; Mochizuki et al. 2006), and MEG (Tamura et al. 2005). A combination of TMS with these methods offers an opportunity to localize the target of stimulation, to measure local and distal responses to the stimulation (*i.e.,* to study reactivity and connectivity the stimulated brain areas), to assess long-term (hours, days, weeks) effects of rTMS, and to investigate the pathophysiology of neuropsychiatric disorders.

TMS holds great promise in therapeutic and clinical settings. In addition to studying alterations of cortical excitability in neurological diseases (Di Lazzaro et al. 2004b; Kühn et al. 2004; Fisher et al. 2008) and task-related cortical excitability changes (Bestmann et al. 2002; Nixon et al. 2004; Ellison and Cowey 2008; Gallasch et al. 2009), the treatment of psychiatric disorders have been the focus of many studies (Cohen et al. 2004; Fitzgerald et al. 2003; Amiaz et al. 2009; Baumer et al. 2009; Kleinjung et al. 2008; Thickbroom et al. 2008). Even though it is unlikely that rTMS will restore function to specific sets of synaptic connections affected by the disease, it may be possible for rTMS to confer compensatory interaction with the normal processes of brain plasticity that accompany damage or chronic disease (Ridding and Rothwell 2007).

3.2 Physical aspects of TMS

Magnetic brain stimulation follows the fundamental physical principles of electromagnetic induction: if the conducting medium (*e.g.,* brain) is adjacent to a rapidly changing magnetic field, the current will be induced in that conducting medium. According to the Lenz's law, the flow of the induced current will be parallel but opposite in direction to the current in the coil (Fig. 3.1). The magnetic field pulse is generated by driving a current pulse $I(t)$ through an induction coil (Polson et al. 1982; Cadwell 1990; Barker et al. 1991; Jalinous 1991). Even though a TMS pulse must have a peak amplitude of up to 10 000 amperes within less than 100 microseconds, the basic electrical circuit of the magnetic stimulator is simple (Fig. 3.2). It consists of a capacitor (capacitance C), a thyristor (switch S), and the stimulating coil (inductance L). The circuit forms an RLC oscillator with a series resistance R in the coil, cables, thyristor, and a capacitor. The capacitor, charged to several kilovolts, is discharged through the coil by gating the thyristor into the conducting state (left panel in Fig. 3.2). In case of rapid-rate stimulators, during the second-half cycle of the oscillation (right panel in Fig. 3.2), the current in the circuit flows in the opposite direction, thus returning the charge to the capacitor through the diode D. If the thyristor gating is terminated during the second half-cycle, the oscillation ends

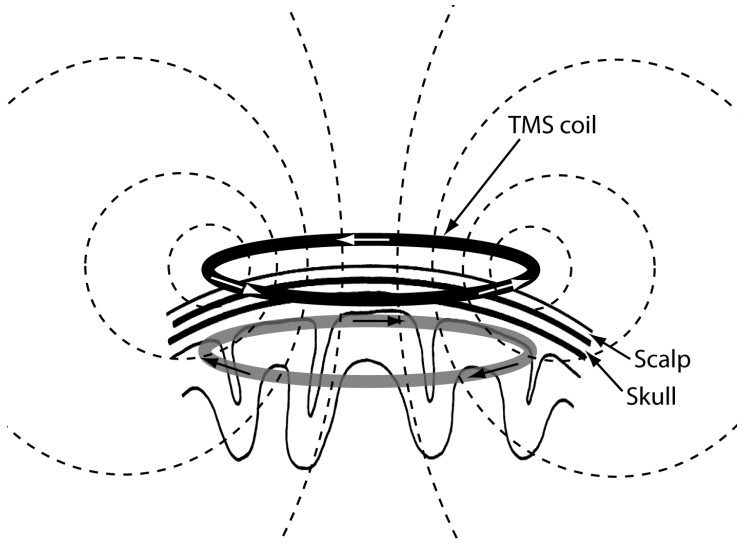

Figure 3.1: Lenz's law: when strong current flows through the magnetic coil placed over the scalp, as a consequence of electrochemical events, underlying cells generate weak electrical currents. Currents induced in the tissue obey Lenz's law - they are parallel to, but in a direction opposing the current flow in the coil.

when the cycle is completed.

Both the electric field **E** and current density **J**=ρ**E** (ρ being conductivity) induced in the neural tissue are proportional to dI/dt:

$$\mathbf{E}(\mathbf{r}) \sim \mathbf{J}(\mathbf{t}) \sim \frac{dI}{dt} = \frac{U_0}{L\omega} e^{\alpha t} = (\omega cos\omega t - \alpha sin\omega t). \tag{3.1}$$

When the electrostatic energy stored in a capacitor bank is discharged, it is transformed into the coil's magnetic energy - the peak energy is dependent on the coil's

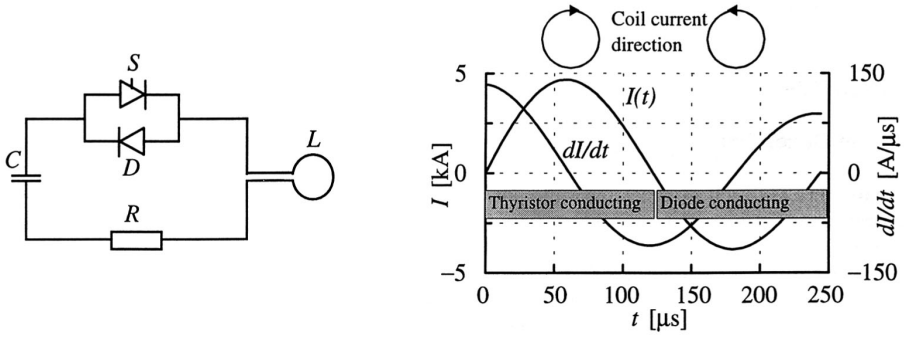

Figure 3.2: Left: basic electrical circuit of the magnetic stimulator. Right: when capacitor is discharged, the oscillating RLC circuit sets up a brief exponentially decaying sinusoidal current pulse I(t). The energy is returned through the diode D from the coil back to the capacitor, which reduces coil heating and power consumption (biphasic pulse). Without D or with great R, the current polarity reversal is absent or suppressed (monophasic pulse). Figure adapted from Ilmoniemi et al. 1999.

inductance L and the peak current in the coil:

$$W = \frac{1}{2} L I_{max}^2. \tag{3.2}$$

The efficacy of the coil can be improved if the inductance and the peak current can be lowered without affecting the strength of the induced electric field. After the electronic sequence of firing the TMS pulse is initiated, the energy is dissipated as a Joulean heating in the coil, cables and electronic components. Most of the circuit's total resistance is in the coil (*e.g.,* 15 out of 20 mΩ); hence most of the heat dissipation is on the coil. This brings into focus coil warming, which is limited by the Safety Standards for Medical Equipment (IEC-601) to temperatures below 41° Celsius. The optimal temperature rise should be limited to about 0.1° per pulse.

The electric field vector $\mathbf{E}$ and magnetic field vector $\mathbf{B}$ can be determined from a scalar potential Φ and a vector potential $\mathbf{A}$ (Jackson 1975; Reitz et al. 1980):

$$\mathbf{B} = \nabla \times \mathbf{A} \tag{3.3}$$

$$\mathbf{E} = -\frac{\partial \mathbf{A}}{\partial t} - \nabla \Phi \tag{3.4}$$

The scalar potential Φ is the same as voltage $\mathbf{V}$ and its source is the charge, while the source of vector potential $\mathbf{A}$ is the current. The vector potential only contributes to the induced electric field if it changes with time (coming from the changing current in the coil and changing magnetic field $\mathbf{B}$). The change of current in the stimulating coil is so rapid that kilo-amperes of current are driven in typically 100 μs, so $\mathbf{A}$ must be considered. Total electric field induced in the tissue, $\mathbf{E}(\mathbf{r}, t)$, is the sum of two terms: $\mathbf{E}_\mathrm{A}$ due to the current integrated over the coil, and $\mathbf{E}_\Phi$ due to the charge integrated over the tissue surface, denoted as primary and secondary electric fields, respectively. The primary electric field $\mathbf{E}_\mathrm{A}$ is induced directly by the changing magnetic field. The electric field produced by magnetic induction forms closed loops concentric with the coil (see Fig. 3.1). In response to this field, charged ions in the tissue move, following the electric field lines until they reach the surface of the tissue or the skull. Thus, $\mathbf{E}$ causes a flow of current according to Ohm's law, $\mathbf{J}{=}\rho\mathbf{E}$, with ρ being conductivity. Since air is an isolator, the charge accumulates on the surface of the skull, *e.g.,* a non-uniform conductivity along the path of the current results in an uneven distribution $\rho{=}\rho(\mathbf{r})$ of electric charges (Ilmoniemi et al. 1999). These charges produce their own electric field, the secondary field $\mathbf{E}_\Phi$, according to Gauss's law, $\nabla{\cdot}\mathbf{E}{=}\rho/\epsilon_0$. The total electric field is then the sum of the fields due to the charge and magnetic induction (Roth and Basser 1990):

$$\mathbf{E} = \mathbf{E}_\mathrm{A} + \mathbf{E}_\Phi = -\frac{\partial \mathbf{A}}{\partial t} - \nabla \Phi \tag{3.5}$$

The induced electric field strength for brain stimulation should be in the order of 100 mV/mm to elicit sufficient motor-cortex activation that would lead to measurable peripheral EMG responses (Ilmoniemi et al. 1999).

3.3 Electrophysiology of excitation in TMS

The activated region under the coil is defined by the strength and direction of the induced electric field with respect to neuronal structures (Komssi and Kähkönen 2006). Macroscopically, the locus of TMS-induced activity is most likely at the maximum of the induced electric field (Krings et al. 1997). Based on experimental evidence in humans (Garnham et al. 1995), it is likely that high effective gradients of the induced electric field ($\partial \mathbf{E}_x/\partial x$) are achieved at axonal bends even in homogeneous $\mathbf{E}$ (Abdeen and Stuchly 1994).

At the cellular level, TMS is thought to excite mostly the corticospinal axons (in M1) close to the axon hillock (Baker et al. 1995; Rothwell 1997), rather than other parts of the neurons (Maccabee et al. 1996). This suggests the pyramidal neurons are activated predominantly transsynaptically, via interneurons in superficial cortical layers (Fig. 2.2; Day et al. 1989; Di Lazzaro et al. 2001a; Nakamura et al. 1996; Sakai et al. 1997; Mills 1991). According to this view, the action potentials are initiated at the initial segment of the axon, close to the cell body (soma) of the neuron and travel both orthodromically and antidromically (Stuart et al. 1997). The most efficient direction of induced current for activation of corticospinal neurons is one along the axis of the neuron (parallel to the apical dendrite) towards the cell body and the initial segment.

However, this view partially contradicts with findings showing that different neuronal structures seem to be preferentially targeted by the different coil orientations (Brasil-Neto et al. 1992; Fox et al. 2004; Mills et al. 1992; Pascual-Leone et al. 1994a; Sakai et al. 1997; Werhahn et al. 1994). It has been hypothesized that these differences may be attributable to different populations of fibres being excited by anterior-posterior (AP) versus posterior-anterionr (PA) directed currents (Di Lazzaro et al. 2001b). It is possible that large afferent axons from premotor and somatosensory areas, which constitute the main cortical input to the motor cortex (DeFelipe et al. 1986; Sutor et al. 2000), may be especially sensitive to AP currents. There, afferents bend into motor cortex (Rockel et al. 1980), and it is known from modelling and peripheral nerve stimulation studies that axonal bends in large fibres have a low threshold for TMS activation (Abdeen and Stuchly 1994; Maccabee et al. 1993; Esser et al. 2005).

Response of the motor cortex to TMS is complex and consists of two major stages. In the first stage, the motor cortical system responds with waves of activity that can last for 5–10 ms after the pulse (Day et al. 1987; Esser et al. 2005). These waves are typically recorded with electrodes positioned in the epidural space in the form of compound action potentials from the axons descending from the motor cortex originating in layer 5 (Fig. 2.2; Di Lazzaro et al. 1998a; Di Lazzaro et al. 2001b; Edgley et al. 1997). The volleys are called direct (D) and indirect (I) waves (Patton and Amassian 1954). D-waves are generated by direct stimulation of corticofugal axons in the white matter, whereas later I-waves come from indirect or trans-synaptic activation of the same corticospinal neurons (Amassian et al. 1990; Edgley et al. 1990; 1997; Burke et al. 1990; 1993; Di Lazzaro et al. 1998b; Houlden et al. 1999).

The second stage of the motor cortical response to TMS is characterized by a longer

period of suppression of ongoing voluntary activity in the EMG, lasting 100–200 ms, which most probably comes from long-lasting inhibitory input mediated by γ-aminobutyric acid (GABA) neurotransmitters responsible for regulating neuronal excitability throughout the nervous system (Ridding and Rothwell 2007; Werhahn et al. 1999; Di Lazzaro et al. 2007; Florian et al. 2008).

3.4 Electrophysiological state-dependency of TMS

One of the fundamental concepts in brain physiology is the functional state of the cortex, which has important electrophysiological consequences for neuronal activity during TMS. Both D- and I-waves were shown to be affected by the current state of the cortex. Since the D-waves arise from the initial segment, their generation will be affected by the overall excitability of the neuron. Hence, D-wave response is likely to be affected by factors influencing the cortical excitability - a fact demonstrated in numerous studies (*e.g.,* Di Lazzaro et al. 2003; Di Lazzaro et al. 2004a). Similarly, the number and the amplitude of the I-waves increase with the level of neuronal activity, showing that they are also strongly influenced by the overall level of excitability (*e.g.,* Cash et al. 2009; Di Lazzaro et al. 2008). This result was expected, because I-waves require transmission through a larger network of neurons (Bohning et al. 2000). The important point here is that any neuronal processes affecting the cortical excitability are likely to be reflected in the efficacy of TMS to stimulate neuronal networks. Indeed, an initial functional neuronal state plays a major role in the modulation of the MEP amplitude, being specific for the type of performed task (Cracco et al. 1999; Nielsen et al. 1999; Bestmann et al. 2004; Tamburin et al. 2005; Gallasch et al. 2009; Bikmullina et al. 2009).

TMS-induced neuronal activity spreads beyond the directly stimulated area to anatomically connected sites (Bohning et al. 2000; Ilmoniemi et al. 1997; Komssi et al. 2002; Paus et al. 2001; Strafella et al. 2001). This implies an important reverse: namely, the anatomically connected sites can equally exert an exogenous influence on the stimulated cortical area, thus making the effect of TMS a function of the activity in anatomically interconnected sites (not the case, though, it could be unidirectional connections). To support this, there is growing electrophysiological evidence also from stimulation of cortical areas other than motor, indicating that the neural impact of TMS is not determined only by the properties of the stimulus, but also strongly by the initial state of the activated interconnected brain regions (Amassian et al. 1989; Ramos-Estebanez et al. 2007; Silvanto et al. 2007; Silvanto and Muggleton 2008).

4 Electrophysiological assessment of cortical excitability

4.1 Concurrent TMS with EEG

Measuring the neuronal electrical activity elicited by TMS is a relatively new modality of functional brain mapping. It enables experimenters to stimulate many regions of the cortical mantle, thus providing real-time information about the state of the cortex (the state of the cortex is usually understood as the distribution of chemicals in intra- and extracellular spaces, membrane potentials, and overall configuration of the cells at the time of stimulation). With an excellent time resolution at a millisecond level, TMS-EEG provides a measurement and mapping of cortical excitability and reactivity (Publications I–IV), monitors how brain oscillatory activity is modulated by targeted stimulation (Publication VII), measures functional connectivity between brain areas and between central and peripheral parts of the nervous system (Publication III), monitors the effects of rTMS during and after treatment, or monitors the safety of magnetic stimulation and alerts if epileptiform activity appears in the EEG (Publication VII). In order to effectively measure the EEG response induced by the TMS, it is necessary to consider several major technical challenges. A successful solution will require various aspects of engineering, combined with electrophysiological and anatomical knowledge, and even electrochemical reactions. TMS-EEG was utilized in Publications I–V of this thesis.

4.1.1 Technical aspects

Figure 4.1 describes the general technical setup that was used for acquisition of all TMS-EEG data sets for this thesis. The central hardware units consisted of an *Experiment-controlling computer* and *Stimulus generation device*, since the whole experimental paradigm is programmed using these two devices, and they control the rest of the hardware. An important aspect of our protocols is integration of peripheral and central responses within TMS paradigms studying the primary motor cortex. Some parts of the data presented here combine both types of activity by using EEG and EMG recordings obtained concurrently with the TMS of the motor cortex. For these measurements, it is important that event-triggers in all recording devices are registered for later off-line data analysis. Fig. 4.1 also schematically describes the triggering scheme between the devices. It is important to note that the output triggers from the magnetic stimulator were collected (for indication that TMS pulse was actually fired) in both the EEG and EMG recording devices (in Fig. 4.1 denoted as 'ST2' and 'EMG trigger collector' inputs, respectively). This is essential in faster oddball paradigms in which events (*i.e.,* stimuli) are interchanged rapidly, and the TMS pulse accompanies only some of these events.

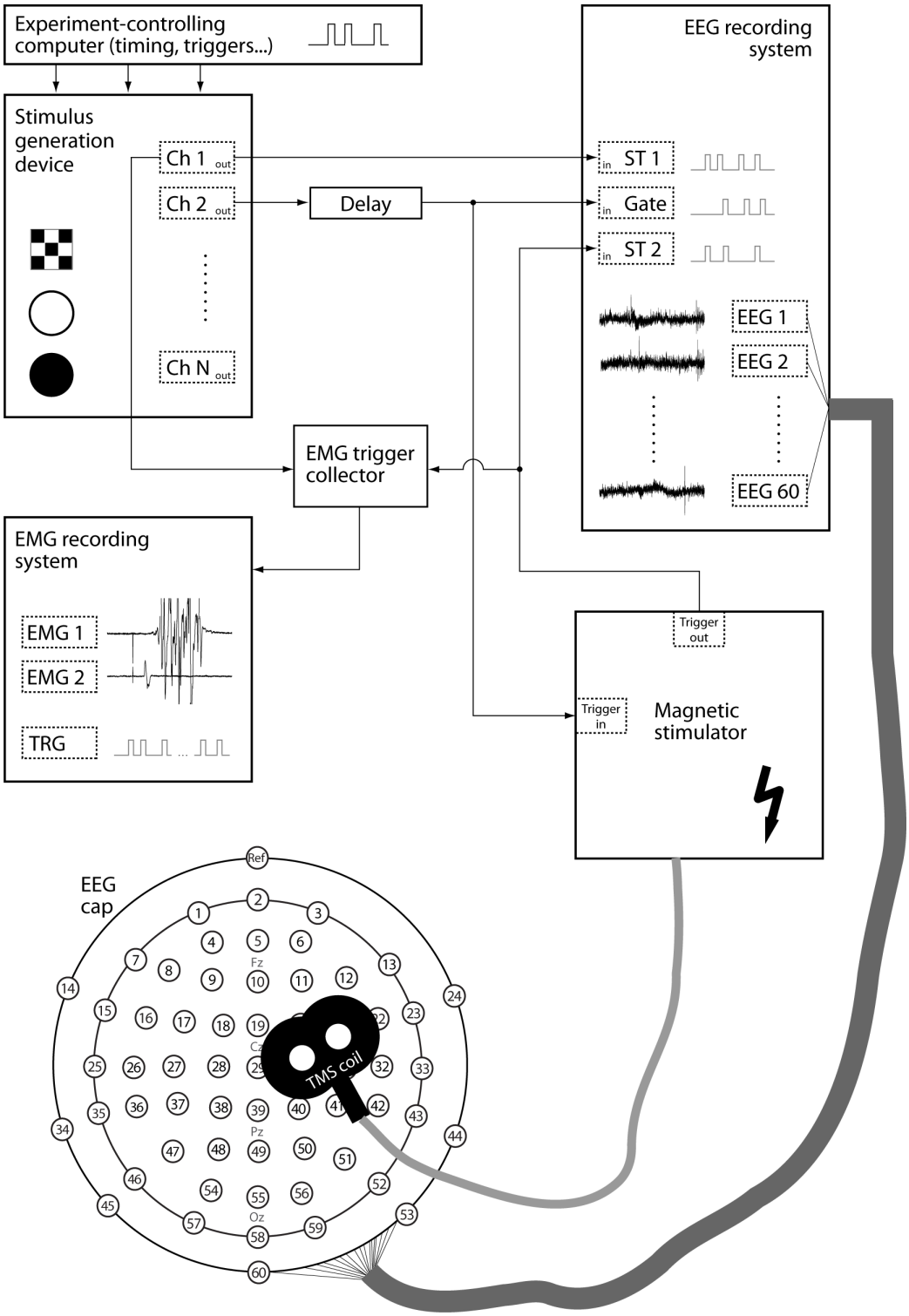

Figure 4.1: A comprehensive experimental setup for recoding the EEG responses to TMS, with parallel recording of the peripheral MEPs.

Because of the fairly long recharging periods of some of the TMS devices, it might happen that the TMS pulse might fail to fire 'on time' within the sequence of other

stimuli. In such cases, the input trigger from the *Experiment-controlling computer* would indicate this as a 'TMS event', even though the TMS was actually not delivered. If present in abundance, such epochs could substantially influence the results. Recording output TMS triggers overrides this problem.

4.1.2 TMS-evoked EEG responses

The first measurement of complete scalp distribution of ERPs following TMS was reported by Ilmoniemi et al. (1997) with an EEG amplifier that was specifically designed to operate in the harsh electromagnetic environment of TMS pulses (Virtanen et al. 1999). Similarly to MEPs, the TMS-evoked EEG responses had until that time mostly been investigated in the motor cortical system (Ilmoniemi et al. 1997; Komssi et al. 2002; Bender et al. 2005a; Massimini et al. 2005; Esser et al. 2006). In this thesis, EEG (with concurrent TMS) was measured with 60 electrodes covering the whole scalp (see Figs. 4.1 and 4.2). A typical topographical plot of TMS-evoked

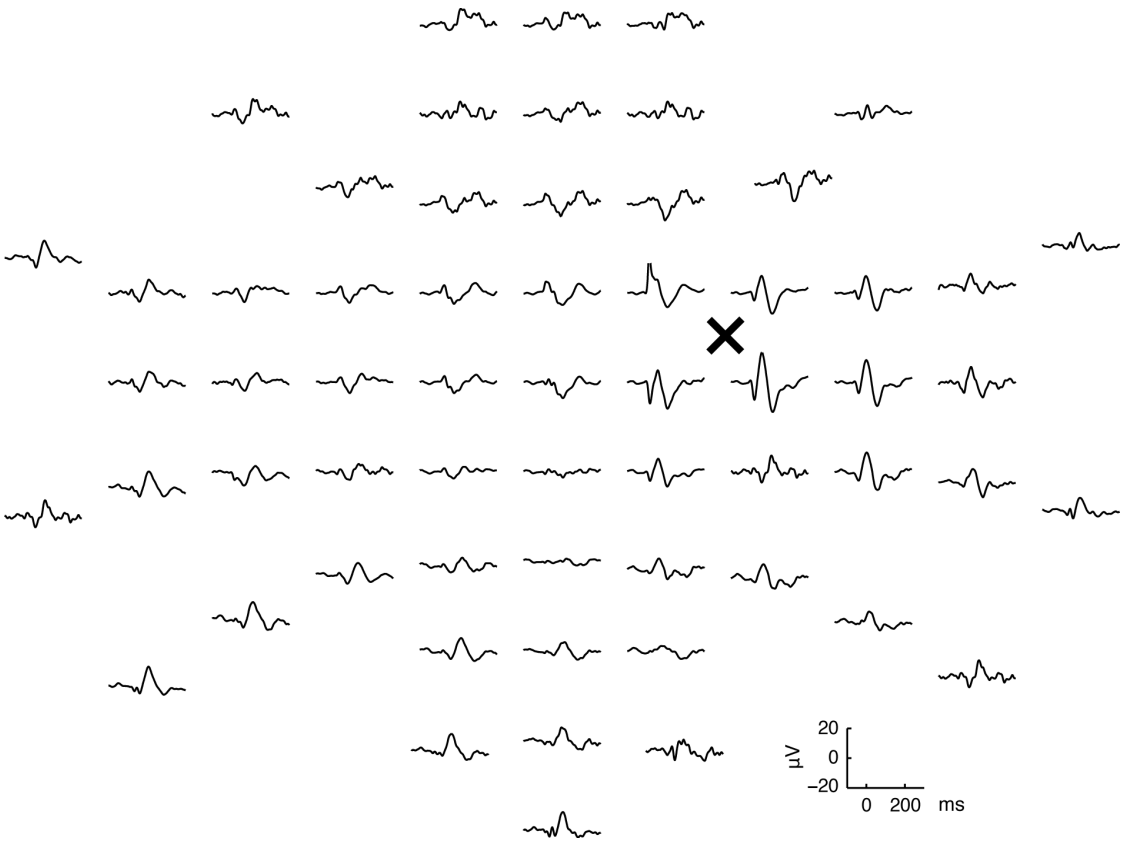

Figure 4.2: Averaged responses evoked by the TMS in one subject. The signals are arranged according to the layout of the electrodes (the view is from the top of the head, nose pointing upward). Prominent response amplitudes at latencies of approximately 50 to 100 ms are dominant in the vicinity of the stimulated point (denoted with 'X'). Note the lateralization of responses: in the vicinity of the stimulated site, the amplitudes are the highest, attenuating with increasing distance from the coil.

EEG responses after stimulation of the right motor cortex is shown in Fig. 4.2. The purpose of such a measurement is to detect both the local and distal effects of TMS: both to measure local excitability of the stimulated patch of the cortex, as well as to assess the spreading of TMS-evoked activity in a broader cortical network.

Fig. 4.2 also shows that the overall responses amplitudes are the highest right under the coil, diminishing with increasing distance from the stimulation point. An important feature of TMS-evoked EEG topography is that even though only one cortical hemisphere was stimulated, clearly bilateral EEG responses are evoked with different features. This confirms interhemispheric connectivity, which was proposed previously to be transcallosal (Ilmoniemi et al. 1997; Komssi et al. 2002; 2004). Locally, within one hemisphere, an increased EEG activity can be seen in a number of neighbouring electrodes, suggesting the spread of TMS-evoked activity to anatomically interconnected cortical areas (Bohning et al. 2000; Fox et al. 1997; Ilmoniemi et al. 1997; Komssi et al. 2002; Paus et al. 1997; 2001; Siebner et al. 2000; Strafella et al. 2001).

The averaged response of approximately 60 single trials from a single channel in the immediate vicinity of the stimulating coil is shown in Fig. 4.3. The investigators have been able to identify several components of the EEG response to a single-pulse TMS in the motor cortex: N15 (negative EEG deflection peaking approximately 15 ms post-stimulus), P30 (positive EEG deflection, 30 ms post-stimulus), N45, P55, N100, P200 (Komssi et al. 2002; 2004; Nikouline et al. 1999; Paus et al. 2001; Bender et al. 2005b; Massimini et al. 2005; Esser et al. 2006). However, it should be noted that the component structure may vary depending on the subjects (*e.g.,*

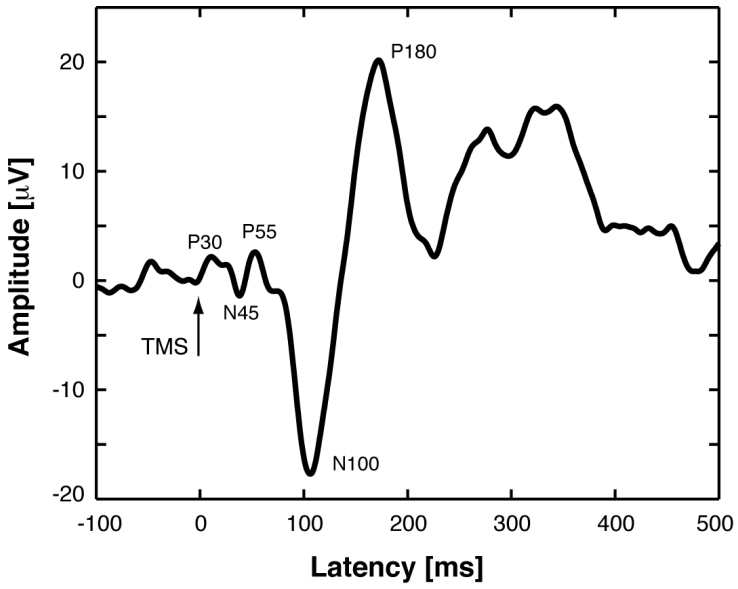

Figure 4.3: TMS-evoked EEG response from the motor cortex: single channel response in the vicinity of the stimulated cortical site. The names of the components relate to the polarities and latencies. The structure and latencies of the peaks may vary slightly between subjects and measurements.

healthy volunteers vs. patients), experimental setup (*e.g.,* no-task or performing the task), or pharmacological manipulation. Indeed, several reports have indicated large variability in the responses at latencies from 0 to 70 ms (Komssi et al. 2002; Bonato et al. 2006; Kähkönen et al. 2004).

In our measurements, the most pronounced and reproducible component across subjects and conditions was the TMS-evoked N100 component (Fig. 4.3), in agreement with other reports (Paus et al. 2001; Bender et al. 2005a; Massimini et al. 2005). This component peaks at about 100 ms after the TMS, with channels having the highest N100 amplitudes being located in the vicinity of the stimulated cortical site. This component was shown to be a robust TMS-evoked EEG response sensitive to subtle changes in cortical excitability (Bender et al. 2005a; Kähkönen and Wilenius 2007), and was suggested to represent the inhibitory response after activation of inhibitory interneurons, reflecting the activation of GABA-B receptors (Connors et al. 1988; Werhahn et al. 1999; Tamas et al. 2003; Markram et al. 2004). In order to maintain compatibility with the large pool of TMS-MEP studies, the EEG responses are also typically referred to as TMS intensity during their recording (*e.g.,* 90% MT). Importantly, it has been shown that clear EEG responses were elicited even at subthreshold TMS intensities, when no peripheral muscle activity was observable (Komssi et al. 2004; Kähkönen et al. 2005). These findings have been previously indicated by combined TMS and fMRI measurements (Bohning et al. 1999; Nahas et al. 2001) and confirm the TMS-EEG as a sensitive method for assessment of cortical excitability.

4.1.3 Reliability of TMS-EEG recordings

The amplitude of EEG signals is typically within a 1–100 microvolt range. Their quality and reliability in the harsh electromagnetic environment of TMS is not an easily achievable goal. Electric disturbances arising from the electronics or the subject may appear in parallel with responses reflecting the real neuronal activity, making the analysis and the interpretation of results difficult. Amplifier saturation is the greatest technical challenge for recording the EEG concurrent with TMS (Izumi et al. 1997; Virtanen et al. 1999; Fuggetta et al. 2005). For example, if the distance between two EEG electrodes on the scalp (*e.g.,* mounted on the EEG-cap) is approximately 20 mm, the induced voltage caused by applied magnetic TMS pulse is in the order of 50 V (Virtanen et al. 1999). An effective TMS-compatible EEG amplifier has to recover from the 50-V pulse fast enough to record the six orders of magnitude smaller ERPs (measured in microvolts) following the pulse.

EEG electrodes have a general purpose of establishing good electrical contact between the skin and the amplifier input, via an electrolyte. The resistance of the electrode contact should be low compared to the input impedance of the amplifier, and sufficiently low to avoid thermal noise in the contact resistance. Heating of the electrodes is caused by the eddy currents induced by the changing magnetic field and is proportional to the square of TMS intensity and the square of the electrode diameter, but independent of the thickness (Roth et al. 1992). The most commonly used electrodes in modern commercial TMS-EEG systems are small Ag/AgCl pellet

electrodes, which exhibit less electric noise than equivalent metallic Ag electrodes (Geddes and Baker 1980). Ag/AgCl pellet electrodes are electrically very stable and can also efficiently remove the direct-current shift that may appear in signals recorded just under the coil (Virtanen et al. 1999). However, they are not without defects. For example, an electro-deposited chloride coating can be relatively easily removed by abrasion - then the level of exhibited noise is far higher than with the AgCl layer. Moreover, Ag/AgCl is photosensitive, *i.e.,* changes its potential slightly when exposed to light (Geddes and Baker 1980).

Sources of disturbances in the EEG signal are numerous. Movement of the electrodes due to TMS coil vibration causes a disturbance of the electrical double layer at the skin-electrode interface, reflected usually as a DC shift in the recorded signal. This event is in the frequency range of many bioelectrical events (Geddes and Baker 1980) and may have a decay constant as high as 300 ms (Virtanen et al. 1999). It has been quite problematic (Paus et al. 2001; Komssi et al. 2004), though the filtering may be employed with success (Komssi et al. 2002). Electrical stability of an electrode in TMS recording is considerably enhanced by stabilization of the electrode-electrolyte (*i.e.,* skin) interface (Virtanen et al. 1999). Preparing impedances for all electrodes in an array to an equal level minimizes coupling of the mains voltage to the recording circuitry. It is useful to place the reference electrode into a relatively electrically inactive position, such as forehead, nose or mastoids.

A very common and strong artefact (at the millivolt scale) may result from direct stimulation of cranial muscles. This usually occurs when the coil is held above the lateral aspects of the head, or near the neck for stimulation of directly underlying cortical neurons. These artefacts are very strong and may last tens of milliseconds, masking the real neuronal activity. Scalp movements can also cause disturbances and are transferred to the EEG signal through electrode contacts (Paus et al. 2001).

Each TMS pulse is associated with a loud click (up to120 dB), which inevitably activates the subject's auditory system, giving rise to an auditory-evoked potential. These middle-latency auditory evoked potentials, such as P30 or P55, usually demonstrate a fronto-central distribution (Cohen 1982; Woods et al. 1987; Deiber et al. 1988) and may partially arise from auditory activation due to the coil click (Komssi and Kähkönen 2006). Sometimes, good hearing protection may be sufficient to deal with the coil click, but one has to be aware that a large part of the effect may be due to bone-conducted sound (Nikouline et al. 1999), which is difficult to mask. More complete suppression of the auditory activation due to coil click can be obtained by playing acoustic noise through headphones during TMS (auditory masking) in addition to hearing protection (Paus et al. 2001; Fuggetta et al. 2005; Massimini et al. 2005).

TMS also activates the sensory terminals at the scalp, giving rise to a somatosensory brain response, which may affect data interpretation. The latency of the N45 response coincides with that involving the conduction of a motor command to the hand muscles and the return of subsequent sensory afferent to the cortex (Tokimura et al. 2000). The potential pattern of N45 remains unchanged regardless of sub- or suprathreshold TMS intensities, strongly indicating that N45 is not generated by afferent input from peripheral muscles (Nikouline et al. 1999; Paus et al. 2001).

4.2 TMS combined with MEG

Recent years have seen enormous interest in the use of rTMS for both clinical research (treatment of neurological and psychiatric disorders) and basic brain research. This has sparked methodological and technical investigation in an effort to find paradigms that could induce strong, long-lasting effects using lower stimulation intensity and a shorter period of stimulation compared to conventional rTMS protocols (Cardenas-Morales et al. 2009). Here, MEG becomes increasingly relevant for mapping the effects and efficacy of rTMS protocols, since it offers far better time resolution than conventionally used techniques, such as fMRI, or PET. Furthermore, advanced source localization methods combined with artifact removal solutions (Taulu et al. 2004) enable the recording of subjects with implants and even life support and other assisting devices (Taulu and Simola 2006) - the patient groups that were up to several years ago unthinkable as participants in MEG studies.

Technically, the only possible combination of TMS and MEG at present is that in which the two measurements are separated in time, usually referred to as 'TMS combined with off-line MEG'. Figure 4.4 describes the off-line MEG protocol for mapping the effects of a single rTMS treatment, utilized in Publication VI of this thesis. With TMS applied first, off-line MEG imaging is usually used to study the

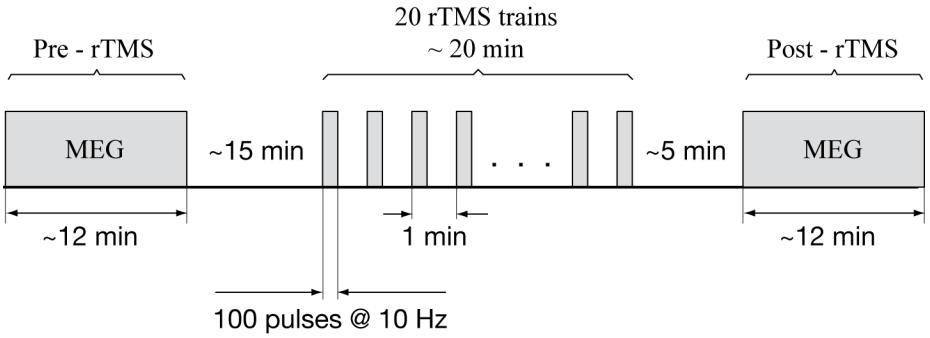

Figure 4.4: Technical setup of TMS-MEG combination. Spontaneous brain oscillations were recorded with MEG before and after the rTMS treatment in which 20 blocks of 100 TMS pulses were delivered to the patient's motor cortex.

long-lasting effects of rTMS on brain function, or spontaneous brain oscillations. If MEG measurement precedes the TMS, it is most probably used to define appropriate cortical sites to be targeted by TMS. Although the neurobiological mechanisms of rTMS are not fully understood at present, they may involve long-term potentiation (LTP)- and depression (LTD)-like processes, as well as inhibitory mechanisms modulated by GABA-ergic activity. The development of optimized rTMS protocols for directing the effects to specific cortical circuits, such as the thalamo-cortical motor loop of interest in Parkinsonian patients, and afterwards utilizing MEG to precisely track the time course of excitability fluctuations in a studied neuronal network have been the focus of contemporary rTMS-MEG studies (Tamura et al. 2005). Obtained data can be subsequently used to further study functional aspects of selected cortical networks.

5 General methods

5.1 Instrumentation and methodology of TMS

5.1.1 Magnetic stimulators

Single pulse TMS in Publications I, II, and IV was performed with Magstim 200 (The Magstim Company Ltd, Whitland, UK) device connected to a coplanar figure-of-eight coil (NP 9925) with an average diameter for each wing of 70 mm. In Publications III and VII, the Nexstim stimulator (Nexstim Oy, Helsinki, Finland) was used in combination with a figure-of-eight coil with a 70-mm outer diameter for each wing. Publication V utilized the Magstim Rapid stimulator.

Rapid rate TMS was used only in Publication VI. Twenty trains consisting of 100 pulses at 10 Hz were delivered at 1-min inter-train intervals. A coplanar figure-of-eight coil was used to deliver trains of rTMS pulses produced by the Magstim Rapid Stimulator.

Sham TMS for control conditions was systematically performed in Publication I and Publication VI in order to check the effect of auditory stimulation alone, and to measure the auditory responses associated with the residual coil click.

5.1.2 Navigated TMS

At the micro-level, the motor cortex consists of spatially discrete clusters of neurons primarily responsible for activation of specific lower motor neurons (Asanuma et al. 1976; Cheney and Fetz 1984). Thus, even small errors in coil placement might lead to a difference in the neurons excited in cortical neuronal clusters, and thereby contribute to variation in the MEPs. Therefore, precise targeting of the TMS coil is needed to accurately repeat the cortical stimulation. This is possible by tracking the location and orientation of the TMS coil relative to the subject (Fig. 5.1).

The navigated TMS targeting system should provide essential information regarding the relationship between the functional aspects of the stimulated cortex, cortical surface anatomy and/or pathological lesions as they exist in individual subjects/patients. The work described in this thesis benefits from the use of navigated TMS (eXimia, Nexstim Ltd., Helsinki, Finland) for enhanced precision and reproducibility of the stimulation. Relative positions of the subject's head and the TMS coil are determined and tracked in real-time by means of an optical locating system, which has a precision of less than 1 mm. In practice, however, this precision can be affected by imperfect registration of the subject's head (with her/his MRI), fixation of the trackers on the coil, errors in optical tracking, and possible head movements. The nTMS system also takes into account the stimulation intensity, coil parameters, and the individual brain anatomy. The intracranial electric field calculation based on the spherical model (Sarvas 1987) is visualized and matched to the 3D reconstruction of individual subject's brain MRIs. The induced electric field is visualized on a colour-coded map based on individual MRIs, enabling the operator to see in

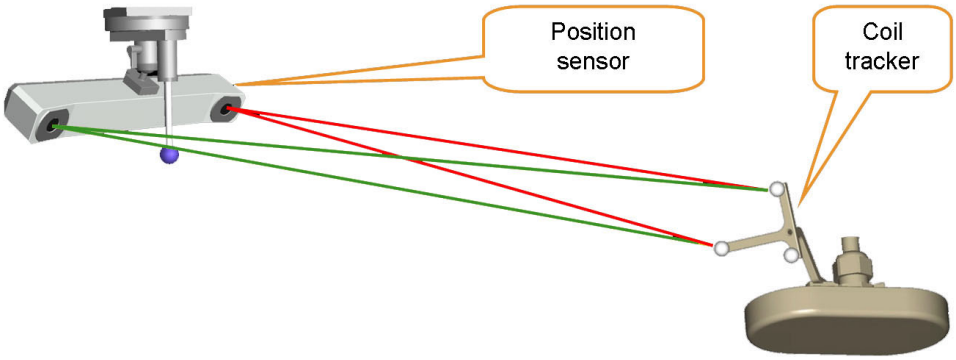

Figure 5.1: Navigation system for the TMS coil. The beam of light is emitted from the emitting diode to be reflected from the coil trackers back to the coil position sensor. The same principle works for the tracking of the subject's head movement. Figure courtesy of Jarmo Ruohonen, Nexstim Oy - Helsinki, Finland.

advance the exact cortical location being stimulated (see, Fig. 1A in Publication III and Fig. 1 in Publication IV). Using this system, the cortical target as well as the coil position, direction, and the angle of the stimulator were monitored in real time throughout the sessions. The device also allows the user to digitize the locations of the EEG electrodes for each subject (see, the lower panel of Fig. 1 in Publication V). The system records the orientation of the coil, its location, and induced electric field for every stimulus pulse. These recorded parameters can be recalled to reproduce the location and orientation (direction and angle) of the coil in subsequent stimulation sessions.

5.1.3 Cortical targets

Classical studies involving direct electrical stimulation of the cortex (Penfield and Boldrey 1937) consistently and repeatedly showed a defined myotopic organization of M1 where stimulation of a small cortical patch leads to activation of a specific effector. Surprisingly, a recent anatomical study (Rathelot and Strick 2006) showed that direct corticomotor neuron monosynaptic connection has a wide distribution in M1 for a specific muscle, *e.g.,* finger muscles. One should note, however, that direct monosynaptic cortico-spinal (CS) projections constitute only a minor part of the CS tract. When electric or magnetic stimulation of the cortex is used, a large number of output pyramidal cell are activated which have mono- and oligosynamptic connections with alpha-motor neurons in the spinal cord. It appears that such heterogeneous activation of the CS tract has the virtue of activating distinctly specific muscle groups. Specifically, muscle representations in M1 were the stimulation targets in the present thesis. For the hand area, they are localized in the anterior bank of the central sulcus, approximately between the two junctions - one is between the precentral and superior frontal sulcus, and the other is between the postcentral and intraparietal sulcus (Rumeau et al. 1994; Sastre-Janer et al. 1998) - and are distinguished by a knob-like form (Yousry et al. 1997) that resembles the letter 'omega' (present in 90% of population), or 'epsilon' (present in 10% of population). This

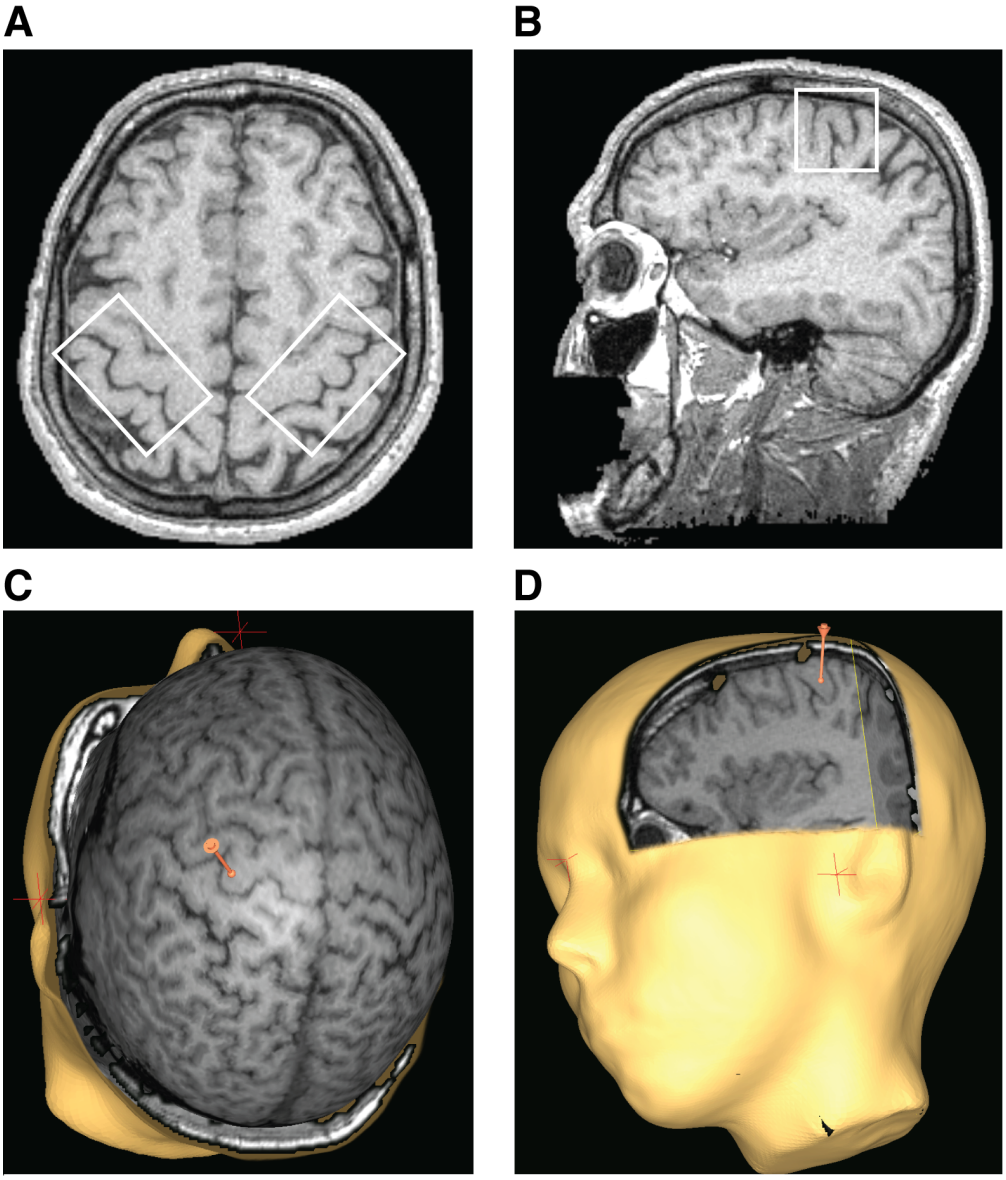

Figure 5.2: Localization of the functional hand area in the motor cortex. A) An omega-shaped segment on the anterior bank of the central sulcus containing hand projections on the motor strip in coronal MRI slices. C) The same structure in 3D nTMS visualization. B) The specific hook-like form of the precentral gyrus within the hand area in saggital MRI slices. D) The same structure in 3D nTMS visualization.

form was evident in the axial slices visualized by the neuronavigational system, as shown in Fig. 5.2. In saggital slices, the precentral gyrus within the hand area has a specific hook-like form (Fig. 5.2B). These cortical forms could be distinguished in all of our subjects and patients. A practical procedure for targeting the motor cortex consisted of two steps: (i) using MR images, we identified the hand area on the anterior bank of the central sulcus (Fig. 5.2A); and (ii) in the vicinity of the hand area, we performed a search for the optimal position where TMS evokes the

strongest MEPs.

The primary motor cortex was stimulated in all studies (I–VII), with the coil being placed tangentially to the scalp and the handle pointing backward and laterally at approximately a 45-degrees angle away from the midline in order to achieve the strongest stimulation of the motor cortex (Thielscher and Kammer 2002; Ziemann et al. 1999). Thus, the current induced in the brain had a posterior-to-anterior orientation, approximately perpendicular to the orientation of the central sulcus (Brasil-Neto et al. 1992).

Apart from motor cortical representations, Publication IV included magnetic stimulation of the dorsolateral prefrontal cortex (DLPFC) in the left middle frontal gyrus, located from a 3D MRI reconstruction, based on anatomical sketches (Yousry et al. 1997). In Publication V, targets other than the primary motor cortex were selected according to source locations (and their time courses) as identified by MEG modelling after electrical somatosensory stimulation of the median nerve of the dominant hand.

5.1.4 Protocol: functionally specific cortical excitability

Figure 5.3 presents the general design of the experimental setup used for investigating functionally specific changes in cortical excitability in this thesis.

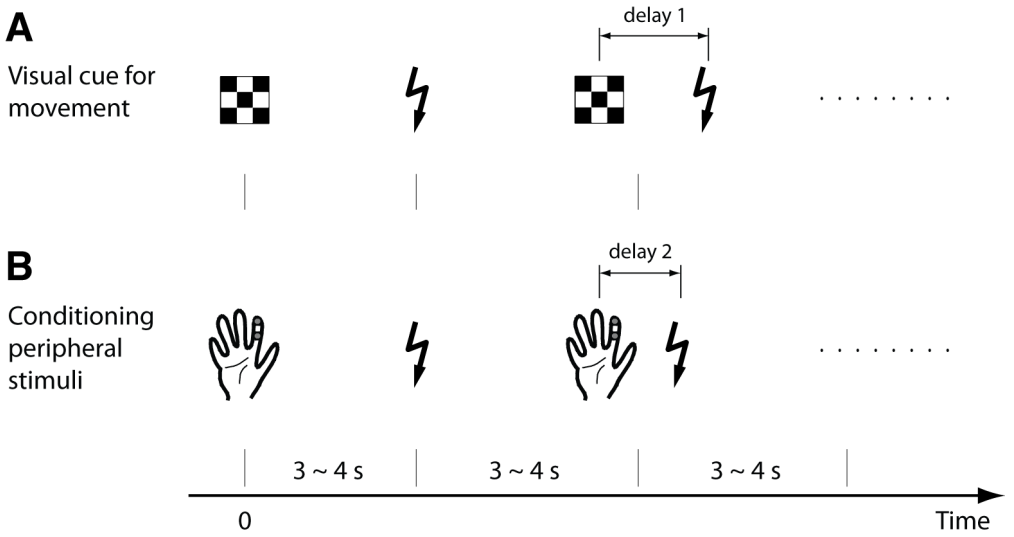

Figure 5.3: General experimental setup for assessing functionally specific cortical excitability. In the presented examples, transient modulation of cortical excitability was achieved prior to TMS by: A) brisk finger movements in response to the visual cue, or B) peripheral electrical stimuli.

The subjects were seated comfortably in an armchair, fully relaxed, with eyes opened with a fixed gaze. TMS was applied time locked to a specific event, which transiently modulated cortical excitability at the target cortical network, such as the instruction to make a brisk unilateral finger movement triggered by a visual cue (Fig. 5.3B,

Publications I and II), the peripheral somatosensory electrical stimulus (Publicatoin III), or a unilateral motor reaction to the peripheral somatosensory electrical stimulus (Publicatoin IV). Both EEG and EMG were recorded continuously and simultaneously during the whole duration of each session.

Depending on the exact experimental paradigm, there were several stimulation categories (normally 2 or 3) distributed across several measurement sessions and conditions. For example, Publication II (see Fig. 5.3A) used three categories of stimuli (visually-cued movements alone, TMS alone, and visually-cued movements followed by TMS) separated into three conditions (ipsilateral motor response, contralateral motor response, and no motor response), each repeated in two sessions. We took special care to balance between overall duration of the sessions and the total number of epochs in order to prevent the measurements from becoming biased by the subject's fatigue or unwanted peripheral tonic muscle activity.

5.1.5 Analysis of TMS-evoked neuromodulation in EEG

In Publications I–V, the ERPs were obtained from the electrophysiological recordings of brain potentials synchronized with delivery of TMS. The analysis of multichannel ERPs focused on time segments from –100 ms up to +500 ms with respect to the TMS. The N100 component was emphasized, since it was hypothesized to reflect functionally specific changes in cortical excitability. In Publications I and II, the modulation of cortical excitability was achieved by performing a unilateral

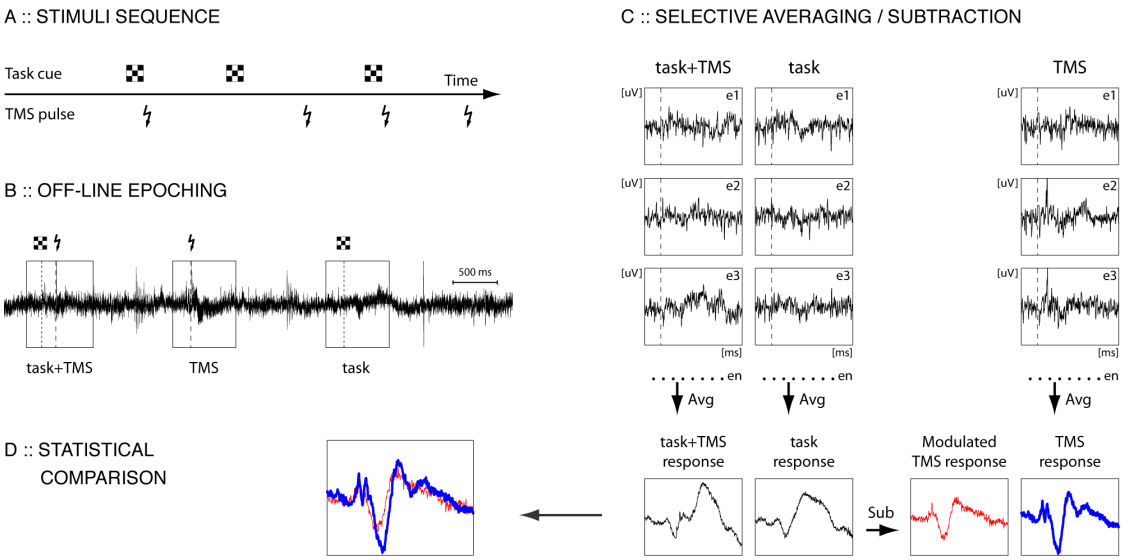

Figure 5.4: Results revealing functionally specific changes in cortical excitability. A) During the data acquisition, stimuli are presented in a specific order. B) Post-processing starts with a grouping of epochs and continues with C) their selective averaging. Averaged potentials for TMS alone, and for a combined presentation of TMS with other stimuli (visual or somatosensory) are calculated separately. D) Statistical comparison of the results reveals the influence of functional states of the cortex on the measured TMS-evoked EEG signals.

movement in response to a visual cue presented on a computer screen, while in Publications III and V, modulation was obtained by delivering peripheral electrical stimuli prior to TMS. In an offline analysis, the EEG data were re-referenced with respect to the common average potential. Data processing is schematically described in Fig. 5.4. After the rejection of the EEG segments containing mechanical and muscle artefacts, responses were grouped into several sets according to their experimental protocol (Fig. 5.4B) and selectively averaged (Fig. 5.4C). Then, the amplitude and latency of the modulated N100 response were obtained from a difference curve (Fig. 5.4C) as follows. In Publications I and II, the responses to visual stimuli alone were subtracted from the responses to the combined presentation of visual stimuli and TMS. Similarly, in Publication III, evoked responses to D2 stimuli alone were subtracted from the evoked responses to a combined presentation of TMS and D2 stimuli. Finally, statistical comparison revealed the influence of the functional states of the cortex on the measured TMS-evoked EEG signals (Fig. 5.4D). The cortical regions of interest (ROI) were sensorimotor areas in both hemispheres. With the goal of investigating local cortical excitability changes, we selected a ROI covered by several electrodes (ranging from 4 to 10) in the vicinity of the point of stimulation. A similar ROI was also selected from the homologous area of the opposite hemisphere in order to demonstrate interhemispheric differences. An average trace was obtained from these electrodes, and the amplitude and latency characteristics of N100 were assessed from it. The amplitude of N100 integrated in the time window of ± 5 ms around the peak latency was calculated separately for each session and condition.

It is important to note that EEG epochs were inspected by taking into account EMG activity, *i.e.,* whether an EMG epoch was rejected, the corresponding EEG epoch was rejected from further analysis as well, and vice-versa.

5.2 MEG instrumentation, data acquisition and analysis

In Publications V–VII, the 306-channel MEG data were recorded with an Elekta neuromagnetometer (Elekta Neuromag Ltd, Helsinki, Finland) in a magnetically shielded room (Euroshield, ETS Lindgren, Eura, Finland). During the MEG recording of spontaneous interictal and ictal brain activity and somatosensory evoked fields (SEFs) to median and tibial nerve stimulation in Publication VII, the head movements were continuously monitored by four coils on the scalp (Medvedovsky et al. 2007; Uutela et al. 2001). This setup provided very accurate ictal recordings, which is important for clinical study. MEG was recorded at a 0.03–172 Hz frequency band and sampled at 600 Hz. MEG data in Publication V were recorded at frequency band of 0.01–330 Hz and sampled at 1 kHz.

In order to reveal ERFs, MEG data in Publication V were averaged with respect to the somatosensory stimuli. Epochs of activity containing electro-oculogram (EOG) signals exceeding ± 150 μV were discarded. The generators of the ERFs were located using dipole modeling. The dipole amplitudes were allowed to vary in a multidipole model as a function of time while keeping their locations and orientations fixed. This resulted in millisecond-accuracy time courses of the activated brain areas. These

time courses were utilized to identify target brain areas for subsequent magnetic stimulation.

In Publication VII, in two epilepsy patients, single equivalent current dipoles (ECD) were first computed for the separate dipolar fields (with limited number of sensors) at different post-stimulus time points. Subsequently, these dipoles were used as initial guesses for a single- or multi-dipole fit using all 306 channels. Finally, the analysis period was extended to cover the entire signal of interest, and the optimal dipole strengths were computed by assuming fixed dipoles. The used dipole had to explain the signal of interest (most commonly, a spike), but not other MEG signals (*e.g.,* posterior alpha activity). Thereafter, the MEG results were co-registered with MRI data and compared with nTMS and the electro-cortical stimulation (ECS) results.

6 Results and discussion

6.1 Time course of movement-related cortical excitability

Publication I demonstrated that EEG responses to TMS are modulated by preparation and execution of visually cued unilateral movements compared to responses when TMS was delivered alone, and subject was not performing any task (Fig. 6.1).

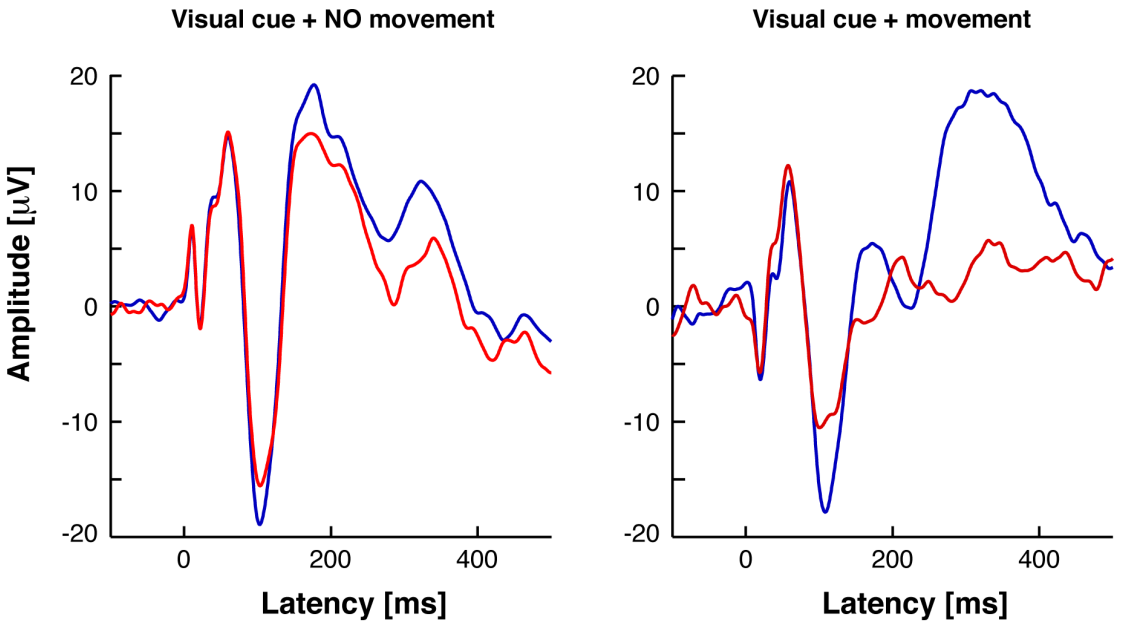

Figure 6.1: Comparison of the EEG responses evoked by TMS pulses delivered alone (blue traces) vs. the TMS-evoked N100 response modulated by observing the visual cues (red trace, left panel) and visually-cued motor cortical processing (red trace, right panel). The right panel shows that modulation is markedly stronger when the subject was reacting to visual cues with brisk thumb twitches. All signals were recorded over the motor cortex contralateral to the moving hand.

At the same time, the amplitude of MEPs was clearly modulated during the movement condition, showing a significant increase. These results are in agreement with accumulated published data suggesting that the time interval immediately before the onset of the movement, during, and immediately after the movement is characterized by the highest cortical excitability, which is seen as lowered motor thresholds to TMS (Rossini et al. 1988; Starr et al. 1988), and/or increased MEP amplitudes (Chen et al. 1998b; Leocani et al. 2000). During that time window, there is an increased rate of neuronal firing in the motor cortex (Evarts 1966; 1974; Fetz and Finocchio 1971; Gribova et al. 2002) corresponding to increased cortical excitability. The experimental paradigm was designed so that the visual cue preceded the TMS by 180 ms. Taking into consideration that the average reaction time of our subjects ranged from 150 to 200 ms (using the onset of the EMG of the moving muscle), the

observed effect of MEP facilitation also covers the increase in motor cortex excitability by approximately 50–80 ms before the voluntary movement had commenced, *i.e.,* pre-movement excitability. The highest MEP facilitation was previously reported in this interval (Starr et al. 1988). The time course of facilitation approximates that found in monkey pyramidal neurons of primary and supplementary areas, which begin to discharge between 120 and 50 ms before movement onset and show increasing rate of firing as the interval to the onset of movement shortens (Evarts 1966; Brinkman and Porter 1979; Kubota and Hamada 1979). Publication I demonstrates that the increasing discharge rate of pyramidal neurons found in animals also occurs in humans (Lee et al. 1986) and is accompanied by lowered neuronal thresholds to TMS applied over the scalp projection of primary motor cortex.

MEP responses could be detected reliably in each individual trial. This was not possible to do for the EEG responses, since signal-to-noise ratio (SNR) was very low (as is the case for practically all EEG studies with or without TMS). Therefore, a detailed temporal dynamics of the responses, with respect to the movement onset, was only feasible for MEPs. However, for Publication I, we subdivided all EEG responses into two groups - fast and slow with respect to the median value of RTs. The results of this analysis are described as a peripheral aspect of the temporal evolution of increased cortico-spinal excitability related to the preparation and execution of movement, as presented in Fig. 6.2. This figure shows the MEP amplitude changes as a function of the speed of the motor response in latency bins of 70 ms. Amplitudes of the MEPs associated with fast reactions up to approximately

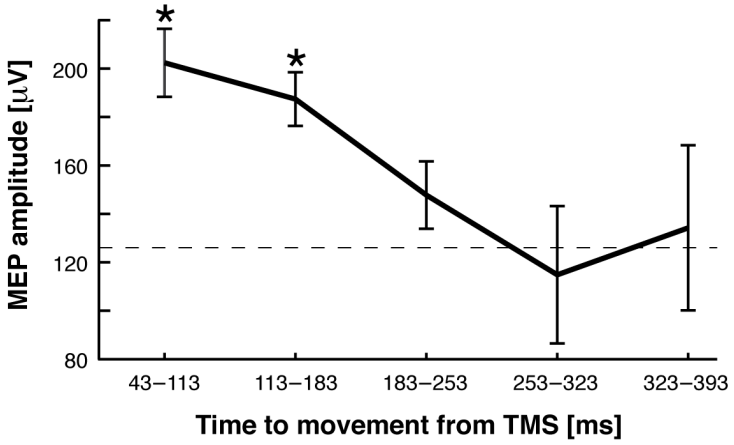

Figure 6.2: Amplitudes of the MEPs as a function of time from the TMS to the onset of motor response. The time on the x-axis indicates the beginning of the movement with respect to the TMS pulse (adding 180 ms to numbers in the horizontal axis gives the reaction time after the visual stimulus). The MEPs were grouped with a bin of 70 ms. The horizontal dashed line shows the average amplitude of the MEPs to the TMS alone. The asterisks indicate a significant enhancement of the MEPs preceding the movement with respect to the MEPs produced by the TMS alone.

200 ms after the TMS were enhanced, while MEPs associated with slower reactions remained unchanged. Enhancement of the 'fast MEPs' is likely to come from an increased amount of synchronously descending impulses along the fast propagating

corticospinal tracts as both the number of pyramidal tract neurons engaged by the stimulus and their firing rates increase. We have related the presented peripheral manifestations of motor cortico-spinal excitability to its EEG (central) counterpart of changes in neuronal activity by selectively averaging the EEG epochs according to the speed of reaction to a visual cue. The EEG epochs were, however, grouped into only two bins: according to whether the reaction time was shorter or longer than the median value. This procedure allows correlating the specific stage of the cortical motor processing with the parameters of the EEG response. According to the literature (Evarts 1966; 1974; Fetz and Finocchio 1971; Gribova et al. 2002; Gottlieb et al. 1970; Hayes and Clarke 1978; Ruegg and Drews 1991), the slow group N100 responses would be associated with the less pronounced cortico-spinal excitability, compared with the fast-group N100 responses. However, the modulation of N100 was similar in both groups, indicating that EEG can detect the onset of excitability modulations even earlier than MEPs. It is important to note that the N100 component was also diminished significantly when TMS was preceded by the visual stimulus, and no motor response was executed (left panel in Fig. 6.1). The origin of this change in motor cortex excitability is not entirely clear, and at least two mechanisms have been suggested to explain this. One scenario is that visual stimuli alone could produce this modulation, since anatomical studies suggest only two synaptic connections from the eye to the motor cortex through the mesencephalic reticular formation (MRF): one form retina to MRF and one from MRF to the motor cortex (Leichnetz 1986; Nakagawa et al. 1998). The second scenario suggests that modulation of N100 might have occurred due to previous association of visual stimuli with the motor reactions. Here, visual stimuli would trigger subthreshold (for generation of motor output) processes in the motor cortex, which

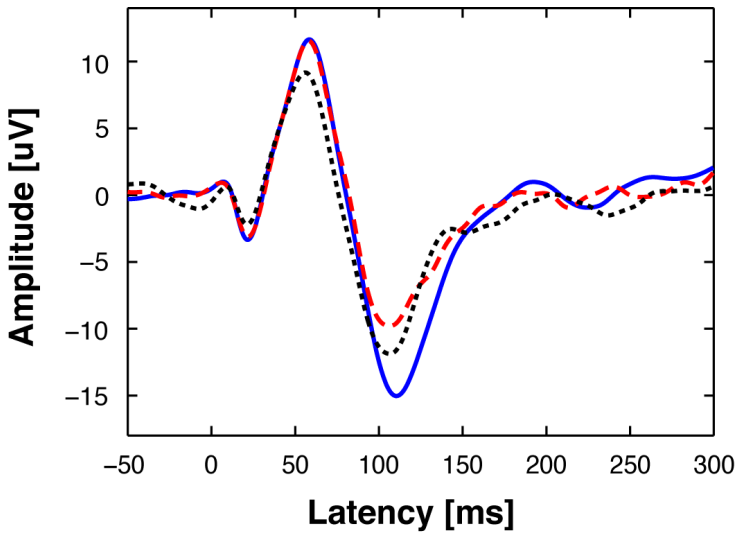

Figure 6.3: Functionally specific modulation of TMS-evoked N100 component. During movements with hand contralateral to the stimulated hemisphere (red trace), the attenuation was stronger compared to the condition when the response was given with ipsilateral hand (black trace). Blue trace represents responses to TMS pulses alone, without visual cue or motor response.

would modulate the N100 amplitude. Future experiments will determine which of these scenarios is more plausible.

6.2 Role of the ipsilateral hemisphere in motor control

Neurophysiological correlates of unilateral movement in sensorimotor areas of both hemispheres were explored in Publication II, with an emphasis on the role of the ipsilateral hemisphere. We aimed at studying bilateral activation of motor areas during the performance of unilateral movements (Kristeva et al. 1991; Rao et al. 1993; Salmelin et al. 1995; Kim et al. 2004). For this purpose, we contrasted the MEP and EEG results and closely analyzed the mirror movements in healthy subjects occurring during unilateral movements. This study successfully repeated the results of Publication I showing that the TMS-evoked N100 component is significantly attenuated during performance of unilateral movements. Furthermore, we demonstrate that a similar attenuation occurs in the ipsilateral hemisphere during the same motor action. Both of these results are shown in Fig. 6.3. Then, we compared the degree of attenuation between the sessions in terms of the percentage of N100 decrease: during contralateral movements, the attenuation was 36%, while during the ipsilateral movements it was 25%. To evaluate the spatial extent of N100 attenuation, we calculated it for each of the 60 recorded EEG channels and plotted a topographical plot (Fig. 6.4). It can be seen that during unilateral movements, the attenuation of N100 is strongest in contralateral hemisphere (left panel in Fig. 6.4), most likely due to the elevated neuronal activity associated with the preparation and generation of motor output

However, only in the contralateral hemisphere were these changes associated with modulation of peripheral muscle responses, as shown earlier in Publication I. This dissociation implies the presence of additional inhibitory mechanisms in the ipsilateral hemisphere responsible for the suppression of motor output discharges. This,

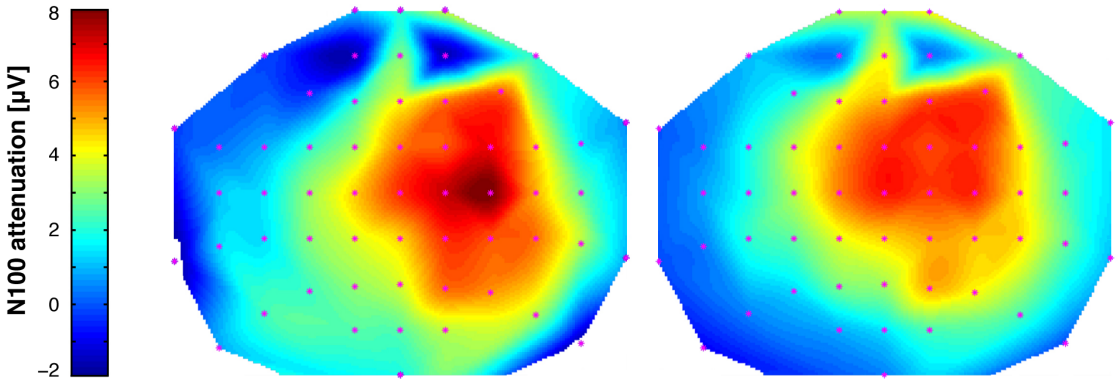

Figure 6.4: Topographical plot of attenuation of the TMS-evoked N100 component during movements with contralateral (left panel) and ipsilateral (right panel) hand. Attenuations of TMS-evoked N100 in both hemispheres have very similar spatial character, being stronger in the hemisphere contralateral to the moving hand.

for example, could be the mechanism that controls the occurrence of mirror movements (Fig. 6.5), which were indeed present in all subjects, at a rate comparable with known literature (Verstynen et al. 2007). These results point to the possibil-

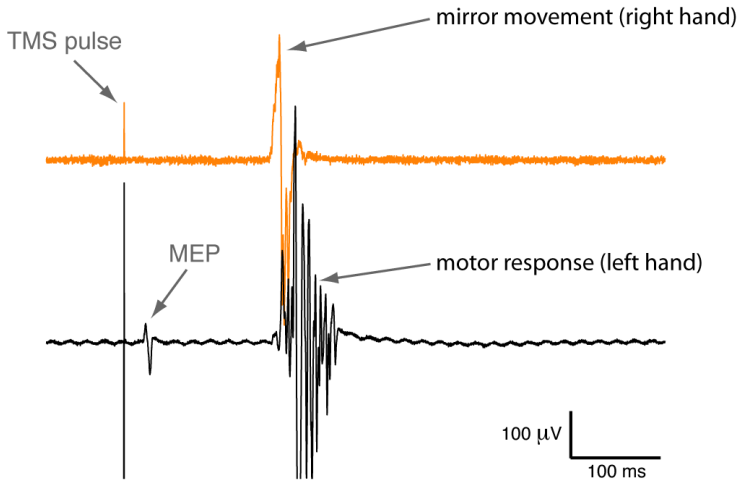

Figure 6.5: An example of EMG recording of a mirror movement. Subject responded with the left hand, but the EMG was clearly recorded also in the right hand, the so-called mirror movement. The MEP response is clearly visible at about 22 ms after the TMS. Vertical lines on the left side of the plot indicate the TMS pulse.

ity of bilateral activation of sensorimotor cortices during the execution of unilateral movements, most probably related to the occurrence of mirror movements (Mayston et al. 1999; Verstynen et al. 2007), or to its suppression (Kristeva et al. 1991; Leocani et al. 2000; Perfiliev 2005). Both processes are possible and might lead to the generation of undesired MMs, which should, nevertheless, be suppressed. The likely mechanism of suppression is transcallosal inhibition from the contralateral hemisphere (Ferbert et al. 1992; Wassermann et al. 1994; Mayston et al. 1999; Ziemann et al. 1999). Publication II shows that most probably these two processes are occurring concurrently in the ipsilateral hemisphere, one being related to the initiation of the unwanted MMs, and another to its suppression (Kobayashi et al. 2003; Perfiliev 2005). By this scenario, the amplitude of N100 in the ipsilateral hemisphere should demonstrate a smaller decrease compared to N100 decrease in the contralateral hemisphere, since MMs-related excitatory activity is to be counterbalanced by inhibitory activity. These are exactly the results of Publication II presented in Fig. 6.3 on page 47.

6.3 Central reflections of periphery

Short-latency afferent inhibition refers to the attenuation of upper limb MEPs evoked by TMS due to preceding stimulation of peripheral digital nerves or the median nerve at the wrist. Based on previous suggestions that SAI reflects primarily cortical processing (Classen et al. 2000; Tamburin et al. 2001), Publication III aimed to further investigate its cortical mechanisms using experimental tools

already tested in Publications I and II. In accordance with previous TMS studies on SAI (Tokimura et al. 2000; Cucurachi et al. 2008; Nardone et al. 2008), we show that MEPs to TMS applied 25 ms after index finger (D2) stimulation (see Fig. 5.3 on page 41) were significantly attenuated. Moreover, the attenuation of MEPs due to SAI is associated with the amplitude attenuation of the TMS-evoked N100 EEG component. We demonstrate for the first time that the attenuation of MEPs is positively correlated with the amplitude attenuation of the N100 response, shown in Fig. 6.6. In that figure it can be seen that even small individual changes in periph-

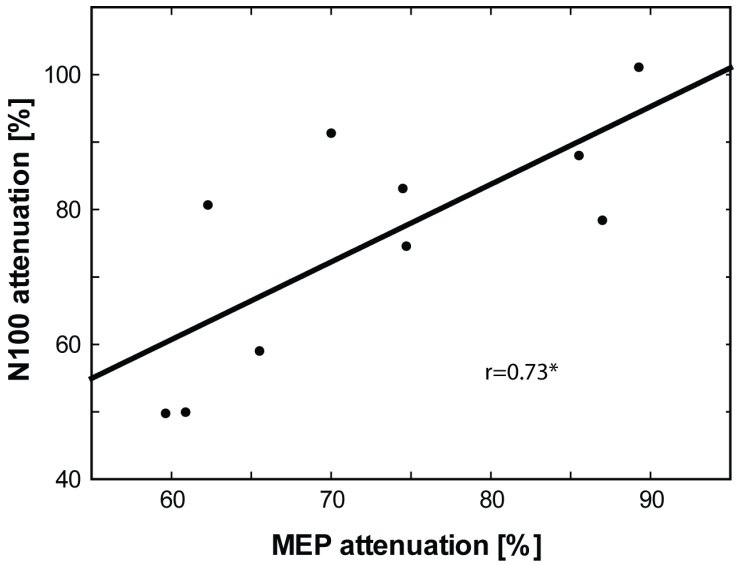

Figure 6.6: Demonstration of a positive correlation between central and peripheral manifestation of the SAI phenomenon. Correlation plot of the amplitude attenuation of the TMS-evoked N100 vs. the amplitude of MEPs due to D2 electrical stimulation. The black line represents the least-squares fit to the data. An important message of this plot is that even small individual changes in the amplitude of peripheral responses are paralleled by amplitude changes in cortical responses.

eral activity are paralleled by changes in cortically probed excitability. This is most probably achieved through an interaction between two inhibitory processes, partially coinciding over time. The first inhibition, due to incoming peripheral electrical stimulus (SAI), is directed at pyramidal cells and should produce hyperpolarization of the neuronal membrane, thus leading to a decrease in the MEP amplitude (the same mechanism can also lead to a decrease in I-waves recorded epidurally, Tokimura et al. 2000). At the time when the second, TMS-induced, inhibition starts, the neurons are already hyperpolarized due to SAI, resulting in a smaller amplitude of N100. Conclusions about probable neuronal assemblies responsible for manifestation of later stages of inhibitory influences of SAI were drawn based on analysis of early EEG responses to TMS. Negativity peaking at 15 ms (N15) indicates initial recruitment of neurons directly activated by TMS, which as such have to be located superficially, where the induced electric field is strongest. The N15 was not affected by D2 stimulation, thus leaving the deeply located pyramidal cells as the most likely candidates responsible for the late inhibitory influences of SAI.

Pursuing further the influences of peripheral stimulation on cortical circuits in combination with TMS-probing of cortical excitability, Publication V studied cortico-cortical communication between the areas receiving parallel sensory input from one side of the thalamus to primary projection areas, and from the other side directly to hierarchically higher-order cortices, bypassing the primary sensory cortices. This Publication utilizes an integrative multimodal approach for studying this effective connectivity. Source locations (together with their time courses) identified by MEG,

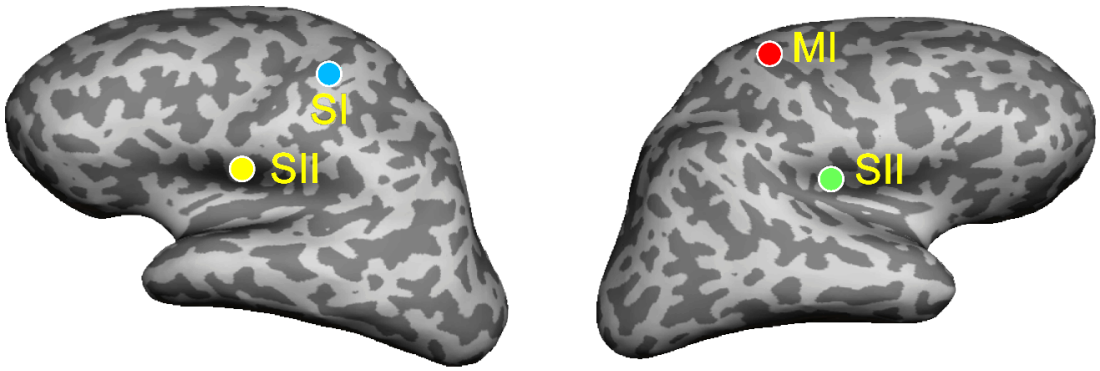

Figure 6.7: MEG source locations used as TMS targets in Study V. During MEG measurement, the subject was instructed to respond to right median nerve stimuli with the left index finger. This resulted in four evoked MEG responses: 1) the primary somatosensory cortex in the hemisphere contralateral to the median nerve stimulus (SI, blue dot), 2) the secondary somatosensory cortices bilaterally (SII, yellow and green dots), and 3) the primary motor cortex contralateral to the motor response, but ipsilateral to the median nerve stimulus (MI, red dot).

after electrical somatosensory median nerve stimulation with a reaction time task, were used in a subsequent session as targets to be modulated with TMS at different latencies after the somatosensory stimulus. As shown on the inflated cortex in Fig. 6.7, these included the contralateral primary somatosensory cortex (cSI), bilateral secondary somatosensory cortices (cSII and iSII), and the ipsilateral primary motor cortex (iMI). Interestingly, MEG data showed the activation of cSII several milliseconds earlier than cSI, confirming previous reports that higher-order cortices may become activated even earlier than primary sensory cortices (Barba et al. 2002; ffytche et al. 1995; Karhu and Tesche 1999). This is inconsistent with serial processing and suggests that SII receives a direct, early parallel sensory input independent of the pathway via SI (Karhu and Tesche 1999). The rest of our results supported this view. First, RT was significantly faster when TMS was given to cSII, than to cSI, or to iSII, with the largest facilitatory effects being observed when the TMS pulse was targeted at the contralateral SII at about 20 ms post-stimulus. Second, peak latency analysis of the TMS-evoked responses revealed that TMS pulses at 15–40 ms speeded up the 140-ms ERP component by 8±8 ms compared to the no-TMS condition, as shown in Fig. 6.8.

Publication V proposes that the speeded RTs could be best explained if the somatosensory-evoked physiological SII activation at about 20 ms normally exerts a top-down SII → SI influence that facilitates the reciprocal SI → SII pathway. TMS

to SII at a latency of approximately 20 ms appears to facilitate the natural brain-speeding mechanism already in place. It appears that fast thalamocortical parallel sensory inputs to multiple cortical sites could decrease the activation thresholds of the cortico-cortical connections between the areas (Ullman 1995).

6.4 Background oscillations and cortical excitability

In addition to the role of the ipsilateral hemisphere in unilateral movement control, Publication II also investigated the fine-tuning of background (*i.e.*, ongoing spontaneous) neuronal activity related to performance of a specific task. The classification as 'ongoing spontaneous' or 'background' implies that the neuronal activity is not evoked or induced by the stimuli. The main idea of this approach is that neurophysiologic TMS-evoked EEG responses during time intervals in which the subject is not performing any task, but merely sitting relaxed, should reflect specific fine-tuning of the neuronal activity broadly related to an experimental condition. When TMS pulses were delivered alone (without preceding visual stimuli) the N100 component

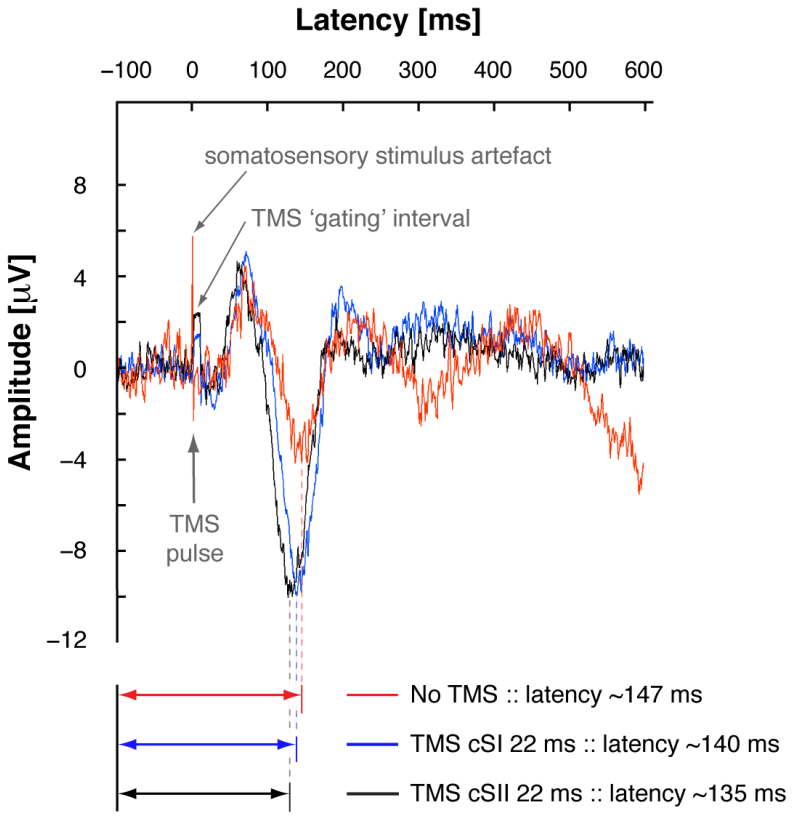

Figure 6.8: Somatosensory ERPs recorded when the subject responded to right median nerve stimuli with the left index finger (unfiltered averaged traces in one subject). Compared to the condition without TMS (red trace), conditions with TMS (blue and black traces) show earlier and stronger SII activity at latencies around 140 ms. The ERP peak shifts appear to correspond to faster RTs.

was significantly larger in sessions requiring a motor response with the ipsilateral hand than in sessions with contralateral responses. This finding may reflect an involvement of inhibitory processes implemented already at the level of spontaneous activity, which are functionally fine-tuned in the ipsilateral hemisphere to prevent the occurrence of MMs during unilateral movements. This is supported by animal studies showing that effective performance in motor tasks is related to a specific fine-tuning of ongoing neuronal firing in the motor cortex (Favorov et al. 1988; Cisek et al. 2003; Perfiliev 2005). In experiments with cats, Perfiliev (2005) demonstrated that background neuronal firing in the ipsilateral hemisphere might contribute to correct selection of the unilateral response. For the first time, here we provide electrophysiological evidence for the existence of a similar mechanism in human sensorimotor cortices. In our TMS-EEG demonstration, the N100 receives a larger contribution from already pre-activated tonic inhibitory processes recruited for the suppression of undesired MMs, indicating that being engaged in a specific motor task differently affects the ongoing background neuronal activity in the contra- and ipsilateral sensorimotor cortices.

Another aspect in the assessment of spontaneous activity in humans is to perturb

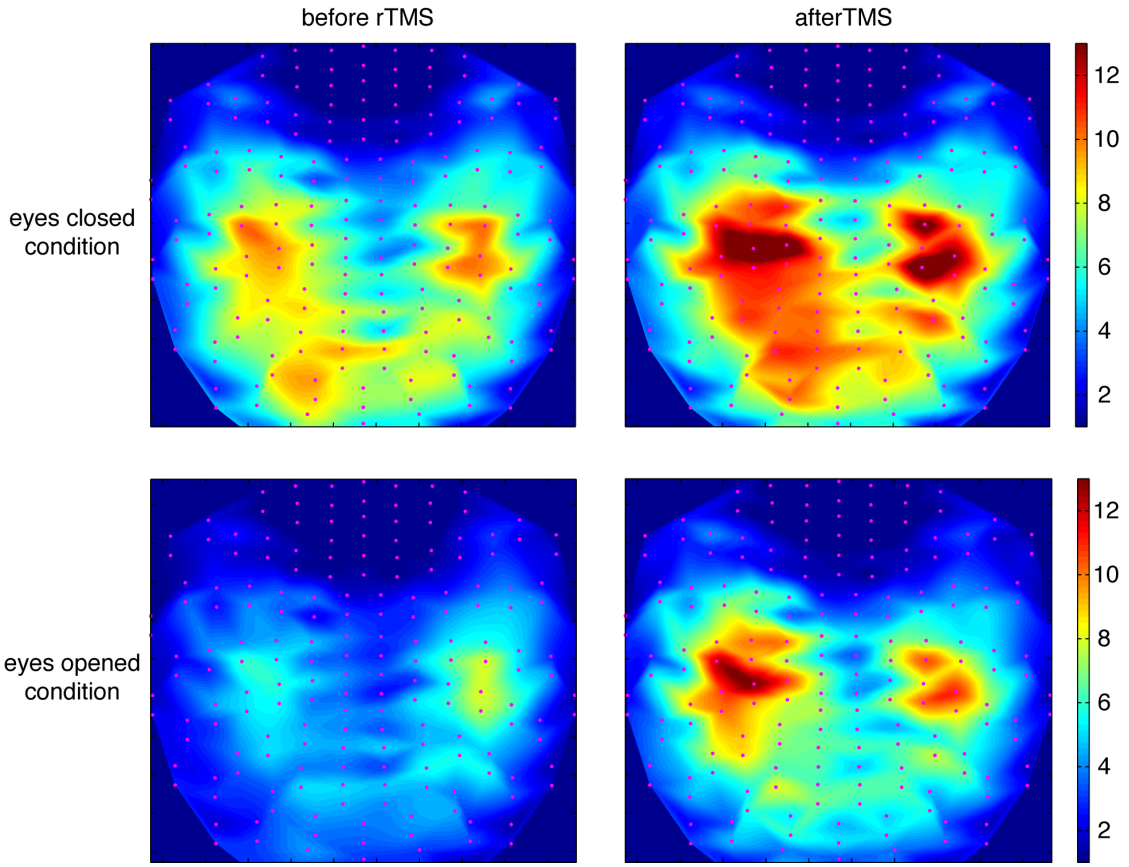

Figure 6.9: An example of changes in 22 Hz beta oscillations after rTMS treatment in one PD patient. A clear bilateral enhancement of the oscillation power over rolandic regions is visible.

its dynamics and observe the changes occurring in response to that perturbation. This approach was utilized in Publication VI, in which we investigated whether a single treatment with subthreshold rTMS to M1 affects the spontaneous cortical oscillations in PD patients. Furthermore, we wanted to explore the correlation between observed features of spontaneous oscillations and improvements in the motor symptoms of PD. A broader goal of this study was to contribute to the development of rTMS protocols that could affect specific cortical circuitry. Based on the basal ganglia–thalamocortical circuit model (Alexander et al. 1990), we targeted the network responsible for functional deafferentation of the primary motor cortex.

After two daily subthreshold rapid rate stimulations of the motor cortex in the hemisphere contralateral to the more affected limb, the total unified Parkinson's disease rating scale (UPDRS) scores were significantly improved, both after first-day and second-day treatments. Specifically, improvements were observed in rigidity and hypokinesia. Hypokinesia, however, led to significant improvement only after the second rTMS treatment. MEG beta spectral power (SP) was calculated over a broad range (14–30 Hz). Measurements performed approximately 20 minutes after the rTMS treatment showed significantly increased beta SP in Rolandic regions, as shown in Fig. 6.9.

The post-stimulation elevated power of oscillatory activity in the beta range demonstrates primarily the effectiveness of rTMS to excite thalamocortical circuits (Rothwell 2007). It has been proposed that beta oscillations are related to a resting (idling) state of the motor cortex (Pfurtscheller 1992; Stancak and Pfurtscheller 1995). The observed beta SP changes may reflect positive alterations in the abnormal synchronization of spontaneous activity generated by the thalamocortical-basal ganglia circuitry in Parkinson's disease (Pollok et al. 2004, Timmermann et al. 2003).

In contrast to studies reporting the placebo effects of rTMS (Strafella et al. 2006), the MEG results were consistent with total UPDRS motor scores, which generally improved only after the first treatment. However, no general significant correlation was detected between these two measures. Relief of rigidity suggests that beta oscillations may be related to akinetic features of PD. Because of the short duration of the measurement sessions (total approximately 2 h), it is unlikely that the observed changes could have been caused by medication withdrawal effects.

Although this is the first study investigating subthreshold TMS stimulation on the motor symptopms in PD patients, the results encourage further studies to determine optimal parameters for effective stimulation. Such parameters could include the intensity and the frequency of TMS pulses, as well as the total amount of stimulation.

6.5 Mapping precision and response repeatability

In Publication VII, the nTMS was used to determine the location and the extent of the primary motor cortical representations for preoperative surgical motor mapping. The novelty of the approach is that it combines nTMS with MEG for use in guiding subdural grid deployment, as well as subsequent comparison with results from ECS and validation by an actual surgery outcome. Furthermore, this study revisited the

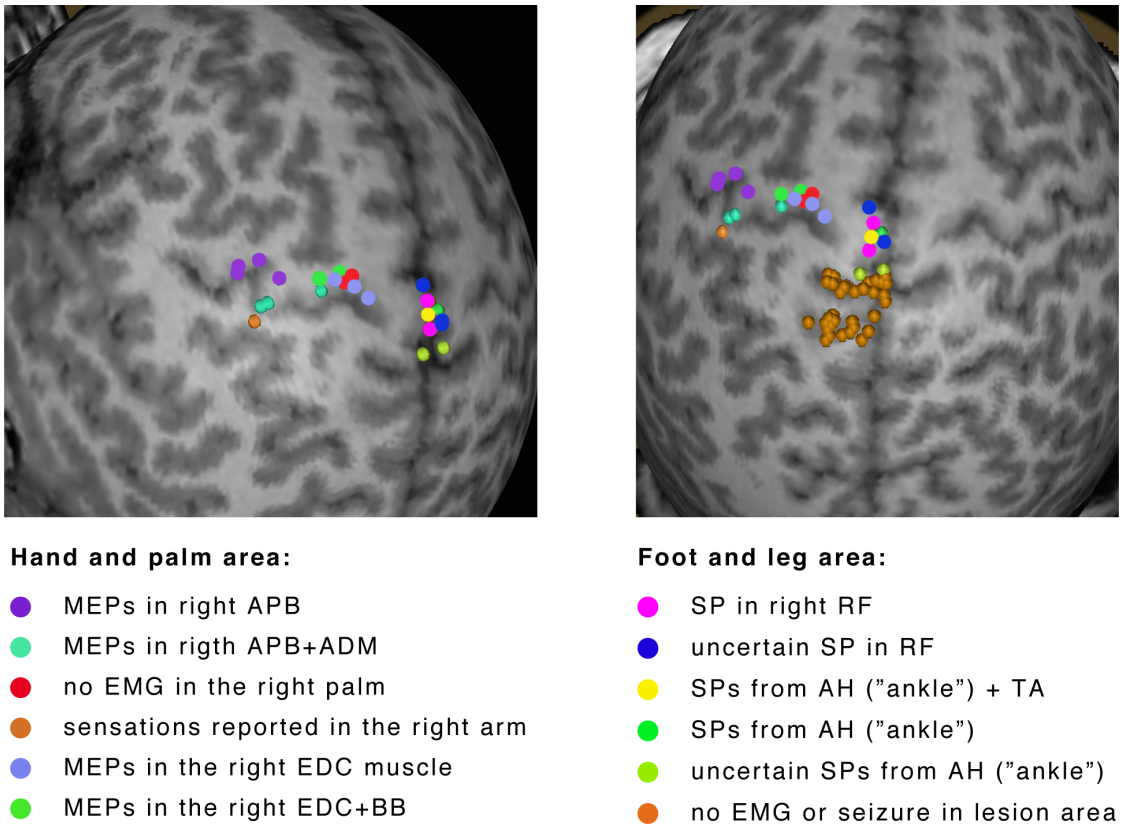

Figure 6.10: Outcome of clinical motor mapping in one epilepsy patient. Left panel (hand and palm area): lilac - MEP response from right hand APB; turquoise - MEP responses from right hand APB and ADM muscles at the same time; red - no EMG responses from the right palm (medial limit); orange - feeling sensation in the right arm reported by the patient (could not be repeated); blue - MEP response from the right extensor digitorum communis (arm); green - MEP response from the right extensor digitorum communis (arm) and biceps muscles at the same time. Right panel (foot and leg area): pink - silent period (SP) and MEP-like wave form in the EMG response from the right side rectus femoris muscle (thigh); blue - unsure SP response from the rectus femoris muscle; yellow - SP response from the abductor hallucis ("ankle") and tibialis anterior muscles at the same time; green - SP response from the abductor hallucis musle alone; light green - unsure SP response from the abductor hallucis muscle; orange - the patient had small lesion in the left medial parietal lobe, close to foot S1, visible in the 3-T MRI (lesion not shown here). Orange dots represent all the locations stimulated over the lesion area, eliciting no leg or foot EMG responses, nor any other responses or seizure activity.

safety issues in the use of single-pulse TMS for epilepsy patients - at intensities close to the MT, epileptiform or ictal EEG activity was not elicited in either of our two studied patients, even though the stimulated sites occasionally overlapped with the MEG-estimated localizations of the epileptogenic cortical region or lesion. Figure 6.10 shows the nTMS mapping results of the foot and leg cortical representations in one epilepsy patient. The orange points below the motor representations in the right panel are those points that produced no measurable response in peripheral muscles. Some of these points lie within the 'lesion' area of this patient, and are

valuable in seizure induction assessment.

This figure is especially important in terms of spatial accuracy, since it gives an impression of how accurately nTMS can distinguish the motor representations. MEG is superior to EEG in locating the interictal epileptic discharges (Shibasaki et al. 2007) and generally in satisfactory agreement with both the intraoperative localizations (for references see Mäkelä et al. 2001), and fMRI-localized primary activation areas (Korvenoja et al. 2006). However, neither MEG nor fMRI can reliably detect the extent of the motor representation provided by nTMS, thus advancing nTMS as a potentially new useful tool for preoperative surgical planning. Indeed, in this study, nTMS produced spatially more precise mapping than ECS (ECS having spatial separation limited by a 1-cm inter-electrode distance). The obtained representations were in line with MEG results adding a new dimension of reliability to the preoperative localization of the primary motor and somatosensory cortices.

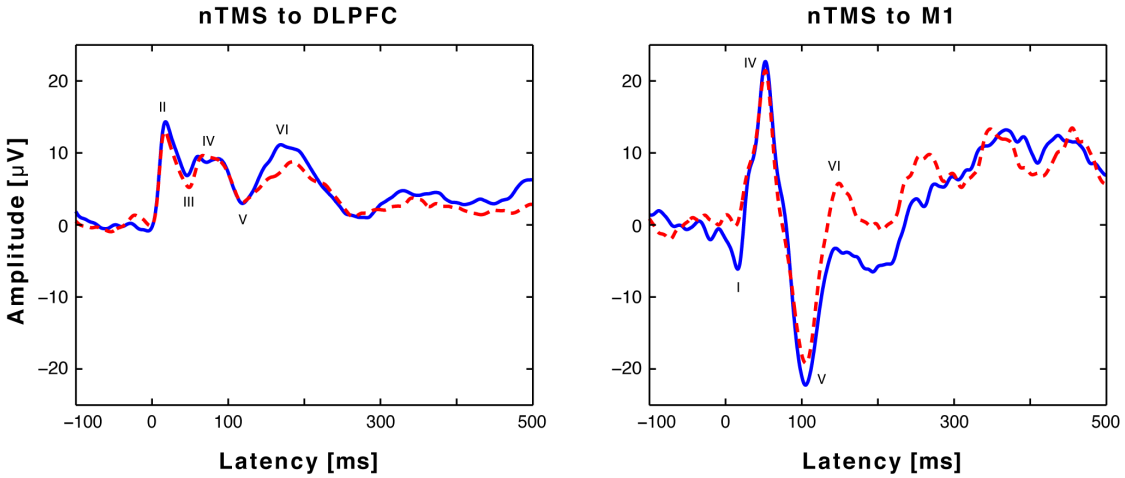

Figure 6.11: Averaged EEG TMS-evoked responses from ROI electrodes after stimulation of DLPFC (left) and the M1 cortex (right) in one subject. Note the difference in general shape of the response and higher amplitudes after stimulation of M1.

An electrophysiological extent of nTMS-based cortical mapping was done in Publication IV, which also investigated mapping precision in term of the repeatability of nTMS EEG measurements. In addition to motor cortical representations and motor threshold, the repeatability of prefrontal TMS-evoked EEG responses was assessed as well. The reproducibility of the TMS-evoked EEG responses is an essential prerequisite for studies with test-retest design. TMS evokes a specific pattern of EEG activity - averaged EEG responses after TMS to primary motor cortex were already presented in Fig. 4.2 on page 33. The amplitudes of responses demonstrated high interhemispheric asymmetry, being most pronounced in the vicinity of the stimulated site. Generally, the response amplitudes were significantly smaller for magnetic stimulation of the prefrontal cortex than M1, indicating the different reactivity of the two regions (Kähkönen et al. 2003; 2004). We have also repeated the results of previous studies by showing that subthreshold TMS to M1 elicits clear EEG responses in healthy humans (Komssi et al. 2004; Kähkönen et al. 2005).

In all subjects, six peaks from the averaged responses were identified after both M1 (right panel in Fig. 6.11) and DLPFC (left panel in Fig. 6.11) nTMS in ROIs over both hemispheres, at all three applied stimulation intensities. Amplitudes of peak II elicited by M1 nTMS, and peak VI elicited by DLPFC nTMS were less replicable than the other deflections. Caution is needed in signal analysis and interpretation of results for peak I (negativity at 15 ms), since it might be considerably contaminated with remains of the stimulus artefact. A very important result within the scope of this work is the high repeatability of both the amplitude and the latency of the TMS-evoked N100 component, enhancing its value as a marker of cortical (inhibitory) processing for both basic and clinical brain research. However, cautious interpretation of N100 results is also needed, especially when analyzing the N100-P180 complex, since it may contain a significant auditory contribution due to bone-conducted sounds (Nikouline et al. 1999). A high correlation was found between repeated measurements of motor thresholds.

7 Summary and conclusions

The main findings of Publications I–VII are:

I The combination of nTMS and EEG provides a sensitive tool for studying changes in cortical excitability related to motor preparation and execution. The increase in pre-movement cortical excitability, manifested by enlarged MEPs, is associated with an amplitude decrease in the N100 component.

II The ipsilateral hemisphere exerts inhibitory control in the human sensorimotor system during the performance of unilateral motor action.

III The attenuation of peripheral MEPs by cutaneous stimulation has its counterpart in the attenuation of the TMS-evoked cortical N100 response, thus providing further support for the cortical origin of SAI.

IV The fact that it offers high overall reproducibility of responses over both hemispheres makes the combination of nTMS and EEG a reliable tool for studies implementing test-retest designs.

V The human brain may utilize direct thalamo-cortical parallel inputs to facilitate long distance cortico-cortical connections, resulting in accelerated processing and faster reaction times.

VI rTMS in Parkinsonian patients modulates spontaneous brain activity, probably by altering cortico-thalamo-basal ganglia networks.

VII Preoperative MEG and nTMS localizations of primary motor representational areas were highly consistent with ECS results and provided improved spatial precision.

7.1 Scientific value of results

This thesis presents an integrative technological and methodological account of a multimodal approach to problems in modern system neuroscience. The most important result is the demonstration that the TMS-EEG approach might be a complementary method for evaluating the cortical effects of TMS, being the only method allowing us to measure TMS-induced neuronal activation at the millisecond time scale. We hypothesized and experimentally proved the combination of TMS with high-resolution multichannel EEG as a very precise and sensitive tool to study transient and fast changing alterations in cortical excitability related to specific functional cortical processing. The presented studies of the motor system in humans bring into focus the recordings of macroscopic cortical neuronal responses to TMS, which, in combination with peripheral measures such as MEPs, allow a more direct evaluation of the cortical excitability without additional contributions from the spinal cord processes. Additionally, we show that this approach produces highly

repeatable results - test-retest correlation of all mapped peak amplitudes ipsilateral to nTMS for both an M1 and DLPFC stimulation exceeded factor of 0.83, revealing a highly significant correlation between repeated measurements. Another very important aspect of this thesis is that it provides an electrophysiological correlate for the state dependency of TMS: the TMS effects were measured and correlated at the level of interaction between the applied stimulus and the functional states of both the central and peripheral neuronal networks under investigation. Together with co-workers, I have been able to raise and at least partially answer important physiological questions, such as which (local and remote) brain areas are affected by TMS over a particular site, or how does TMS over a particular brain area affect interconnected areas, in relation to a particular cortical processing or clinical pathology? Even though the concept of the state dependency of TMS has a very strong spatial basis, this thesis provided important answers in its temporal domain. Probing the functional cortical excitability with TMS requires delivering it at the correct time during the cortical processing of interest. Here, we demonstrated how the TMS could be effectively assisted through prior MEG imaging of the subject performing the same task that will be performed in a subsequent TMS experiment, and how the source time courses and dipole locations delineated by MEG provide precise TMS targeting at the millisecond time scale and millimetre spatial scale. The neural basis of effects induced by rTMS is likely to be very different from those of online single-pulse stimulation. rTMS has evidently a prolonged effect on brain activity, exerting effects on cortical excitability lasting for up to 30–60 minutes (Ridding and Rothwell 2007; Rothwell 2007). Considering the tremendous interest in using rTMS for clinical treatment, as well as the present trend in clinical neuroscience toward finding optimized rTMS protocols able to affect specific cortical circuitry, the new technological solutions and paradigms are more than welcome in this arena. The present thesis has explored a new technological and methodological approach for clinical assessment of rTMS-induced plastic changes in a group of Parkinson's patients (Rothwell 2007), by introducing the MEG as a far more precise temporal monitor compared to standards such as fMRI, or PET. Last but not least, another contribution of this thesis is to show that concurrent TMS-EEG can be reliably mapped outside the motor areas, in repeatable sessions. Almost all studies involving electrophysiological assessment of cortical excitability using TMS with a test-retest design will benefit from our findings, as an essential prerequisite. Response changes elicited by, *e.g.,* rTMS over the dorsolateral prefrontal gyrus in healthy subjects or patients with depression, as well as changes elicited by M1 TMS in patients with movement and degenerative disorders, can be tracked precisely in test-retest designs to gain information on the pathophysiological mechanisms of the disease.

7.2 Clinical relevance of the study and future avenues

Publication VII clearly showed the practical clinical need for detailed nTMS mapping in epilepsy patients in cases when the epileptogenic focus is located near the sensorymotor cortex, or in cases when the malformation might alter the anatomical organization of the motor representation. This brings nTMS close to one of its

potential key applications - pre-surgical mapping of the cortical areas that should be preserved (Picht et al. 2009). In addition to improved surgical planning and mapping of the motor cortical representations in a range of patient groups, nTMS might also be effectively used for non-invasive detection and verification of eloquent cortical areas that should be protected during surgery. A potentially very important clinical application might emerge from stroke neurodiagnostics. There, nTMS can be used to rapidly assess the status of the human central nervous system (central and peripheral) to reveal changes in the acute phase of a stroke. If the peripheral hand or leg MEPs could be observed and evoked by nTMS during this acute stroke phase, it might provide an indication of an elevated chance of good motor recovery (for example, Peurala et al. 2008). This very early indication of the state of the human motor system after stroke might be very useful in rehabilitation planning and follow up. Increased interest has focused on assessment and functional basis of sleep disorders, such as insomnia, restless leg syndrome, and narcolepsy. Thus far, nTMS has successfully been utilized for studying sleep by providing evidence for a breakdown of transcallosal and long-range effective connectivity during NREM sleep (Massimini et al. 2005), which was explained as a transient impairment in the brain's ability to integrate information among specialized thalamocortical modules. For example, it would be important to determine whether cortical effective connectivity recovers in part during late-night sleep, especially during the REM phase - a time at which conscious reports become long and vivid (Stickgold et al. 2001) - and relate these electrophysiological measurements to general sleep quality. Thus, probing the brain's effective connectivity directly with nTMS-EEG may become useful in determining the optimal pattern of sleep stages and contribute to better clinical assessment/therapy of sleep disorders. Finally, an important contribution of the present work is that it provides empirical confirmation of the TMS-evoked N100 component as an inhibitory process induced by the TMS, as well as several important implications for basic brain research and clinical applications arising from this knowledge. First, our findings offer a plausible neurophysiological interpretation of activity during TMS. TMS effectively activates the inhibitory interneurons whose activity is associated with a long-lasting inhibition. These evoked inhibitory processes last up to a few hundred milliseconds and reflect the activation of the GABA-B receptors (Connors et al. 1988; Werhahn et al. 1999; Tamas et al. 2003; Markram et al. 2004). GABA-B receptors can be activated by repetitive firing of interneurons or their cooperative co-activation (Tamas et al. 2003). Simultaneous activation of many neurons can be easily achieved with TMS, with the net effect being a long-lasting inhibition, such as that observed in the present experiments. Second, this thesis has opened up new possibilities for basic brain research in the study of the cortical mechanisms underlying interaction between cognitive and motor functions in the living brain. For example, one could investigate the relation between the anticipatory changes in cortical excitability (Bender et al. 2005b; Brunia and van Boxtel 2001) and the cortical inhibitory processes as revealed by the TMS-evoked N100 component. Third, the known electrophysiological data and proposed phenomenology of TMS-evoked N100 component can provide a sound basis for studies with pharmacological agents modulating GABA-B and/or GABA-A receptors.

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Publication I

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Modulation of electroencephalographic responses to transcranial magnetic stimulation: evidence for changes in cortical excitability related to movement

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Abstract

Transcranial magnetic stimulation (TMS) and multichannel electroencephalography (EEG) were used for the investigation of cortical excitability preceding voluntary movement in human subjects. The study showed the practical value of the combined TMS–EEG approach in differentiating between cortical and spinal-cord mechanisms, which is difficult with conventional electromyographic measures alone. TMS induced a pronounced negativity (N100) lasting for 150–200 ms, with the amplitude maximum in the stimulated hemisphere. When TMS was applied just before the onset of the visually triggered movement, N100 was markedly attenuated, although motor evoked potentials (MEPs) became larger. We suggest that the N100 component represents an inhibitory response following TMS. This interpretation is in agreement with intracellular recordings in animals, paired-pulse TMS studies and experiments showing increased premovement excitability on the basis of MEPs. N100 was not affected only by the subsequent movement, but also by the switching from rest to the motor-task condition, which caused a slight attenuation of the N100 component; no changes, however, were found in the amplitude of MEPs, suggesting that modified excitability did not affect the output of the corticospinal pyramidal cells. By contrast to MEPs, N100 was modulated also by the presentation of the visual stimulus alone, i.e. when no movement was required. This attenuation suggests that even in a rest condition visual stimuli have an access to the sensorimotor regions of the cortex, most probably through ascending arousal brain systems.

Introduction

The aim of the present study was to identify electroencephalographic correlates of increased cortical premovement excitability using transcranial magnetic stimulation (TMS) as a probe. Although TMS has been used in studies of cortical excitability in combination with electromyography (EMG), no direct electroencephalographic evidence has been obtained so far for the quantification of cortical excitability. Below, we show that the traditional TMS–EMG approach is complemented by using electroencephalography (EEG) to record TMS-evoked activity.

Initially and most commonly, TMS has been used in studies of the motor system by observing peripheral muscle activity, in particular by recording compound motor evoked potentials (MEPs). MEPs caused by TMS are used routinely in research; abnormalities in the latency or size of MEPs, or in the duration of the electromyographically observed cortical silent period, are often taken as indicators of cortical pathology (Meyer, 2002; Ziemann, 2002). The problem with MEPs (and electromyography in general) is, however, that they are affected by a combination of cortical, subcortical and spinal-cord mechanisms. These effects often coincide in time, thus making their separation very difficult. Therefore, conclusions about cortical pathologies or in general about involvement of the motor cortex in a given task might be unreliable when based on MEP recordings only. The present study provides an alternative approach, utilizing EEG to reveal a cortical involvement in TMS paradigms.

TMS has been recently combined with EEG (Ilmoniemi *et al*., 1997), positron emission tomography (PET; Paus *et al*., 1997), functional magnetic resonance imaging (fMRI; Bohning *et al*., 2000) and near-infrared spectroscopy (NIRS; Nissilä *et al*., 2002). The combination of TMS with these methods allows one to study reactivity and connectivity of the stimulated brain areas. Among the above-mentioned techniques, EEG has a millisecond temporal resolution, being thus the only suitable method for the study of transient neuronal responses.

In TMS experiments, cortical excitability has been most extensively studied at motor areas. To incorporate findings from these experiments, the present study addressed the issue of cortical excitability related to movement. The enhancement of MEP amplitude has been observed in numerous studies when TMS was applied before, during and after the movement. It has been shown for triggered movements (Rossini *et al*., 1988; Starr *et al*., 1988; Chen *et al*., 1998), for self-paced movements (Chen *et al*., 1998) and in a paradigm with go/no-go responses (Leocani *et al*., 2000; Yamanaka *et al*., 2002). This modulation of MEPs has been interpreted in terms of increased cortico-spinal

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excitability just prior to movement execution. The term 'cortico-spinal excitability' involves the very difficult issue of differentiating between cortical and spinal-cord processes. Thus, the possibility to differentiate between these two very distinct anatomical levels is both important and challenging. The present study aimed at electroencephalographic demonstration of increased cortical excitability preceding voluntary movement on the basis of the changes in the electroencephalographic evoked responses to TMS.

Materials and methods

Subjects

Seven healthy male subjects (23–31 years old) were investigated. The experimental procedures were approved by the local Ethical Committee of the Helsinki University Central Hospital. All subjects gave written informed consent.

Task and stimuli

The subject was seated comfortably in a chair, wearing a combination of special ear-plugs and head-phones in order to attenuate the acoustic clicks caused by TMS.

The task for the subject was to make a quick abduction with the right thumb in response to a black-and-white checkerboard pattern presented on a computer screen. The duration of the visual stimulus was 50 ms. In the control condition, no motor response was required; the subject merely watched the visual stimuli. In total, there were four sessions with the motor response to the visual stimulus (movement condition) and two sessions with no response to the visual stimulus (no-movement condition, Fig. 1). The order of the sessions was different across the subjects. In each session, in random order, there were three types of stimulation categories with 35 stimuli in each: (i) visual stimuli only, (ii) TMS only and (iii) combined presentation of visual stimuli and TMS. In the combined presentation, the delay between the onset of the visual stimulus and TMS was 180 ms. The interstimulus interval between consecutive presentations of stimuli varied randomly between 3300 and 4000 ms. The subjects were asked to respond quickly to the presentation of the checkerboard, so that the latencies of the motor responses would be between 200 and 400 ms. This latency range allowed us to study the modulatory effects of movement preparation on evoked responses in the time range 20–220 ms prior to the onset of voluntary EMG. Before starting the experiment, the subject was allowed to practise in order to achieve the required performance. The onset of each motor response was determined on the basis of visual inspection of the rectified EMG traces.

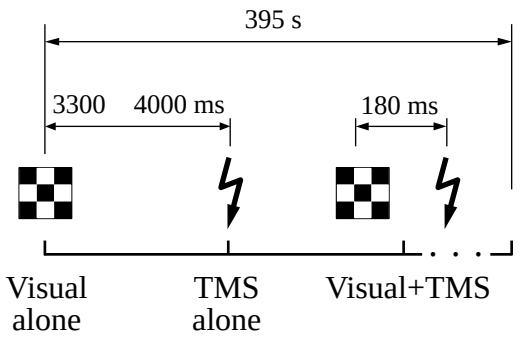

FIG. 1. The scheme of the experimental sessions. There were three types of stimuli ($n = 35$ for each type): TMS alone, visual stimulus alone, and combined application of visual stimulus and TMS (visual + TMS, 180 ms delay from visual stimulus to TMS). The interstimulus interval varied from 3300 to 4000 ms. The order of the stimuli was random. In four sessions, the subject responded to the visual stimulus (movement condition); in two sessions, no motor response was required (no-movement condition).

Stimulation

The magnetic stimulation was performed with a Magstim 200 (Magstim Co.) device and figure-eight coil (average diameter of the windings in each wing is 70 mm). To elicit the strongest response in the right *abductor pollicis brevis* (APB), the coil was placed tangentially over the left hemisphere, the handle pointing backward and laterally at a 45° angle away from the midline. The stimulus intensity was 20% above the subject's motor threshold at rest, determined as the intensity capable of eliciting MEPs with the amplitude of at least 50 μV in four out of eight trials. EMG was recorded from bipolar surface electrodes.

EEG

The EEG was recorded continuously with 60 Ag/AgCl electrodes; the pass-band was 0.1–500 Hz and sampling rate 1450 Hz. The TMS-compatible amplifiers recovered from each TMS pulse in a few milliseconds (Virtanen *et al.*, 1999). Multichannel recordings enabled us to select electrodes showing the strongest reactivity in the present paradigm. For the following data analysis, the average reference was used. After the rejection of the epochs contaminated by mechanical or muscle activity, the responses were averaged and low-pass filtered with the cut-off frequency of 40 Hz.

Analysis

EEG

We focused on the TMS-evoked N100 component, which is a pronounced and the most readily reproducible component across the different conditions and subjects. The name of this component reflects the fact that it has negative polarity above the stimulated area and peaks at about 100 ms after the TMS. Earlier (<50 ms) and later components (>200 ms) had significant variability in a shape across the subjects, thus making their evaluation problematic. Because of the wide distribution of the N100 component over the stimulated sensorimotor area, ten electrode sites with the most pronounced N100 component were chosen for the analysis. Although, in general, N100 was much smaller in the contralateral (right) hemisphere, symmetric ten channels from this hemisphere were also included, mainly for the demonstration of interhemispheric asymmetry in the amplitude of the N100 component. The peak latency and the average amplitude around the peak (±10 ms) were calculated on the basis of averaged activity from these electrodes in each hemisphere. The N100 latency and amplitude were calculated for TMS alone and for TMS when the visual stimulus was delivered. In the latter case, in order to assess the TMS-evoked N100 parameters, the following procedure was applied. The responses to visual stimuli alone were subtracted from those to visual stimuli + TMS. This subtraction was made for the two experimental conditions separately: for the movement and no-movement conditions (see above). These two conditions are needed to compare the modulatory effects of movement-related activity and/or visual processing alone.

The EEG epochs were also averaged according to whether the reaction times to the visual stimuli were shorter or longer than their median value. This procedure allows a comparison of the modulatory effect of the speed of the reaction on the parameters of the N100 component.

Experiments and analysis related to control conditions

In order to check the effect of ear-plugs and ear-phones and to measure the auditory responses associated with the residual of the click, the

experimental paradigm was run in two subjects as described above, but with a 2-cm-thick plastic piece between the coil and the scalp. This procedure allows one to mimic the acoustic and somatosensory sensations on the scalp produced by the coil, while significantly reducing the induced electric field in the brain (Nikouline *et al*., 1999).

In all subjects, additional data analysis was performed for the possible effect of peripheral somatosensory afferentation from MEPs. The MEPs were divided into two groups on the basis of whether they were smaller or larger than the median value. EEG epochs corresponding to each group were averaged in order to detect a possible correlation between EEG and MEP responses. In one subject, another control was performed for peripheral somatosensory afferentation. Both acoustic stimulation as described above and right median nerve stimulation were applied, the latter producing MEPs similar to those caused by TMS. The median nerve was stimulated so that APB contracted at the same time with respect to the onset of the click as did APB when TMS was applied. Therefore, this procedure mimics both auditory and peripheral somatosensory effects of TMS.

Electromyography

The MEP amplitude was represented by the mean value of the rectified electromyogram in the time interval 18–43 ms after the TMS pulse. This time interval is sufficient to cover the major part of the MEP. In the movement condition, MEPs preceding cued movement were sorted on the basis of the median value of the reaction times, thus creating two groups of MEPs: slow- and fast-reaction groups.

Statistical analysis

Analysis of variance was used for the EEG data with the factors: Hemisphere (left and right), Condition (movement or no movement) and Stimulus (presence or absence of visual stimulus before TMS), a $2 \times 2 \times 2$ design. For MEPs, Condition and Stimulus factors (2×2 design) were used. One-way ANOVA was used for the evaluation of: (i) effect of the amplitude of MEPs on the amplitude of N100 in the movement condition (Amplitude factor with three levels: all MEPs, small MEPs and large MEPs); (ii) dependency of the N100 amplitude on the speed of the reaction in the movement condition (Movement factor with three levels: fast reaction, slow reaction and no reaction – TMS alone) and (iii) the amplitude of MEPs depending on the latency of the reaction (Movement factor with three levels: fast reaction, slow reaction and no reaction – MEPs to TMS alone only). Newman–Keuls *post hoc* (p.h.) analysis was performed. Paired *t*-tests were used when appropriate for the comparison of two means only.

Results

The reaction times to the visual stimuli (mean ± SEM) were 304 ± 16 and 290 ± 18 ms with and without TMS, respectively, the difference not being statistically significant.

Figure 2 shows responses to TMS and their modulation in the movement and no-movement conditions. The amplitude of the N100 component demonstrated high interhemispheric asymmetry, being most pronounced over the left central area in the vicinity of the stimulated site ($F_{1,6} = 12.01$, $P < 0.014$, Hemisphere factor; Figs 2 and 3). The difference in the N100 amplitude between the left and right hemispheres was 5.5 and 5.8 μV in the movement ($P < 0.0003$, p.h.) and no-movement ($P < 0.0003$, p.h.) conditions, respectively. The latency of N100 to TMS alone did not differ systematically between the hemispheres (Fig. 2).

N100 was significantly changed in a situation when TMS was preceded by the visual stimulus ($F_{1,6} = 17.54$, $P < 0.006$, Stimulus factor). There was also a significant interaction (Condition × Hemisphere × Stimulus; $F_{1,6} = 7.02$, $P < 0.04$). In the stimulated hemisphere after the delivery of the visual stimulus, the amplitude of N100 was attenuated by 37% and 15% in the movement ($P < 0.0003$, p.h.) and no-movement ($P < 0.005$, p.h.) conditions, respectively (Fig. 3), this attenuation being stronger in the movement condition ($P < 0.04$). The amplitude of N100 to TMS alone was larger in the no-movement than the movement condition ($P < 0.04$, p.h.; Fig. 3). The latency of N100 was significantly affected by the visual stimulus ($F_{1,6} = 7.21$, $P < 0.04$, Stimulus factor). Compared with N100 caused by TMS alone, the latency of N100 to TMS after the visual stimulus was delayed by 32 ms and 30 ms in the movement

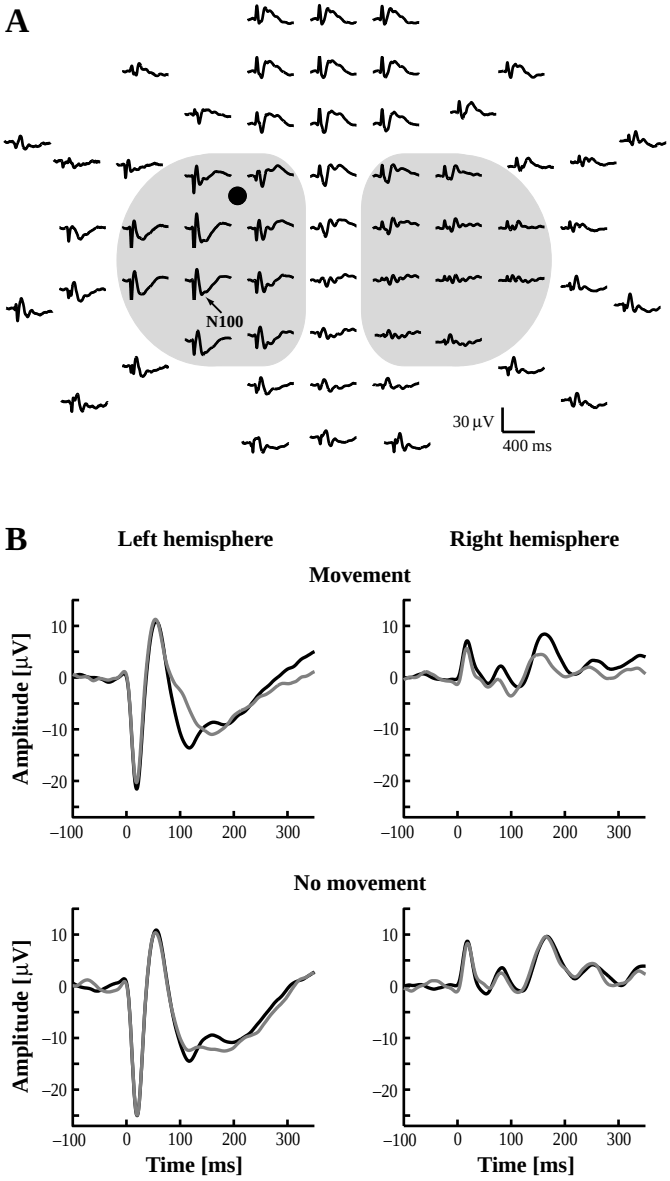

FIG. 2. EEG responses to TMS in one subject. (A) Averaged responses evoked by TMS alone arranged according to the layout of the electrodes (the view is from the top of the head, nose pointing upward). Note the prominent negativity (N100) following TMS. The shaded areas show the electrode sites used for the analysis. The dot shows the centre of the figure-eight coil. (B) EEG responses averaged across ten channels in the left and right hemispheres for different stimuli and conditions. Upper row: movement condition. Lower row: no-movement condition. Black line: EEG responses to TMS alone. Grey line: subtraction of the responses to visual stimuli from the responses to visual stimuli + TMS.

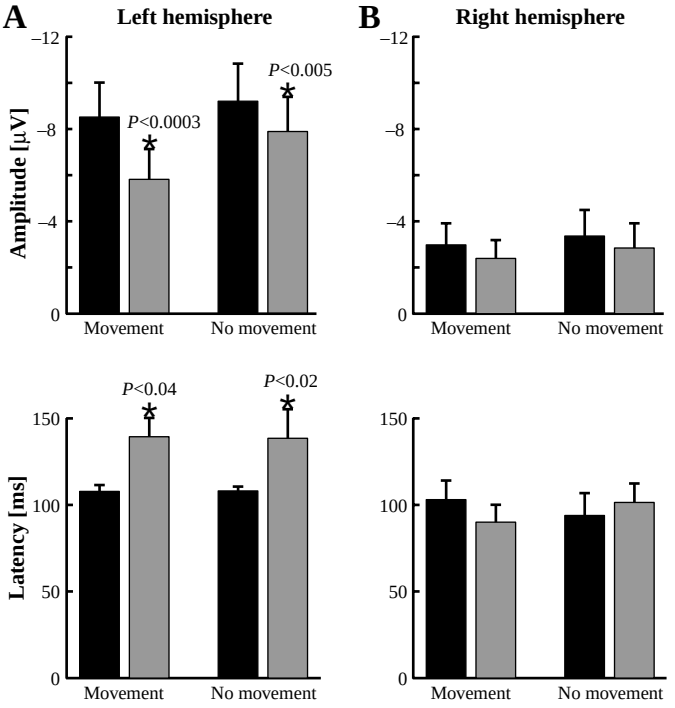

FIG. 3. Amplitude and latency of the N100 component in the left (A) and right hemispheres (B). Black bars: EEG responses to TMS alone. Grey bars: subtraction of the responses to visual stimuli from the responses to visual stimuli + TMS. Asterisks mark a significant drop of the N100 amplitude and an increase in the N100 latency.

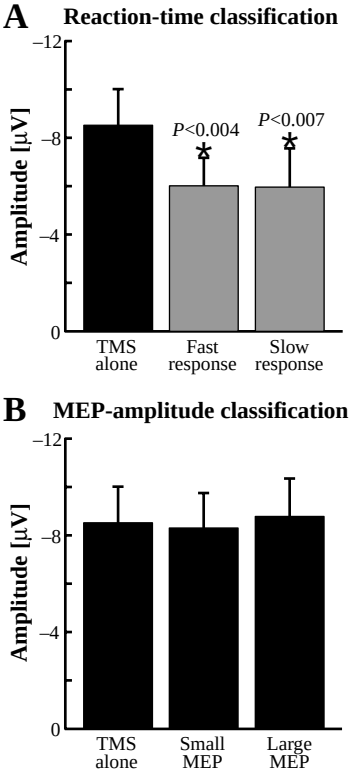

FIG. 4. Amplitude of selectively averaged N100. (A) EEG epochs averaged with respect to the latency of the movement (fast and slow responses). Black bars: EEG responses to TMS alone. Grey bars: subtraction of the responses to visual stimuli from the responses to visual stimuli + TMS. Asterisks indicate significant drop of N100 amplitude for both groups. (B) EEG epochs averaged with respect to the amplitude of MEPs (small and large MEPs).

($P < 0.04$, p.h.) and no-movement ($P < 0.02$, p.h.) conditions, respectively.

Pursuing further the attenuation of N100 in the stimulated hemisphere, the EEG responses were averaged selectively on the basis of the reaction times. The results of this analysis show that the amplitude of the N100 component in the stimulated hemisphere did not differ significantly between the slow- and fast-groups of EEG responses (Fig. 4A). However, N100 belonging to both groups differed significantly from N100 caused by TMS without subsequent movement (one-way ANOVA, $F_{2,12} = 9.5$, $P < 0.004$).

Figure 4B shows also results of the comparison of the EEG responses, when the amplitude of MEPs was taken into account. The amplitude of large-group MEPs was 272% greater than that of small-group MEPs. However, no difference was detected in the amplitude of N100 when the responses were averaged on the basis of either small or large amplitude of MEPs (one-way ANOVA, $F_{2,12} = 1.38$, $P < 0.29$). This fact suggests that N100 is not likely to be caused by the peripheral afferentation from the MEP.

Figure 5 shows results of the control experiments with auditory and somatosensory stimulation (see Methods), indicating that the responses in the control conditions are very small and different from those evoked by TMS.

The amplitude of the MEPs was clearly modulated in the movement condition (Fig. 6A) and affected by the Stimulus factor ($F_{1,6} = 6.87$, $P < 0.04$) and Condition factor ($F_{1,6} = 19.1$, $P < 0.005$). The following *post hoc* analysis revealed that MEPs to TMS preceding movement were increased compared with TMS alone in the movement condition ($P < 0.017$, p.h.). However, amplitude of MEPs to TMS with the preceding visual stimulus did not change in the no-movement condition. Moreover, there were no differences in the amplitudes of MEPs to TMS alone between the movement and no-movement condition.

Figure 6B shows that the amplitude of MEPs was affected differently depending on how fast the cued motor response was performed ($F_{2,12} = 7.53$, $P < 0.008$). Further *post hoc* analysis showed that only MEPs belonging to fast-group responses are significantly increased in amplitude compared with MEPs to TMS alone ($P < 0.008$, p.h.).

Discussion

In the present study, changes in cortical excitability were probed on the basis of TMS-evoked EEG. TMS induced a pronounced negativity (N100) with the amplitude maximum in the stimulated hemisphere. This negativity was significantly reduced by the visually triggered movement, although MEPs became larger.

Considering the novelty of the approach used, some methodological issues should be discussed first.

Methodological issues

TMS of the motor cortex is accompanied by auditory clicks and by peripheral somatosensory afferentation from the contracting target muscle. These sensory stimuli should be considered when interpreting the evoked EEG responses.

Special care was taken in order to prevent the sound click from being heard. Although the sound was attenuated, there was some acoustic residual heard by the subjects. In the control experiments, the EEG response to the click was very small and different from the real TMS-evoked potentials (Fig. 5). Moreover, published data suggest that auditory responses tend to be largest over the hemisphere contralateral to the source of the sound (coil in our case), yet in our experiment N100

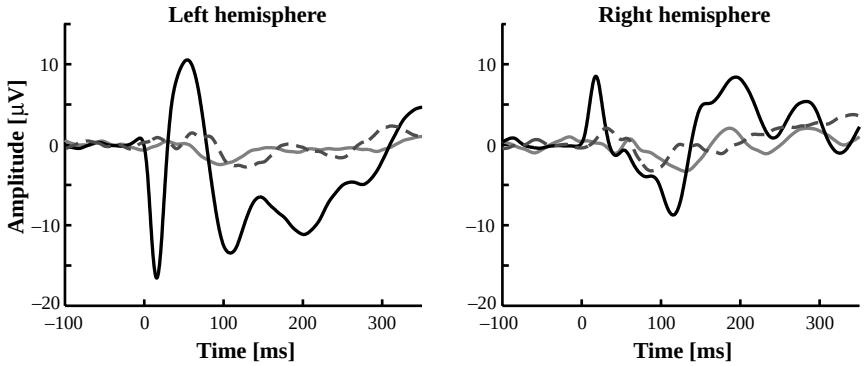

FIG. 5. EEG responses to TMS and control stimuli in one subject. The traces represent EEG activity averaged across ten channels in each hemisphere (see Fig. 1). Solid line: responses to TMS. Grey line: responses to acoustic click. Dotted line: responses to combined acoustic and peripheral somatosensory stimulation.

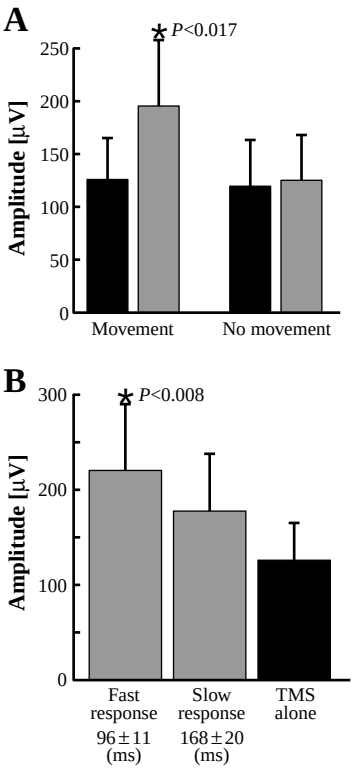

FIG. 6. Amplitude of MEPs. (A) Amplitude of MEPs in the movement and no-movement conditions. (B) MEP amplitudes sorted on the basis of the median value of the reaction times for the cued movements, thus creating two groups of MEPs: slow- and fast-response groups. For these two groups, time values below show how early with respect to the beginning of the cued movement TMS was applied. For both A and B, black bars – MEPs to TMS alone, grey bars – MEPs to TMS after the presentation of the visual stimulus (on B only for the movement condition). Only epochs with the latency of the movement not earlier than 43 ms after the TMS are plotted. The earlier epochs, although representing enhancement of MEPs, might be contaminated by the EMG from the motor response.

was largest over the stimulated hemisphere. Paus *et al*. (2001), using 90-dB white-noise masking (instead of soundproof ear-plugs and headphones), also arrived at the conclusion that N100 was evoked primarily by TMS.

As Fig. 5 shows, somatosensory responses are very different from the TMS responses, although their small contribution to the EEG responses at about 100 ms is evident. Moreover, Fig. 4 demonstrates that the amplitude of the N100 component was similar for the small and large MEPs, implying that this component is not due to the peripheral afferentation. This same observation had been made by Paus *et al*. (2001).

It seems plausible then that N100 predominantly reflects neuronal activation caused by the direct stimulation of the cortex.

Nature of N100

Despite the wide use of TMS in brain studies, only few reports have addressed the nature of macroscopic stimulation of the brain on the cellular level. The most pronounced effect of electrical stimulation applied to the cortical surface is inhibition, which is associated with long-lasting IPSPs (>100 ms) in intracellular recordings (Krnjević *et al*., 1966; Rosenthal *et al*., 1967). Electrical stimulation activates inhibitory interneurons, which in turn produce inhibitory responses in other cells (Krnjević *et al*., 1966). It is important to note that the inhibitory effect of electrical stimulation can be overcome by the iontophoretic application of glutamate (Krnjević *et al*., 1966). By analogy with the above-mentioned animal experiments, TMS should also activate inhibitory interneurons; their post-synaptic effect appears to be represented as the N100 component, which is the most pronounced and long-lasting TMS component. This component seems to be the candidate for the electroencephalographic equivalent of long-lasting IPSPs evoked by brain stimulation. N100 should be then smaller during the time period associated with movement because of the increased firing rate of neurons in the motor cortex. Thus, the attenuation of the N100 response appears to be an indication for the increased level of excitatory activity in the cortex during the preparation to move.

Another confirmation for the inhibitory nature of N100 stems from the experiments with the paired-pulse paradigm using suprathreshold conditioning stimulus. Results of these studies show that the amplitude of MEPs to the test stimulus is suppressed for the time period of 200 ms, with a peak at about 100–150 ms after the presentation of the conditioning stimulus (Valls-Solé *et al*., 1992; Roick *et al*., 1993; Matsunaga *et al*., 2002). In fact, the time course of the inhibitory effect in those experiments is remarkably similar to the shape of N100 obtained in the present study. Accumulated published data suggest that this inhibition originates at the cortical level (Ziemann, 2002).

Modulation of N100 in the no-movement condition

The amplitude of the N100 component was diminished also in the no-movement condition (Fig. 2), indicating that visual stimuli alone could

produce this modulation. In the literature, this type of modulation has not been reported on the basis of MEPs. Anatomical studies suggest that only two synaptic connections are required for visual stimuli to reach from the eye to the motor cortex through mesencephalic reticular formation (MRF): one from retina to MRF and another from MRF to the motor cortex (Leichnetz, 1986; Nakagawa *et al*., 1998). This ascending afferentation projects diffusely to the cortex, causing desynchronization of neuronal activity and increasing neuronal firing (Moruzzi & Magoun, 1949; Steriade *et al*., 1996). This arousal activation caused by visual stimuli might not have been sufficient to change the excitability of the output neurons in the motor cortex, and therefore to affect MEP amplitudes, but it changed the activity in this cortical area sufficiently to affect EEG responses, when tested with TMS pulses. This arousal activation could also be achieved through the activation of the non-specific thalamo-cortical system, which includes midline, medial and intralaminar nuclei (for a review, see Jones, 2003) or, of course, via neuronal connections from the visual cortex to the motor cortex.

The present results thus demonstrate that visual stimuli in a rest condition (subject passively watching the flickering checkerboard) have access to the sensorimotor regions of the cortex, most likely through non-specific ascending arousal brain systems. Internally, the status of the motor cortex is affected upon the arrival of the visual stimuli, yet this change does not lead to changes in the motor output (no changes in MEPs). The presence of this modulation may be beneficial for the organization of quick effects of the arousal systems on the cortical activity when needed, because one would not need to establish new connections, but rather to enhance existing ones.

Another interesting result is related to the differences in the N100 amplitude to TMS alone between the movement and no-movement conditions. Although small (~9%), there was a significant increase of N100 amplitude in the no-movement condition. Donoghue *et al*. (1998) showed that the primary motor cortex may be activated not only during the performance of a task, but also as a result of a transition from a rest state to task engagement, thus reflecting a more global process rather than specific details of the upcoming motor action. By analogy with the above-proposed mechanism for the attenuation of N100 related to the actual movement, N100 to TMS alone could be diminished in the movement condition because of the concurrent excitatory activity related to task engagement. However, this excitatory activity was not sufficient to affect the output function of the corticospinal pyramidal cells, because no MEP changes were observed to TMS alone between the movement and no-movement conditions.

Movement and N100

The advantage of the TMS–EEG method is that EEG gives a direct measure of neuronal activity, whereas MEPs allow the evaluation of neuronal activation only indirectly through EMG responses. MEPs in the present study were modulated significantly only for the responses belonging to fast-group visually cued responses (Fig. 6B). A similar increase of cortico-spinal excitability, preceding voluntary movement, has been found in other TMS studies (Chen *et al*., 1998; Leocani *et al*., 2000; Yamanaka *et al*., 2002). The amplitude of the N100 component was attenuated equally for the fast and slow motor responses. The EEG responses belonging to the fast-response group consisted mostly of the epochs with the movement starting after the application of TMS, although some responses coincided with or preceded TMS. The EEG responses belonging to the slow-response group included epochs with the corresponding latencies of the movement after TMS.

According to published data, the time interval immediately before the onset of the movement, during and immediately after the movement is characterized by the highest cortical excitability. This excitability is seen as a lowered motor threshold (Rossini *et al*., 1988; Starr *et al*., 1988) and increased MEP amplitudes (Chen *et al*., 1998; Leocani *et al*., 2000). During this time window, there is an increase in the rate of neuronal firing in the motor cortex (Evarts, 1966, 1974; Fetz & Finocchio, 1971; Gribova *et al*., 2002), thus indicating increased cortical excitability. By contrast, it is known that the spinal H-reflex is also facilitated in the period of −100 to 20 ms with respect to movement onset; this facilitation is due to the removal of pre-synaptic inhibition at Ia terminals (Gottlieb *et al*., 1970; Hayes & Clarke, 1978; Ruegg & Drews, 1991); thus, the modulation of MEPs is affected both by the spinal-cord and cortical facilitation mechanisms. According to the above-mentioned literature, slow-group N100 responses would be associated with the less pronounced cortico-spinal excitability, compared with the fast-group N100 responses. Yet, the modulation of N100 was similar in both groups. This may mean that EEG can detect the onset of excitability even earlier than MEPs.

Other methods to address the issue of cortical vs. spinal-cord activity

In the literature, the H-reflex is the most commonly used method for ruling out the participation of the spinal cord in the alterations of MEPs evoked by TMS. Unfortunately, as was pointed out by Morita *et al*. (1999) and Nielsen *et al*. (1999), one should be careful about H-reflex being used as a control for the cortical excitability. These authors showed that (1) the set of spinal motoneurons activated by the H-reflexes and TMS are different and (2) TMS might influence the size of MEPs by the activation of spinal interneurons, which makes it difficult to compare TMS stimulation with the H-reflex experiments.

The alternative to the H-reflex can be transmastoidal electrical stimulation at the level of the brainstem (Ugawa *et al*., 1991; Werhahn *et al*., 2002). This technique, however, leads to an activation of other descending tracts and it lacks the complex I-wave pattern related to TMS. Another possibility is to use transcranial electrical stimulation, which is thought to be capable of stimulating axon hillocks of pyramidal cells, without interfering largely with the cortical network. Yet, although this stimulation is good for the activation of D-waves, I-waves might be present as well (Di Lazzaro *et al*., 1999), and thus intracortical and spinal-cord influences would be mixed.

We therefore believe that the TMS–EEG approach might be a complementary method for the evaluation of cortical effects of TMS, being the only method allowing us to measure TMS-induced neuronal activation in the millisecond time-scale.

Conclusions

This study shows that an increase in premovement cortical excitability is associated with the amplitude decrease and latency increase of the TMS-induced N100 component. It is hypothesized that the N100 component can represent an inhibitory response following TMS. This suggestion is in agreement with intracellular recordings in animals, paired-pulse TMS studies and experiments showing increased pre-movement excitability on the basis of MEPs. The amplitude of the N100 component was significantly affected by the visual stimuli alone, thus suggesting that even in a rest condition visual stimuli have access to the sensorimotor cortex, most probably through non-specific ascending arousal mechanisms. The N100 component was also affected by the switching from the rest- to task-condition, thus implying that not only the preparation and execution of the movement, but also engaging to a task-condition has an effect on the excitability of the sensorimotor cortex.

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Abbreviations

ABP, abductor pollicis brevis; EEG, electroencephalography; EMG, electromyography; MEP, motor evoked potential; MRF, mesencephalic reticular formation; TMS, transcranial magnetic stimulation.

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II

Publication II

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BILATERAL CHANGES IN EXCITABILITY OF SENSORIMOTOR CORTICES DURING UNILATERAL MOVEMENT: COMBINED ELECTROENCEPHALOGRAPHIC AND TRANSCRANIAL MAGNETIC STIMULATION STUDY

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Abstract—It remains unclear what neuronal mechanisms in humans are reflected in the activation of the ipsilateral hemisphere during the performance of unilateral movements. To address this question we combined transcranial magnetic stimulation (TMS), electroencephalography (EEG), and electromyographic (EMG) recordings of motor evoked potentials (MEPs). Compared with previous TMS studies, where changes in excitability might be related to both cortical and spinal mechanisms, our setup allowed a more direct evaluation of the cortical processes related to the performance of unilateral movements. EEG responses showed that the unilateral motor reactions were associated with the bilateral increase in the excitability of sensorimotor cortices. However, this increase was smaller in the ipsilateral hemisphere most likely due to the fact that the excitation in ipsilateral hemisphere coincided with additional inhibitory processes related to the suppression of mirror movements. This explanation was further corroborated by showing that only contralateral changes in cortical excitability led to the increase in the amplitude of peripheral MEPs, while neuronal activation in the ipsilateral hemisphere was not associated with the changes in the muscle responses. These results suggest that the increased excitability in the ipsilateral hemisphere was uncoupled from the modulation of the cortico-spinal output. Moreover, we show that the background neuronal activity during unilateral movements was different in the ipsi- and contralateral hemisphere. This difference most likely reflects inter-hemispheric balance between the excitation and inhibition which is required for the optimal performance of the unilateral movement.

Key words: unilateral movement, mirror movement, transcranial magnetic stimulation, electroencephalography.

The present study explores neurophysiological correlates of unilateral movement in sensorimotor areas of both hemispheres, with a special emphasis on the role of the ipsilateral hemisphere. Functional imaging studies have demonstrated that unilateral movements are associated with activations in the ipsilateral sensorimotor cortex (Shibasaki and Nagae, 1984; Rao et al., 1993; Chen et al., 1997, 2002; Alkadhi et al., 2002; Kobayashi et al., 2003; Caramia et al., 2000; Kim et al., 2004). Measurements of movement-related magnetic fields have also shown a bilateral activation of motor areas at about 500 ms prior to self-paced movement (Kristeva et al., 1991; Salmelin et al., 1995; Tandonnet et al., 2003; Vidal et al., 2003). Previous studies with transcranial magnetic stimulation (TMS) have contradicting results showing that the cortical excitability in the ipsilateral hemisphere can be increased (Hoshiyama et al., 1996; Muellbacher et al., 2000; McMillan et al., 2004, 2006), decreased (Leocani et al., 2000; Duque et al., 2005; Koch et al., 2006) or not changed during the performance of unilateral movements (MacKinnon and Rothwell, 2000). It is possible that such different results could be related not only to the specific experimental tasks, but also to the fact that motor evoked potentials (MEPs), used in those studies, reflect contribution of both cortical and spinal cord excitation which might have been differently mixed in each specific experiment. An advantage of our study is the combination of electroencephalography (EEG) and TMS, which allows a more direct evaluation of cortical excitability in both hemispheres during the preparation and execution of the unilateral movements.

Apart from neurophysiological basis of unilateral movement organization, studying of ipsilateral activation might be also valuable in understanding the origins of mirror movements (MM, Cohen et al., 1991; Kristeva et al., 1991; Mayston et al., 1999; Verstynen et al., 2005; 2007). In general MMs have been hypothesized to occur either due to uncrossed cortico-spinal fibers (Konagaya et al., 1990; Cohen et al., 1991; Ziemann et al., 1999), branching of cortico-spinal axons innervating homologous "mirror" muscle (Hess et al., 1986; Tinazzi and Zanette, 1998; Stedman

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Abbreviations: ANOVA, analysis of variance; APB, abductor pollicis brevis; CONTRA, contralateral movement; EEG, electroencephalography; EMG, electromyography; ER, evoked responses; IPSI, ipsilateral movement; MEP, motor evoked potential; MM, mirror movement; MRF, mesencephalic reticular formation; MT, motor threshold; NOMOV, no movement; p.h., post hoc; ROI, region of interest; RT, reaction time; TMS, transcranial magnetic stimulation; VIS, visual stimuli only; VISTMS, visual stimulus followed after 187 ms by a transcranial magnetic stimulation pulse.

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et al., 1998; Muellbacher et al., 2000; Stinear et al., 2001; Ziemann and Hallett, 2001) or due to bilateral activation of sensorimotor cortices (Cramer et al., 1999; Mayston et al., 1999; Verstynen et al., 2007; Perfiliev, 2005). However since MMs do not occur in abundance under the normal conditions, there should exist an inhibitory mechanism, which would prevent excessive ipsilateral cortical activation leading to MMs. By contrasting MEP and EEG results in the present study we show neurophysiological correlates of such inhibition.

Prevention of MMs and in general performance of unilateral movements might involve not only neuronal processing triggered by the sensory stimulus, but also a fine tuning of background/ongoing neuronal activity. A term background/ongoing implies that the neuronal activity is task related but not evoked or induced by the stimuli. It underlies the neuronal processing which is required for the performance of a given task. Animal studies have shown that the effective performance in motor tasks is related to a specific fine-tuning of the ongoing neuronal firing in the motor cortex (Favorov et al., 1988; Cisek et al., 2003; Perfiliev, 2005). In experiments with cats, Perfiliev (2005) demonstrated that background neuronal firing in the ipsilateral hemisphere might contribute to the correct selection of the unilateral response. The present study provides for the first time an evidence for the existence of a similar mechanism in the human sensorimotor cortices. We probed a current state of the neuronal activity in the cortex by applying TMS and simultaneously recording EEG responses. The main idea of such approach is that the neuronal responses to TMS should be different depending on the neuronal activity in the given cortical area. A similar approach was successfully utilized previously by probing the background state of the motor cortex with TMS–MEP combination under different experimental conditions, e.g. face recognition (Keenan et al., 2001), self-induced emotional thoughts (Tormos et al., 1997), and non-motor linguistic processing (Papathanasiou et al., 2004). An advantage of TMS–EEG over TMS–MEPs methods, however, is that the earlier can be applied to any cortical areas, while the latter can only be used for the studies of the motor cortex.

EXPERIMENTAL PROCEDURES

Subjects

Nine right-handed healthy subjects (age 23–32 years, four females) participated in the study after giving written informed consent. The subjects did not have any neurological or psychiatric disorders. Vision in all subjects was normal or corrected to normal. The ethical Committee of the Helsinki University Central Hospital approved the experimental procedures for the study.

Experimental setup

Design of the present study is presented schematically in Fig. 1A. Subjects were seated comfortably in a chair with their hands being placed on a special support so that the arm was bent in the elbow at approximately 110° angle and fully relaxed. Subjects were instructed to make a motor response to a visual stimulus displayed on a computer screen. Before the beginning of the experiment, subjects practiced for several minutes in order to get familiar with the task and to reach a required level of the performance. In two sessions, sub-

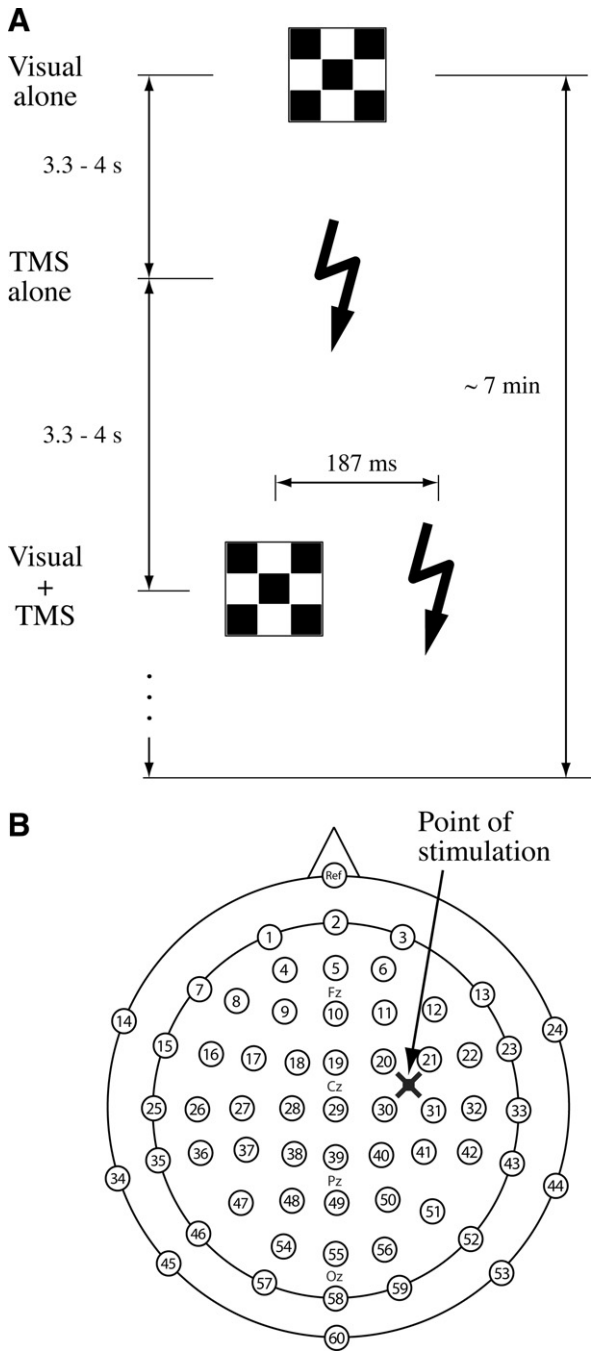

Fig. 1. Experimental setup. (A) Types of stimuli: VIS, TMS pulses only, and combined presentation of visual stimuli and TMS pulses. These three types of stimuli were presented in random order. For each type of stimulus there were 35 repetitions in one session (70 for two sessions). Inter-stimulus interval varied between 3.3 and 4 s. In total, there were six sessions: two sessions with ipsilateral thumb abduction (IPSI session); two sessions with contralateral thumb abduction (CONTRA session), and two sessions where no motor response was required (NOMOV session). One stimulation session lasted for approximately 7 min. (B) Schematic diagram of the 60-channel array used for the recording of TMS-evoked EEG responses. A nose is pointing upwards, cross indicates center of the TMS coil.

jects were instructed to move the thumb contralateral to the stimulated hemisphere (contralateral movement, CONTRA); in two sessions, the ipsilateral thumb had to be moved (ipsilateral movement, IPSI); in two other sessions, subjects were asked not to perform any movement, but merely to observe the stimuli on the screen (no movement, NOMOV). In all subjects and in all sessions only the right hemisphere was stimulated with TMS, but depending on the reacting hand, this hemisphere was either contra- or ipsilateral. This experimental design was deliberately chosen as it allows a precise comparison of both sessions (CONTRA and IPSI) with the same coil position, motor threshold (MT), and electrode placement. Another advantage of the current experimental design is that there was no need to reposition a TMS coil between the hemispheres, which is a procedure prone to targeting variations and significant prolongation of the experiment duration. Another idea for using TMS of the right hemisphere was to compare the results of the present study with the results of our previous study (Nikulin et al., 2003) where left hemisphere was stimulated. Although this comparison is applicable only for the contralateral hemisphere (since it was the only studied hemisphere in Nikulin et al., 2003) it allows a generalization of the obtained results in simple reaction-time paradigm for both left and right hemispheres.

Consecutive presentation of the stimuli was separated by an inter-stimulus interval that varied randomly between 3.3 and 4 s. On the one hand the duration of the experiment should be as short as possible in order to maintain a stable position of TMS coil with respect to the head and on the other hand inter-stimulus intervals should be sufficiently long in order to allow a full relaxation of the performing hand so that the EEG/electromyography (EMG) results are not biased by the remaining muscle activity. Our inter-stimulus interval (3.3–4 s) represents a compromise between the two abovementioned factors. One stimulation session lasted for approximately 7 min; the resting period between the two consecutive sessions was 4–5 min. The stimuli were presented in random order.

Visual stimulation

The visual cue was 5×5 black-and-white checkerboard, each check with a visual angle of 1×1° (vertical×horizontal). In the middle of the checkerboard there was a red fixation cross. The visual pattern was displayed for 50 ms. There were three stimulation conditions (see Fig. 1) with 70 stimuli in each: 1) visual stimuli only (VIS), 2) magnetic stimuli only (TMS), and 3) combined presentation of visual stimulus followed after 187 ms by a transcranial magnetic stimulation pulse (VISTMS). In the present study we wanted to explore predominantly the excitability of the sensorimotor cortices during the preparation to perform a movement. From previous TMS–MEP study (Chen et al., 1998) it was known that in simple reaction-time paradigm the excitability in the contralateral cortex starts changing approximately 100 ms before the movement onset. Given that that the mean of reaction times (RT) in the present study was ~280 ms, the delay of 180 ms would thus correspond to ~−100 ms with respect to the movement onset. For comparative purposes we also wanted to use a delay which would be similar to a delay (180 ms) from our previous study (Nikulin et al., 2003). However, for technical reasons it was not possible to use exactly 180 ms. Instead, a delay of 187 ms was utilized, and 7 ms difference was quite negligible in the context of the present paradigm.

EEG recordings

Electroencephalogram was recorded with 60 C-shaped Ag/AgCl electrodes (outer diameter 10 mm and inner diameter 6 mm) in order to avoid overheating due to eddy currents induced by the magnetic pulse. The electrodes were mounted on an elastic fiber cap to form a multi-channel EEG array (represented in Fig. 1B, Virtanen et al., 1996). The reference electrode was attached to the nose. Continuous EEG was recorded using the TMS-compatible prototype of eXimia (Nexstim Ltd., Finland) EEG amplifier (Virtanen et al., 1999), with sampling frequency 1450 Hz, 16-bit resolution and passband of 0.1–500 Hz. During the 6-ms "gating" period, in which the TMS-pulse artifacts could be present, the amplifier was blocked (by sample-and-hold circuitry, Virtanen et al., 1999).

EMG recordings

Bipolar surface Ag/AgCl electrodes were used for the recordings of compound MEPs from the abductor pollicis brevis (APB) muscles on both hands using the muscle-belly tendon technique. EMG was recorded in the frequency band 10–1000 Hz and sampled at 5952 Hz.

TMS

A Magstim 200 magnetic stimulator (Magstim Ltd., UK) connected to a coplanar figure-of-eight coil (NP 9925, 70 mm wing) was used for the cortical stimulation. We positioned the coil over the right sensorimotor area at the place where the largest MEPs in the left-hand APB could be evoked. The handle of the coil was pointing backward and laterally at an angle of approximately 45° away from the midline (cf. Thielscher and Kammer, 2002; Ziemann et al., 1999) in order to achieve optimal stimulation. MT for each subject was determined as the intensity of TMS pulses sufficient to elicit the MEPs with an amplitude of at least 50 μV in the targeted muscle in ~50% of trials (Rossini et al., 1994, 1999). An intensity of the stimulation in experimental sessions was 20% above the subject's MT. TMS pulse was applied either alone or 187 ms after the onset of the visual stimuli. During the experimental sessions, the magnetic coil was mounted on a tripod stand (Model 58, Manfroto Ltd., Italy) with a flexible extension arm (Model 244, Manfroto Ltd.) that allowed fast and accurate positioning/repositioning and the maintenance of the same coil position during the course of the measurements. A combination of special earplugs and soundproof headphones was used to attenuate the acoustic click produced by the coil.

EEG analysis

For further analysis, the data were re-referenced with respect to the common average potential. After the rejection of EEG segments containing mechanical and muscle artifacts, the responses were averaged and low-pass filtered with a cut-off frequency of 40 Hz. The data were segmented into epochs from −100 ms to + 300 ms with respect to the TMS pulse. Several previous reports have indicated large variability in the responses at latencies from 0 to 70 ms (Komssi et al., 2002; Nikulin et al., 2003; Bonato et al., 2006): variations were pronounced not only among subjects, but also depended on the experimental setup. Similar to other TMS–EEG studies (Paus et al., 2001; Bender et al., 2005; Massimini et al., 2005), in our measurements the most pronounced and reproducible component across subjects and conditions was the TMS-evoked N100. This component peaks at about 100 ms after the TMS and channels with the highest N100 amplitudes are located in the vicinity of the stimulated cortical site. Recent studies (Nikulin et al., 2003; Bender et al., 2005; Kähkönen and Wilenius, 2007) have shown that N100 is a reliable TMS-evoked EEG response and is sensitive to subtle changes in cortical excitability. We focused our analysis on the amplitude and latency of TMS-evoked N100 component.

The cortical regions of interest (ROI) in the present study were sensorimotor areas of both hemispheres. With the goal to address local cortical excitability changes, we selected a ROI covered by six electrodes in the vicinity of the point of stimulation where the most pronounced N100 was observed (shaded areas in Fig. 3A). A similar ROI was also selected from the homologous area of the

opposite (left) hemisphere in order to demonstrate interhemispheric differences in amplitude and latencies.

An average trace was obtained from these six electrodes and the amplitude and latency characteristics of N100 were assessed from it. The following two types of averaged epochs were used: 1) N100 to TMS pulses only and 2) N100 to TMS with the preceding visual cue (VIS). In the latter case, the parameters of N100 were obtained from a difference curve: the responses to visual stimuli alone were subtracted from the responses to the combined presentation of visual stimuli and TMS (i.e. VISTMS minus VIS). These two types of N100 responses (TMS-only and TMS after visual stimulus) were calculated separately for CONTRA, IPSI, and NOMOV sessions. The amplitude of N100 integrated in the time window of ±5 ms around the peak latency was calculated separately for each session (CONTRA, IPSI, and NOMOV) and condition (TMS and VISTMS).

EMG analysis

The amplitudes of MEPs were calculated as the mean value of the rectified EMG in the 18–43 ms time interval, which covers the time course of MEPs evoked by the TMS. Since the interval of interest for measurements of MEP was up to 43 ms after the TMS pulse, and visual stimuli preceded TMS by 187 ms, reactions faster than 230 ms were within the averaging window for MEPs, thus interfering with them. These epochs were discarded from the MEP analysis.

Statistical analysis

When appropriate, the comparison of two means only was performed using paired *t*-tests. Otherwise, repeated measures analysis of variance (ANOVA) was used for EMG and EEG data.

Statistical analysis of EMG data. For overall assessment of RTs to visual stimuli, a 2×2 ANOVA design was used with the factors SESSION (reaction with ipsilateral or contralateral hand) and STIMULUS (presence or absence of TMS after the visual stimulus). Analysis of MEP amplitudes was performed using 3×2 ANOVA with factors: SESSION (3 levels; CONTRA, IPSI, and NOMOV) and STIMULUS (presence or absence of visual stimuli before the TMS).

Statistical analysis of EEG data. ANOVA for EEG data had three factors: SESSION (CONTRA, IPSI or NOMOV), STIMULUS (presence or absence of visual stimuli before TMS), and HEMISPHERE (left or right), thus yielding 3×2×2 design. Post hoc (p.h.) analysis was performed with the Neuman-Keuls test.

RESULTS

RTs to visual stimuli

When subjects performed reactions with the hand contralateral to the stimulated hemisphere (i.e. the left hand), RTs to VIS and VISTMS were 273±19 (mean±standard error of the mean) and 288±16 ms, respectively. When reacting with the ipsilateral hand (i.e. the right hand), RTs were 271±13 and 270±12 ms for VIS and VISTMS conditions, respectively. Repeated measures ANOVA revealed significant ($F_{1,8}$=16.12, P<0.004) interaction between SESSION (CONTRA-IPSI) and STIMULUS (VIS vs. VISTMS) factors. P.h. Neuman-Keuls analysis showed that the reactions to visual stimuli in the CONTRA sessions (reactions with the hand contralateral to the stimulated hemisphere) in VISTMS condition were significantly slower than in all other conditions (VIS condition in CONTRA and IPSI, and VISTMS condition in IPSI with P<0.002, p.h. for all comparisons). There was no difference between the RTs to VIS when subjects performed task with the right of left hand (P<0.54, p.h).

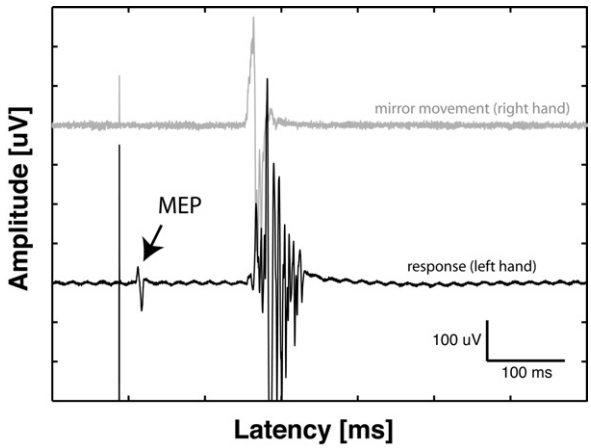

Fig. 2. An example of a MM recorded in CONTRA session (VISTMS condition, i.e., combined presentation of visual stimulus and the following TMS). Subject responded with the left hand, but one can also see an occurrence of EMG in the right hand, so-called MM. The MEP response is present at about 22 ms after the TMS. Vertical lines on the left side of the plot indicate the TMS pulse. The visual stimulus preceded the TMS pulse by 187 ms.

MMs

When subjects moved the thumb in response to the visual cue, EMG activity was occasionally registered in the homologous muscle of the opposite hand. An example is presented in Fig. 2.

The occurrence of MMs was unevenly distributed among subjects and in CONTRA session the range (with respect to the total number of movement with the correct hand) was 0.7–22%. During the IPSI session, the range was 0–1%. The amplitude of MMs was also unevenly distributed among subjects; on average it was about 1/3 of the amplitude of EMG responses from the contralateral hand.

EEG results

N100 amplitudes. A topographic plot of averaged EEG evoked responses (ER) is presented in Fig. 3A. Dashed traces represent the average of EEG responses to TMS stimuli from the sessions not requiring any motor response; solid and dotted traces represent EEG activity to TMS stimuli with the preceding visual stimuli requiring reaction with contra- or ipsilateral hand, respectively. There were approximately 65 epochs for each type of ER. Fig. 3B shows average signals of six highlighted electrodes over the sensorimotor areas from each hemisphere. The amplitude of N100 component was attenuated in both CONTRA and IPSI sessions when TMS was preceded by the visual stimulus requiring a motor reaction.

The amplitude of N100 demonstrated a high interhemispheric asymmetry with the largest values being in the channels located over the right sensorimotor area ($F_{1,8}$=33.35, P<0.0005, HEMISPHERE factor, see Figs. 3 and 4A). In the CONTRA sessions the amplitude of N100 was larger in the right than in the left hemisphere by

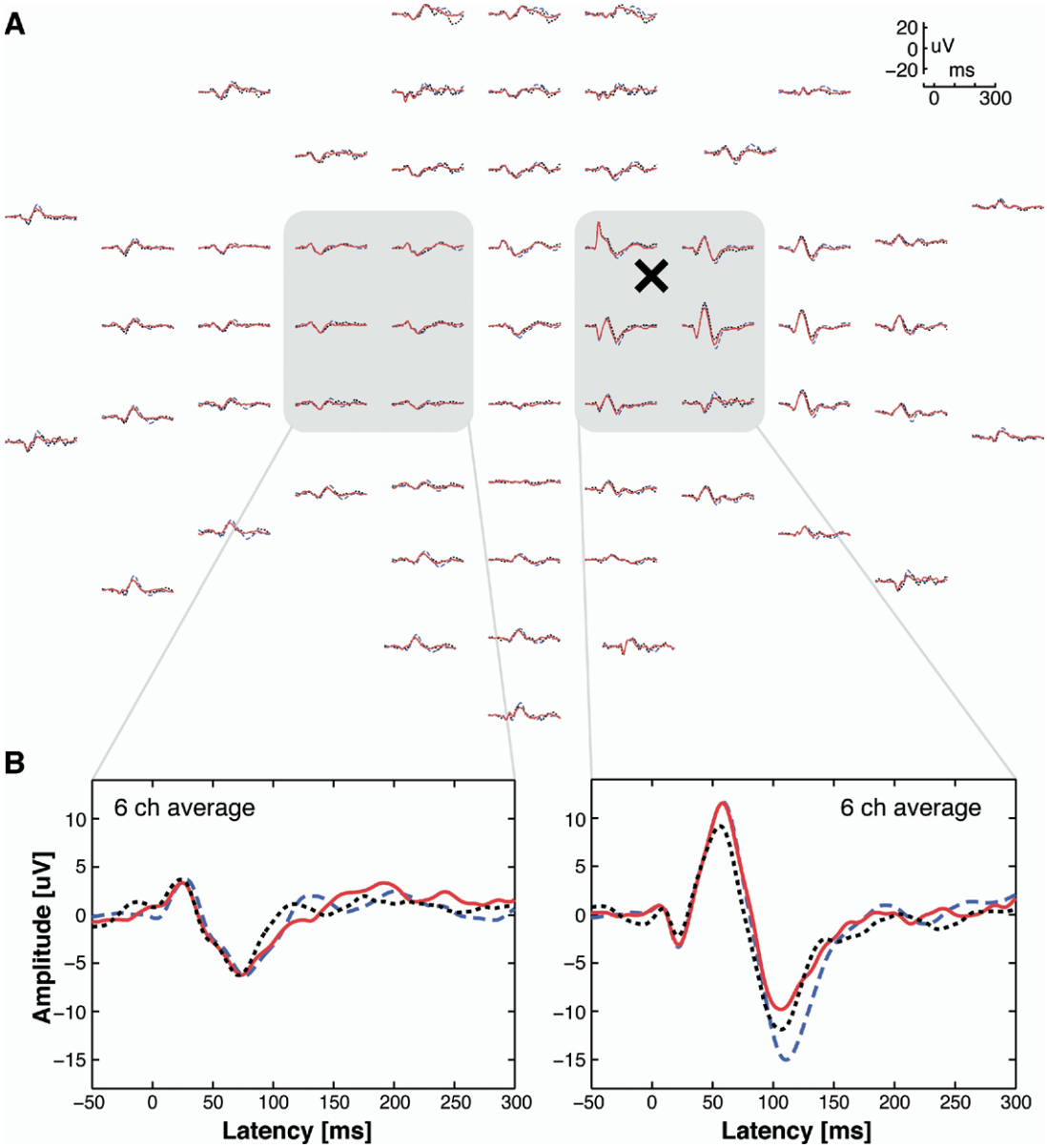

Fig. 3. TMS-evoked EEG responses recorded in one subject. (A) Whole-head plot (nose pointing upwards, "top of the head" view). A cross represents a center of the stimulating coil. Dashed blue traces - EEG responses to TMS-only in sessions without motor responses (NOMOV); solid red line - EEG response calculated as VISTMS minus VIS in CONTRA session; dotted black line - EEG response calculated as VISTMS minus VIS in IPSI session. Note pronounced lateralization of the responses: in the vicinity of the stimulated site the amplitudes are the highest and are attenuated with the distance from the point of the stimulation. The shaded areas depict the electrodes used in the analysis. (B) EEG responses averaged across six highlighted electrodes in the right and left hemispheres. Note that in the stimulated hemisphere (right panel), TMS-evoked N100 responses were stronger attenuated in CONTRA than in IPSI sessions. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

approximately 4.7 μV. This inter-hemispheric difference was even more pronounced for the IPSI session.

N100 and CONTRA. In the right hemisphere, N100 in the CONTRA session was significantly reduced in VISTMS condition compared with TMS-only condition (Fig. 4A, right panel, cVT vs. cT bar). In the left hemisphere, N100 was also significantly attenuated ($P<0.0003$, p.h.) in the VISTMS condition.

N100 and IPSI. In the right hemisphere during IPSI sessions the N100 was significantly attenuated in VISTMS condition compared with the TMS-only condition (Fig. 4A, right panel, iVT vs. iT bar, $P<0.0002$, p.h.). Similar attenuation of N100 was also observed in the left hemisphere (Fig. 4A, left panel, iVT vs. iT bar, $P<0.03$, p.h.).

Task related differences between the hemispheres. As demonstrated above, N100 in the stimulated (right) hemisphere was attenuated both for CONTRA and IPSI sessions in VISTMS condition. We then compared the degree of this attenuation between these sessions. The attenuation was expressed in percentages in order to com-

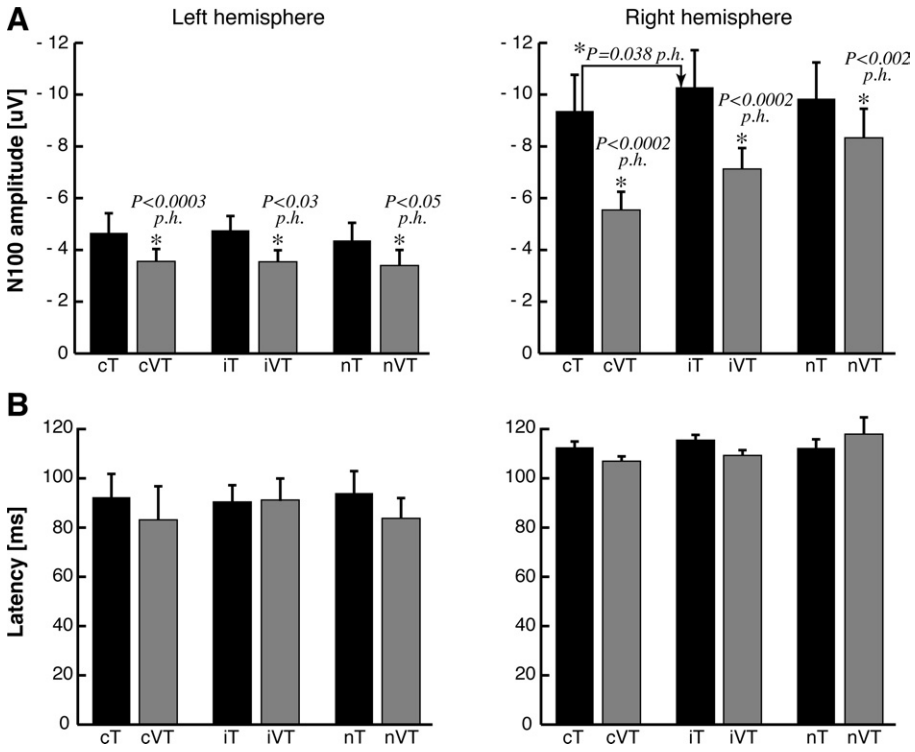

Fig. 4. Grand averages (n=9) of amplitudes (A) and latencies (B) of N100 component in the stimulated (right) and non-stimulated (left) hemispheres. In all plots, black bars are responses to TMS-only, and gray bars represent responses obtained with VISTMS minus VIS calculation. Note a significant decrease of N100 amplitude in VISTMS conditions in both hemispheres and in all sessions. cT, iT and nT - amplitudes of N100 to TMS-only in CONTRA, IPSI, and NOMOV sessions, respectively; cVT, iVT and nVT - amplitudes of N100 to a combined stimulation of visual stimulus and TMS in CONTRA, IPSI and NOMOV sessions.

pensate for the differences in the absolute value of the N100 amplitude. Compared with the TMS-only condition, N100 in VISTMS condition was attenuated stronger in the CONTRA than in the IPSI session ($P<0.03$, paired t-test), the values for attenuation were 36% and 25%, respectively.

N100 in no-movement session. In the right hemisphere during NOMOV sessions, the N100 in VISTMS condition was significantly attenuated (Fig. 4A, right panel, nVT bar) compared with the TMS-only condition (Fig. 4A, right panel, nT bar). Even though this difference appeared to be relatively small, it was statistically significant ($P<0.002$, p.h.). In the left hemisphere, N100 in VISTMS condition (Fig. 4A, left panel, nVT bar) was also significantly attenuated ($P<0.05$, p.h.).

N100 to TMS-only in CONTRA and IPSI sessions. EEG responses to TMS-only in three experimental sessions are presented in Fig. 4A (black bars). In the stimulated hemisphere, the N100 was significantly higher in the IPSI session than in the CONTRA session (right panel, black bars; $P=0.038$ p.h.).

N100 latencies. The ANOVA revealed significant effect of HEMISPHERE factor ($F_{1,8}$=8,84, $P<0.02$) for the latencies of the N100 component. P.h. test showed that the latencies of the N100 component in the right (stimulated) hemisphere were longer compared with the corresponding latencies in the left (non-stimulated) hemisphere. There were no significant differences between the latencies of N100 belonging to CONTRA and IPSI sessions.

EMG results

Only epochs without pre-stimulus EMG were selected for the analysis. The ANOVA showed that there were significant changes in the amplitude of MEPs related to both SESSION (Fig. 5; $F_{2,16}=9.08$, $P<0.003$) and STIMULUS ($F_{1,8}=22.92$, $P<0.002$) factors, as well as significant interaction between SESSION and STIMULUS factors. Neuman-Keuls p.h. analysis showed that MEPs during the CONTRA sessions of VISTMS condition (performance with the contralateral hand) were significantly larger than in all other experimental sessions (CONTRA, IPSI and NOMOV for both TMS-only and VISMTS conditions, $P<0.0002$ p.h.). MEP amplitudes to TMS-only in three sessions (IPSI, CONTRA, and NOMOV) were not statistically different from each other. Fig. 6 shows also an example of enhancement of MEP amplitude related to the preparation to perform the movement in CONTRA session.

Since we were also interested in the correlation of excitability measures from the cortex and periphery, a correlation was calculated between the increase of MEPs and the attenuation of N100 amplitude in VISTMS condition of the CONTRA session. This correlation was not significant ($\rho=0.23$, $P=0.55$).

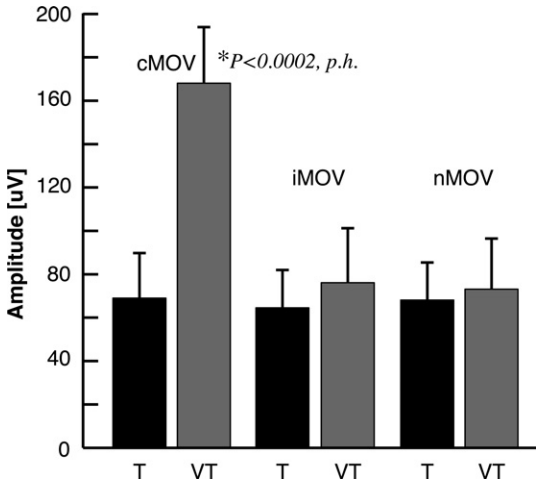

Fig. 5. Grand averages (n=9) of the MEP amplitudes from the left APB in all experimental conditions and sessions. Only in VISTMS condition of CONTRA session (reaction with the contralateral hand), the amplitudes of MEPs were significantly enhanced. cMOV, iMOV, and nMOV - CONTRA, IPSI, and NOMOV sessions, respectively. T and VT - TMS-only and VISTMS conditions, respectively.

DISCUSSION

The present study shows that preparation and execution of unilateral movement is associated with bilateral changes in cortical excitability and only in the contralateral hemisphere these changes were associated with the modulation of muscle responses. Such dissociation implies that apart from some bilateral increase in excitation, additional inhibitory mechanisms in the ipsilateral hemisphere were recruited in order to suppress its output and thus to prevent an occurrence of the MMs. The present study benefits from the recordings of macroscopic cortical neuronal responses to TMS which allow a more direct evaluation of the cortical excitability without additional contribution from the spinal cord excitability known to be modulated by the movements. Below we discuss in detail the implications of our findings for the organization of the unilateral movements.

MMs

All of our subjects showed an occasional occurrence of the MMs recorded in the homologous APB muscle of the non-responding hand. Frequency of MMs was comparable to other studies with similar paradigms in healthy subjects (e.g. Verstynen et al., 2007). In our experiments the effector was APB, which is a thin and superficially located muscle, which makes it an ideal candidate for the detection of the weakest EMG.

Neurophysiological origin of N100

In line with the previous studies (Nikulin et al., 2003; Bender et al., 2005) we suggest that the N100 component reflects an inhibitory process induced by the TMS. A number of experimental approaches support this hypothesis. Electrical surface stimulation of the cortex produces long-lasting (>100 ms) IPSPs (Krnjević et al., 1966; Rosenthal et al., 1967). The time course of such inhibition is similar to the development of TMS induced N100. Another pool of the data supporting an inhibitory nature of TMS-evoked N100 originates from the experiments involving double-pulse paradigm with a supra-threshold conditioning stimulus. These studies showed that the amplitude of MEPs to the test stimulus is suppressed in the interval of about 200 ms after the conditioning TMS, with a peak at about 100–150 ms (Valls-Solé et al., 1992; Roick et al., 1993; Matsunaga et al., 2002). Temporal characteristics of N100 to supra-threshold TMS are remarkably similar to the time-course of inhibitory effects in the abovementioned studies.

Modulation of N100 amplitude in the contralateral hemisphere

Recordings of single cells in the contralateral motor cortex showed that the time period immediately preceding movement was associated with the increased cortical excitability manifested in the high firing rate of neurons (Evarts, 1966, 1974; Fetz and Finocchio, 1971; Gribova et al., 2002). In line with these findings previous TMS studies also showed that the preparation to move is related to increased cortico-

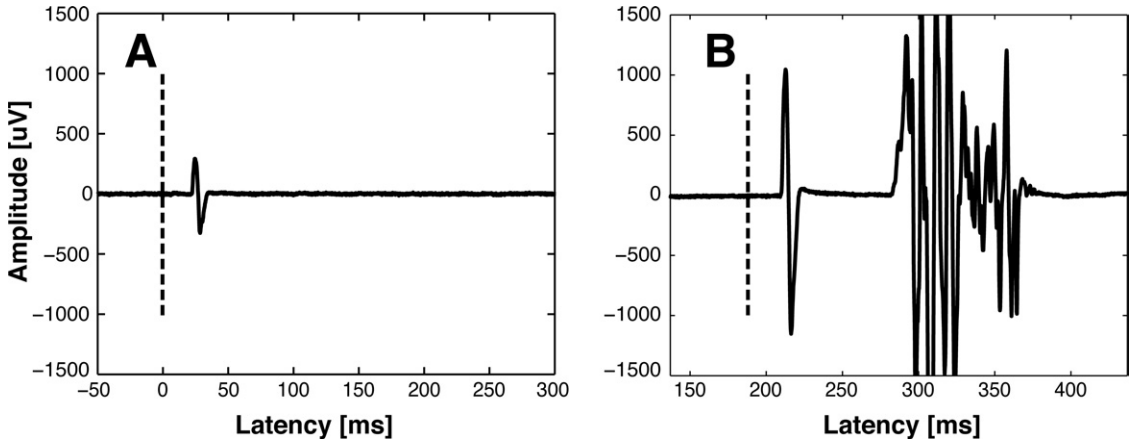

Fig. 6. An example of single-trial MEP enhancement during the contralateral reactions (left hand): (A) MEP to TMS-only pulse, and (B) enhancement of MEP due to preparation to move (VISTMS condition). Voluntary motor response to visual stimulus is visible at approximately 300 ms.

spinal excitability, which is reflected in the augmented amplitude of MEPs (Rossini et al., 1988; Starr et al., 1988; Pascual-Leone et al., 1992; Wassermann et al., 1992; Tomberg, 1995; Hoshiyama et al., 1996; Chen et al., 1998; Leocani et al., 2000; Zaaroor et al., 2001; Burle et al., 2002; Nikulin et al., 2003; McMillan et al., 2004, 2006; Bender et al., 2005). The results of the present study also showed that the amplitude of MEPs was increased when TMS was applied immediately before the movement.

We also show that the amplitude of N100 in the stimulated hemisphere was decreased during motor preparation and execution in CONTRA sessions. In line with the abovementioned studies we hypothesized that the decrease in the amplitude of N100 is caused by the increased excitability in the sensorimotor cortex during motor preparation and execution in CONTRA sessions. Such summation is likely to result in the smaller amplitude of N100 because of inhibitory currents (constituting N100) being to some extent counterbalanced by the excitatory currents related to the movement preparation.

Although MEPs do serve as a measure of excitability, the difficulty is that they reflect state of the neurons both in the motor cortex and spinal cord. Therefore often a term "cortico-spinal excitability" is used to emphasize indiscriminability of MEPs' amplitude to the cortical or spinal cord influences. In the current context it is worth mentioning that the H-reflex, characterizing the spinal cord excitability, can also increase in the time interval preceding the voluntary movement (Hasbroucq et al., 2000; Kato and Kasai, 2000). Moreover, the absence of a correlation between the increase of MEP amplitude and the decrease of N100 amplitude in the present study indicates that additional enhancement of excitability might have occurred at the spinal cord level, thus obscuring the relationship between the amplitude of N100 and MEPs. Such parallel development of cortical and spinal cord excitabilities (revealed with H-reflex) makes it difficult to disentangle them on the basis of MEPs only. On the contrary, EEG measures reflect primarily cortical processes and thus are more straightforward for the interpretation of predominantly cortical processes.

Modulation of N100 amplitude in the ipsilateral hemisphere

We also observed attenuation of the N100 component in the ipsilateral hemisphere, although this attenuation was smaller than in the contralateral hemisphere. In accordance with the discussion above, we hypothesize that the attenuation of the N100 amplitude in the ipsilateral hemisphere is related to the increased cortical excitability. This finding implies that MMs might have a bilateral component (Mayston et al., 1999; Verstynen et al., 2007), and are not restricted to unilateral origin via the uncrossed ipsilateral cortico-spinal pathway as suggested elsewhere (Konagaya et al., 1990; Cohen et al., 1991). An increase in the ipsilateral excitability can occur due to (1) motor irradiation from the contralateral hemisphere (see for rev. Carson, 2005), or (2) can originate directly in the ipsilateral hemisphere (Mayston et al., 1999). Both processes are possible and thus might lead to the generation of undesired MMs, which however should be suppressed. The existence of such inhibitory processes was shown in the previous studies (Kristeva et al., 1991; Leocani et al., 2000; Carbonnell et al., 2004; Perfiliev, 2005). Trans-callosal inhibition from the contralateral hemisphere (Ferbert et al., 1992; Wassermann et al., 1994; Mayston et al., 1999; Ziemann et al., 1999) is the most likely mechanism leading to the suppression of MMs. Thus two processes are happening concurrently in the ipsilateral hemisphere: one is related to the initiation of the MM and another to its suppression (Kobayashi et al., 2003; Perfiliev, 2005). Therefore, the amplitude of N100 should demonstrate smaller decrease of N100 amplitude, since MM-related excitatory activity is to be counterbalanced by the inhibitory activity. And indeed we observed that the attenuation of N100 was smaller in the ipsilateral hemisphere.

We also show that the decrease in the amplitude of ipsilateral N100 did not correspond to the increase of MEP, like in the case of the contralateral N100 (Figs. 5 and 6). This observation is especially interesting considering the fact that there is a heterogeneity in the results from the TMS-MEP studies addressing excitability changes in the ipsilateral hemisphere during the unilateral movements. While a number of studies showed decrease (Leocani et al., 2000; Duque et al., 2005; Koch et al., 2006), other studies showed increase (Hoshiyama et al., 1996; Muellbacher et al., 2000; McMillan et al., 2004, 2006) or no changes (MacKinnon and Rothwell, 2000) in the excitability of the ipsilateral motor cortex during the preparation and performance of unilateral movement. This is most likely due to different tasks used in those studies, stimulation techniques but also due to the fact that MEPs do not provide satisfactory description of a cortical activity only, they are also sensitive to the changes in the excitability of the spinal cord and thus reflect a cumulative effect of cortical and spinal cord excitabilities. This difficulty was addressed in the present study by using direct cortical response produced by TMS.

Reactions to visual stimuli

Significant prolongation of the RTs by the TMS was observed in our experiments only in CONTRA sessions. This is in agreement with previous TMS studies showing similar increase of RT produced by the stimulation of the motor cortex (Terao et al., 2001; Schluter et al., 1999).

The RTs were on average around 280 ms thus implying that TMS preceded the onset of a movement by approximately 100 ms. And thus TMS was applied primarily during the preparation to the movement. But to some extent the development of N100 is also related to the actual execution of the movements. In general, we wanted to see whether ipsilateral hemisphere is active at all during the performance of the unilateral movements similar to other studies using functional imaging techniques (Cramer et al., 1999; Kobayashi et al., 2003; Verstynen et al., 2005). In these studies and in our study the presence of ipsilateral activity during either preparation or execution of movement allows a postulation of a bilateral mechanism for the origin of MMs.

Modulation of N100 in no-movement condition

In agreement with the previous study (Nikulin et al., 2003) we showed that the amplitude of the N100 component was attenuated by the presentation of visual stimuli not requiring any behavioral response. This attenuation is congruent with the anatomical data, which suggests that the motor cortex is only two synaptic connections away from the retina: one from retina to mesencephalic reticular formation (MRF), and second from MRF to the primary motor cortex (Leichnetz, 1986; Nakagawa et al., 1998). The results of the present study show that the visual stimuli modify cortical activity both in ipsi- and contralateral hemispheres. Such bilateral effect is in agreement with the functional findings suggesting that the influences from the visual input via the reticular formation have a diffuse character (Leichnetz, 1986; Steriade et al., 1996; Nakagawa et al., 1998) and reach sensorimotor cortex.

Since NOMOV sessions were interleaved with CONTRA and IPSI sessions, modulation of N100 might also have occurred due to previous association of visual stimuli with the motor reactions. Under this second explanation visual stimuli would trigger some subthreshold (for generation of movement) processes in the motor cortex, which would modulate amplitude of N100. At this stage it is difficult to resolve between the two explanations for modulation of N100 in NOMOV session. One way to resolve it is to perform experiments where NOMOV sessions would be presented in the beginning of the experiment before subjects had a possibility to learn about the behavioral relevance of visual stimuli. A modulation of N100 amplitude in these control experiments would indicate that no association between visual stimuli and motor responses is required for the changes in cortical activation due to visual stimuli. It is also worth mentioning that in principle, be it first or second hypothesis, the changes in the state of the motor cortex in NOMOV session could only be detected with the combined TMS–EEG method by "probing" a neuronal activation in the motor cortex. This is because visual stimuli by themselves cannot evoke production of motor output and MEPs are not sensitive enough to detect these subtle changes in cortical excitability related to visual input to motor cortex.

Changes in background neuronal activity in contra-and ipsilateral hemisphere

When TMS pulses were delivered alone (without preceding visual stimuli) the N100 component was significantly larger in the IPSI than in CONTRA sessions. Based on this fact, we hypothesized that the larger amplitude of the N100 response in the hemisphere ipsilateral to the moving hand reflects background activity involving inhibitory processes which are functionally fine-tuned to prevent occurrence of MMs. In this scenario, N100 will get a larger contribution from already pre-activated tonic inhibitory processes, which are recruited for the suppression of undesired MMs. Similar changes in the background activity of the ipsilateral motor cortex were shown in animal experiments (Perfiliev, 2005). Thus, our data indicate that the readiness to be engaged in a specific motor task differently affects background activity in the contra- and ipsilateral sensorimotor cortices.

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III

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RESEARCH ARTICLE

Electrophysiological correlates of short-latency afferent inhibition: a combined EEG and TMS study

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Abstract Cutaneous stimulation produces short-latency afferent inhibition (SAI) of motor-evoked potentials (MEPs) elicited by transcranial magnetic stimulation (TMS). Since the demonstration of SAI is primarily based on the attenuation of MEPs, its cortical origin is not yet fully understood. In the present study we combined TMS with concurrent electroencephalography (EEG) in order to obtain direct cortical correlates of SAI. TMS-evoked EEG responses and MEPs were analysed with and without preceding electrical stimulation of the index finger cutaneous afferents in ten healthy volunteers. We show that the attenuation of MEPs by cutaneous stimulation has its counterpart in the attenuation of the N100 EEG response. Moreover, the attenuation of the cortical N100 component correlated positively with the strength of SAI, indicating that the transient changes in cortical excitability can be reflected in the amplitude dynamics of MEPs. We hypothesize that the hyperpolarization of the pyramidal cells due to SAI lowers the capacity of TMS to induce the inhibitory current needed to elicit N100, thus leading to its attenuation. We suggest that the observed interaction of two inhibitory processes, SAI and N100, provides further evidence for the cortical origin of SAI.

Keywords Short-latency afferent inhibition · TMS-evoked EEG response · Motor cortex

Abbreviations

D2	Index finger
EEG	Electroencephalography
EMG	Electromyography
ERP	Event-related potentials
FDI	First dorsal interosseous
GABA-A and GABA-B	γ-Aminobutyric acid type A and type B receptors
MEP	Motor-evoked potential

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ROI Region of interest
SAI Short-latency afferent inhibition
TMS Transcranial magnetic stimulation

Introduction

Stimulation of digital nerves and the median nerve at the wrist attenuates upper limb motor-evoked potentials (MEPs) elicited by transcranial magnetic stimulation (TMS) (Delwaide and Olivier 1990; Tokimura et al. 2000). This short-latency afferent inhibition (SAI) starts at about 20 ms and continues for up to 50 ms after the peripheral stimulation. SAI elicited by the stimulation of the index finger (D2) can be observed in several upper limb muscles, and thus it appears to be a rather widespread phenomenon (Helmich et al. 2005). Although SAI is thought to reflect primarily cortical processing, subcortical and spinal inhibition may also contribute to the MEP decrease (Classen et al. 2000; Tamburin et al. 2001). Therefore, the cortical mechanisms of SAI should be investigated with a combination of neurophysiological methods contrasting cortical with peripheral activity.

One such approach is the combination of TMS and electroencephalography (TMS-EEG; Ilmoniemi et al. 1997; Paus et al. 2001; Komssi et al. 2002; Nikulin et al. 2003; Bender et al. 2005; Massimini et al. 2005; Bonato et al. 2006; Esser et al. 2006; Kičić et al. 2008). This approach allows direct electrophysiological measurements of the neuronal responses induced by TMS, instead of inferring cortical functioning only through indirect peripheral measures, such as MEPs. Numerous studies have demonstrated that both behavioural and physiological effects of TMS depend on the functional state of the neuronal populations in the stimulated region (Silvanto et al. 2007; Chambers et al. 2004). Importantly, in the current context, recent studies showed that event-related potentials (ERPs) evoked by TMS are dependent on the minute current states of the stimulated areas of the cortex (Nikulin et al. 2003; Thut et al. 2003; Bender et al. 2005; Fuggetta et al. 2005; Kičić et al. 2008; Raij et al. 2008).

We utilized TMS-evoked EEG responses as a probe of cortical excitability in the time interval when SAI is most pronounced (Tokimura et al. 2000) and aimed at obtaining a direct demonstration of SAI at the cortical level. Moreover, in our study we provide for the first time evidence for a linear relationship between the central (EEG responses) and peripheral (MEPs) counterparts of SAI, thus further consolidating the findings related to the central origin of SAI and, in general, for the notion of using an amplitude modulation of MEPs as a reflection of changes in the cortical excitability.

Materials and methods

Subjects

Ten healthy right-handed subjects (six females, 26 ± 7 years, mean ± standard deviation, range 22–46 years) participated in the study. The experimental protocol was developed in accordance with the "Declaration of Helsinki" and was approved by the local ethics committee. All subjects gave their written informed consent to participate in the study.

Electromyography

Electromyographic activity (EMG) was recorded with Blue Sensor surface electrodes (N-00-S, Ambu, Denmark) using a belly-tendon montage over the right first dorsal interosseous (FDI) muscle. The EMG signals were recorded with a band-pass filter of 5–5,000 Hz and digitized at a sampling rate of 10 kHz (MegaWin 2.4, Mega Electronics Ltd, Finland). Each 130-ms epoch included a 30-ms pre-TMS baseline. Peak-to-peak MEP amplitudes (Rossini et al. 1994; Fitzgerald et al. 2002; Daskalakis et al. 2004; Ziemann et al. 2001) were measured offline for each individual trial using the MegaWin 2.4 system (Mega Electronics Ltd).

Transcranial magnetic stimulation

TMS was performed with a figure-of-eight coil with a 70 mm outer diameter of each wing (Nexstim Ltd, Finland). Monophasic TMS pulses had a 70 μs rise- and 1 ms decay time. The positioning of the TMS coil was performed with the eXimia navigation brain system (Nexstim Ltd, Finland) using a three-dimensional reconstruction of the individual magnetic resonance images (Fig. 1a). The procedure consisted of two-steps: (1) using magnetic resonance images, we identified the hand area on the anterior bank of the central sulcus, and (2) in the vicinity of the hand area we performed a search for the position of the coil where TMS evoked the strongest MEPs in FDI. To obtain a maximal MEP response, the coil was placed tangentially over the left primary motor cortex with the handle pointing backward and laterally at a 45° angle away from the midline (Thielscher and Kammer 2002).

In TMS research, the intensity of the stimulation can be expressed: (1) as a percentage of the motor threshold (Nikulin et al. 2003; Komssi et al. 2007; Talelli et al. 2007), (2) as a percentage of the output of the stimulator (Nikouline et al. 1999; Hortobagyi et al. 2006), (3) on the basis of the neurophysiological effects, such as the amplitudes of MEPs (Tokimura et al. 2000; Komssi et al. 2002; Mochizuki et al. 2004), 4) on the basis of the subjective experience of e.g. phosphenes (Silvanto et al. 2007; Marzi et al. 2008; Romei

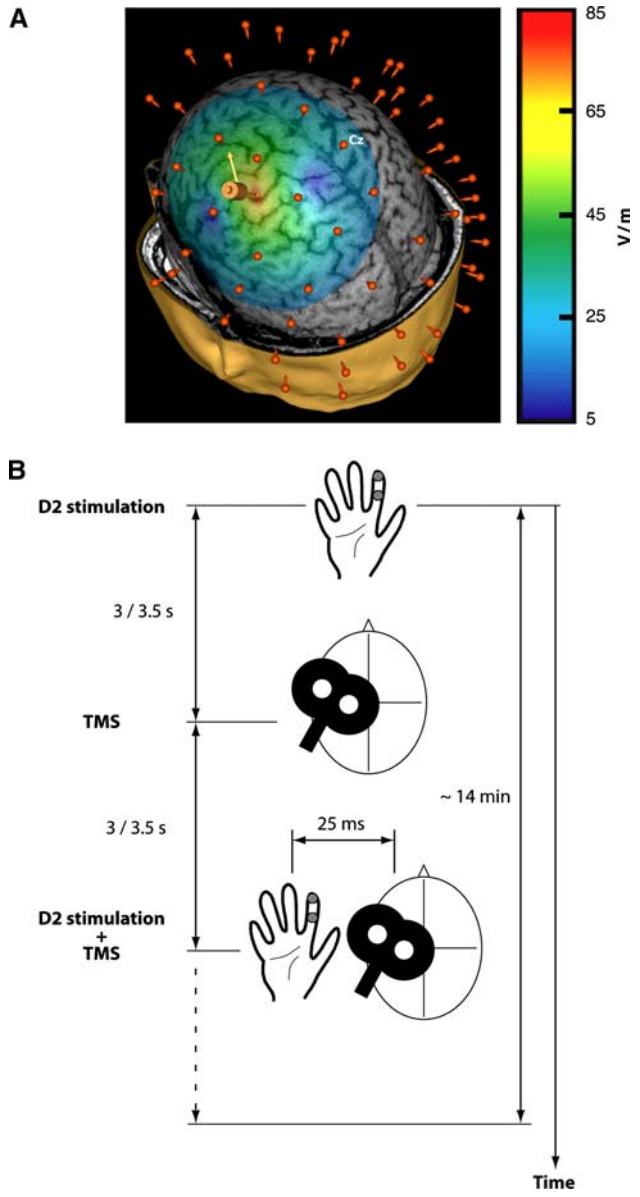

Fig. 1 **a** Positions of EEG electrodes and TMS coil. The cylinder indicates the centre of the TMS coil (projection area of the FDI in the motor cortex) with the cylinder's *arrow* showing the orientation of the intracortically induced electric current. The *colour map* shows the strength of the intracortically induced electric field. The *pins* indicate the locations of the EEG electrodes. Cz electrode position is marked. **b** Experimental design. Three conditions were pseudo-randomized during the experimental session: the index finger stimulation (D2 stimulation), the TMS alone, and a combination of the TMS and the D2 stimulation. The *arrows* on the *left* represent intervals between the three conditions; the *arrows* on the *right* represent the approximate duration of one session

et al. 2008). In accordance with the previous TMS studies on SAI (Tokimura et al. 2000; Cucurachi et al. 2008; Nardone et al. 2008), the strength of the test TMS pulse was adjusted to evoke MEPs with an amplitude of approximately 1 mV. In order to facilitate a comparison of our results with the results from other studies and to provide a clear guidance for other researchers using standard TMS equipment, we also report TMS intensities with respect to the individually measured motor threshold and to the output of the stimulator. The resting motor threshold was defined as the TMS intensity sufficient to elicit MEPs of at least 50 μV in five out of ten consecutive trials in the relaxed target muscle. The motor threshold for the FDI muscle was 61 ± 1% (mean ± standard error of mean) of the maximal stimulator output. The test stimulus intensity was set at 70 ± 1% of the stimulator output, thus corresponding to 115 ± 1% of the motor threshold. In order to study SAI on the basis of MEP attenuation, a suprathreshold intensity was chosen to evoke MEPs in practically all trials.

According to the eXimia brain navigation system, the average depth of the cortical surface across all ten subjects was 18 ± 2 mm from the skull surface (Fig. 1a), and the average of the individually measured maximal intra-cortically induced electric fields at this depth was 81 ± 10 V/m.

Electrical nerve stimulation

Conditioning 1-ms electrical rectangular pulses at an intensity of three times the subject's sensory perception threshold were delivered to the right D2 (Fig. 1b) with the Digitimer Constant Current Stimulator (DS7A, Digitimer Ltd, UK). The bipolar electrodes were placed on the palmar side of the distal and middle phalanges; the cathode was proximal to the anode. The sensory threshold was defined as the pulse intensity that was detected by the subject in two out of four consecutive pulses. The threshold was determined with both ascending and descending changes in the stimulus strength.

Electroencephalography

The EEG was recorded continuously with a 60-channel Ag/AgCl electrode system (eXimia EEG, Nexstim Ltd, Finland). The EEG signals were band-pass filtered from 0.1 to 500 Hz and sampled at 1.450 Hz. The reference electrode was placed on the right mastoid and the ground electrode was located at a distance of approximately 10 cm from the reference electrode, on the right zygomatic bone. Importantly, for the EEG analysis we used a common average reference. The following critical factors were taken into consideration in order to obtain EEG recordings without excessive contamination from TMS (e.g. due to artefacts caused by the currents induced in electrodes or due to possible slight mechanical movements of the electrodes). The skin under the electrode was carefully prepared to keep the impedances of the electrodes below 5 kΩ. A relatively small amount of electrode gel (Grass-Telefactor, EC2 Electrode Cream) was applied to avoid gel bridging between the electrodes. We took also special care to obtain similar imped-

ances across the recording electrodes. A tight and stable contact (between the head/cap and TMS coil) was obtained in order to avoid movements of the electrodes with respect to the coil. To efficiently attenuate the sound originating from the coil click we used a combination of the earplugs and dumping earmuffs. The earplugs had a noise reduction rate of 29 dB (E.A.R. Classic Earplug, UK). Additionally, the dumping earmuffs (E.A.R. Model 4000, UK) attenuated the sound by 30 dB in the broad frequency range.

Experimental procedures

The subjects were seated comfortably in an armchair during the experiments. They were instructed to relax and keep their eyes open with a fixed gaze. In total, there were two recording sessions for each subject. In each session, there were three types of stimulation categories with 40 stimuli in each: (1) non-conditioned trials in which TMS was delivered alone, (2) non-conditioned trials in which the electrical stimuli to D2 were delivered alone, and (3) conditioned trials in which an electrical stimulation of D2 was followed by a TMS pulse after 25 ms (Fig. 1b). The stimuli of each stimulation category were presented in pseudo-random order. A delay between the D2 electrical stimulation and TMS was set at 25 ms because previous studies have shown that this interstimulus interval leads to the most pronounced SAI (Tokimura et al. 2000). The interval between the consecutive TMS pulses varied between 3 and 3.5 s.

Data analysis

We focused on the analysis of the N100 component of the TMS-evoked EEG response, which was previously shown to be highly susceptible to changes in cortical excitability (Nikulin et al. 2003; Kičić et al. 2008; Paus et al. 2001; Bender et al. 2005; Kähkönen and Wilenius 2007). Late EEG responses are less likely to be affected by the TMS artefacts produced by the magnetic pulse. Visual inspection of the EEG responses showed that the N100 was the most stable component, having a similar polarity and latency range across the subjects and conditions. If the N100 component contained a ripple at the peak (two deflections around the peak latency), the one with the highest amplitude was chosen. TMS to the left motor cortex evoked a series of EEG deflections (Fig. 2a, b), which were similar to the responses described previously by other authors (Ilmoniemi et al. 1997; Komssi et al. 2002; Komssi et al. 2004; Paus et al. 2001; Kähkönen et al. 2005). Single EEG epochs contained a 100 ms pre-TMS interval as a baseline period and a 300 ms post-TMS interval. Each set of EEG data was visually inspected epoch-by-epoch and the trials contaminated with eye movements, muscle activity or sharp spikes caused by TMS were removed. The overall rejection level was low with a maximum of 10 epochs being rejected in any one subject. Therefore, the lowest number of epochs included in the averaging was 70. It is important to note here that if an EEG epoch was rejected, the corresponding EMG epoch was also rejected from further analysis.

To eliminate sensory-evoked potentials produced by the D2 stimulation in trials with combined TMS (D2 stimulation + TMS trials), the following procedure was applied (Seyal et al. 1993; Tiitinen et al. 1999; Schürmann et al. 2001; Nikulin et al. 2003): the evoked responses obtained from the epochs with D2 stimuli alone were subtracted from the evoked responses with the combined presentation of TMS and D2 stimuli. In the present study, the cortical regions of interest (ROI) were the sensorimotor areas of both hemispheres. With the goal to address local and selective cortical excitability changes (Tamas et al. 2003), we selected an ROI covered by four electrodes (FC3, FC1, C3, C1) in the vicinity of the point of stimulation where we also observed the strongest N100 in all subjects. A similar ROI was also selected from the homologous area of the opposite (right) hemisphere (electrodes FC2, FC4, C2, C4). A symmetric fronto-central selection of electrodes in the left and right hemispheres was used in order to detect interhemispheric differences in the amplitude of the N100 and its reactivity to the peripheral afferent stimulation. If present, such interhemispheric asymmetry can indicate local changes in the sensorimotor processing and it can also be used for ruling out the effects of the auditory stimulation (see "Discussion"). In addition, we also analysed the amplitude of the N15 component (Komssi et al. 2002, 2004; Bonato et al. 2006) in the stimulated hemisphere in order to study the earliest changes in the cortical excitability after TMS.

Repeated measures two-way ANOVA (2×2 design) was performed for the EEG data (amplitude and latency) with factors CONDITIONING (two levels: presence or absence of D2 stimulation prior to TMS) and HEMISPHERE (two levels: N100 responses in the left or right hemispheres). The EMG data were analysed with *t* test comparing MEPs with and without preceding D2 stimulation. Tukey HSD test was used for the post hoc comparisons. A Pearson correlation analysis was performed for both EEG and EMG data. Offline data processing was conducted with Matlab 6.5 software (The Mathworks, Natick, MA).

Results

Short-latency afferent inhibition

The MEP amplitudes to the TMS alone were 1.02 ± 0.03 mV. MEPs to TMS, which was applied 25 ms after D2, were significantly attenuated to 0.69 ± 0.04 mV (*t* test: $P = 0.008$).

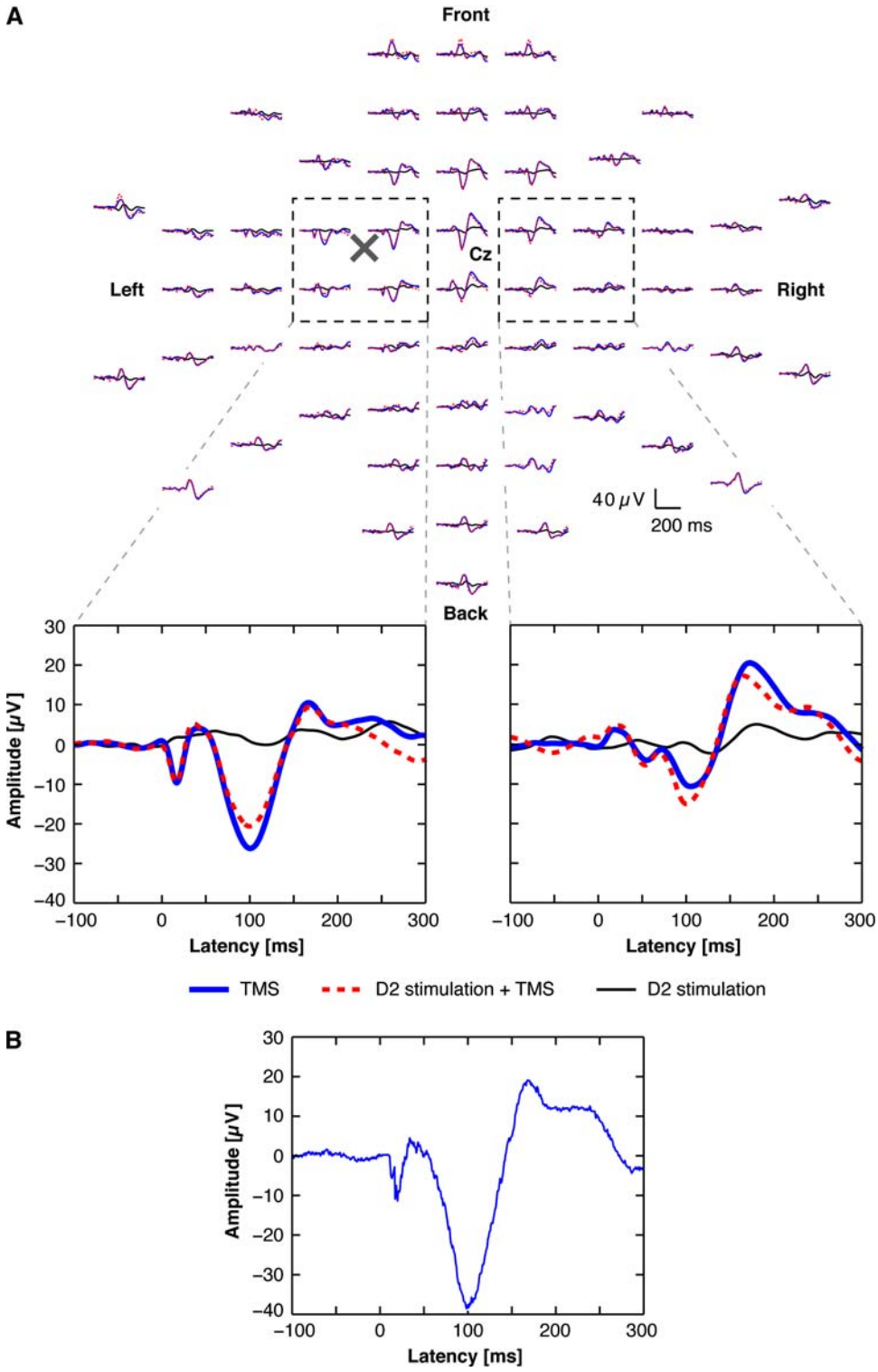

Fig. 2 EEG responses to magnetic stimulation of the motor cortex with and without preceding D2 stimulation (data from a single representative subject). **a** *Top*: EEG responses in topographically arranged channels (Cz electrode position is marked; *X* indicates the centre of the TMS coil). Averaged responses from the four left and four right electrodes (enclosed in the *dashed rectangles*) were used for the analysis. *Bottom*: averaged responses from the highlighted electrodes in the left and right hemispheres. *Black thin lines* EEG response to D2 alone, *blue thick lines* response to TMS alone, *red dashed lines* response to a combined application of D2 and TMS (after the subtraction of the D2 alone EEG response). **b** TMS-evoked response in a channel under the centre of the TMS coil. The response was obtained by averaging 80 EEG epochs without the application of low-pass filters

N100 response to TMS of the motor cortex

There was a significant main effect of the factor HEMISPHERE ($F_{1,9} = 11.01$, $P = 0.009$) showing that the amplitude of the N100 response was stronger in the left (stimulated) hemisphere (Fig. 3a) than in the right (contralateral to TMS) hemisphere (Fig. 3a). In addition, a significance was observed for the factor CONDITIONING ($F_{1,9} = 11.01$, $P = 0.008$) and for the interaction between the factors HEMISPHERE and CONDITIONING ($F_{1,9} = 9.93$, $P = 0.012$). The N100 amplitude was significantly attenuated in the left hemisphere when TMS was

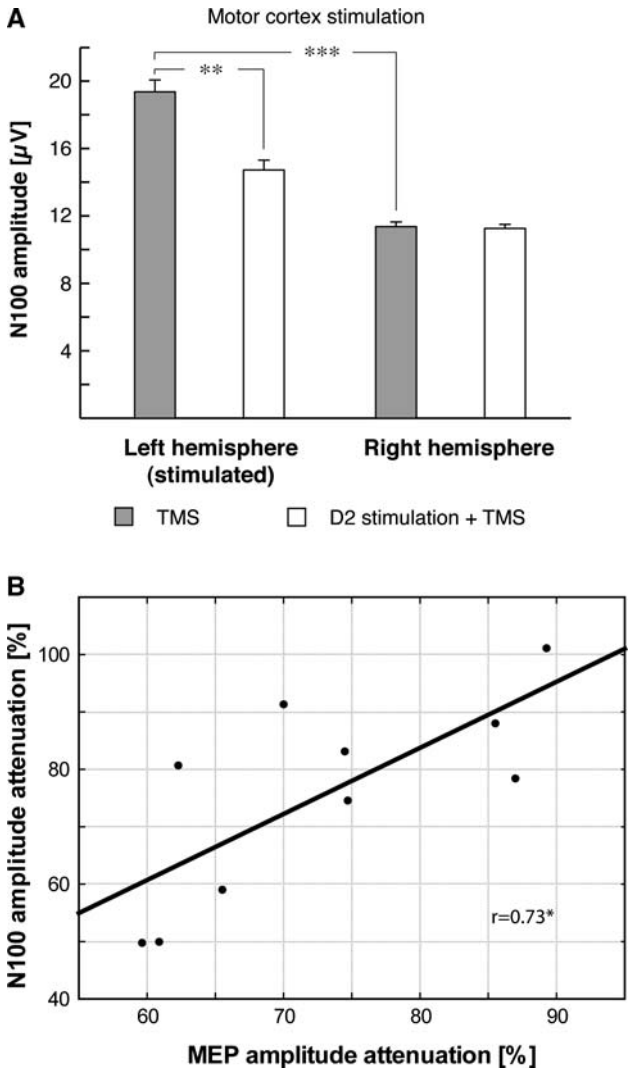

Fig. 3 **a** Amplitudes (mean ± standard error of mean) of the N100 response to TMS of the left motor cortex (average across 10 subjects). *Grey bars* N100 amplitudes to TMS alone, *white bars* N100 amplitudes to TMS with preceding D2 stimulation. $^{**}P < 0.01$ and $^{***}P < 0.001$. **b** Relationship between the attenuation of N100 amplitude and the attenuation of MEP amplitude due to D2 stimulation. The data represent a relative decrease of N100 and MEP due to SAI with respect to the amplitude of these measures to TMS alone. This normalization was needed to avoid inter-individual dispersion of absolute values. Each *point* corresponds to one subject. *Solid line* represents a least-squares fit. $^{*}P < 0.05$ and r is Pearson correlation coefficient

preceded by the conditioning afferent stimulation ($P = 0.006$, Figs. 2, 3a). No significant changes of the N100 amplitude were observed in the right hemisphere (Fig. 3a). Thus the changes in the N100 amplitude appeared to be local and restricted to the same hemisphere where afferents from D2 project.

To quantify the relationship between the peripheral and central correlates of SAI we calculated the correlation between the attenuation of MEPs and that of N100. This correlation was positive and significant ($r = 0.73$, $P = 0.016$, Fig. 3b), indicating that a stronger attenuation of the N100 corresponded to a stronger attenuation of MEPs.

There was no significant effect of D2 stimulation on the latency of the N100. In the left hemisphere, the latencies were 102 ± 2 ms to TMS alone and 98 ± 2 ms to TMS with preceding D2 stimulation. In the right hemisphere, the respective latencies were 101 ± 3 and 103 ± 2 ms.

N15 response to TMS of motor cortex

To monitor early changes in the cortical excitability we compared the amplitudes of the N15 component of TMS-evoked EEG response (latency ~ 15 ms) in the left (stimulated) hemisphere with and without preceding D2 stimulation. The N15 amplitude to the TMS alone was 10.63 ± 1.57 μV. The N15 to TMS applied 25 ms after D2 remained unchanged: 10.40 ± 1.52 μV (*t* test: $P = 0.972$).

Discussion

We explored the electrophysiological cortical correlates of SAI by combining EEG/EMG with TMS of the motor cortex. The attenuation of MEP amplitudes due to SAI had its counterpart in the attenuation of the N100 amplitude. We also discuss the neurophysiological aspects of the TMS-induced EEG responses and their relationship to SAI.

Neurophysiological origin of N100

Several previous studies (Nikulin et al. 2003; Bender et al. 2005; Kičić et al. 2008; Kimiskidis et al. 2008) argued that the N100 component of the TMS-evoked EEG response most probably reflects a progressive inhibitory process induced by the TMS. Various experimental approaches support the hypothesis of the inhibitory origin of the N100 component. Stimulation of the cortical surface produces long-lasting (>100 ms) inhibitory postsynaptic potentials (Krnjević et al. 1966; Rosenthal et al. 1967). Intracortical recordings and detailed current source-density analysis by Barth and Sutherling (1988) showed a sequence of excitatory and inhibitory events after electrical stimulation of the cortical surface in rats. The authors concluded that the earliest (5 ms after the stimulation) responses were excitatory and reflected soma depolarization of the cells in the upper layers. These responses were followed (20 ms after the stimulation) by the inhibitory responses. The latter were characterized by a prolonged hyperpolarization of the soma of deep pyramidal cells and lasted more than 200 ms. The time course of these responses is strikingly similar to the development of the TMS-evoked N100. Other supporting evidence for the inhibitory nature of N100 originates from the experiments applying a paired-pulse paradigm with a

supra-threshold conditioning TMS stimulus. These studies demonstrated that the amplitudes of the MEPs to the test stimulus were mostly suppressed at about 100–150 ms after the conditioning TMS (Valls-Solé et al. 1992; Roick et al. 1993; Matsunaga et al. 2002). Importantly, the temporal characteristics of the N100 to supra-threshold TMS used in our study are remarkably similar to the time-course of the inhibitory effects in the studies mentioned above. In a recent study, Kimiskidis et al. (2008) showed a significant positive correlation between the length of the EMG silent period and the amplitude of N100, thus providing more evidence for the inhibitory nature of the N100.

It was shown previously that the long-lasting inhibition up to a few hundred milliseconds after the activation of inhibitory interneurons reflects activation of GABA-B receptors (Connors et al. 1988; Werhahn et al. 1999; Tamas et al. 2003; Markram et al. 2004). GABA-B receptors can be activated by repetitive firing of interneurons or cooperative coactivation of several of them (Tamas et al. 2003). Simultaneous activation of many neurons can be easily achieved with TMS. The net effect of such activation can indeed be a long-lasting inhibition, such as has been observed in the experiments with strong electrical extracellular stimulation (Krnjević et al. 1966; Rosenthal et al. 1967; Barth and Sutherling 1988).

The neurophysiological considerations presented above seem to indicate that the time course and latency of the N100 component reflect the presence of inhibitory processes, which are most likely mediated through GABA-B receptors.

N100 and TMS-produced acoustic click

TMS is accompanied by an acoustic click that induces auditory activation (Nikouline et al. 1999; Tiitinen et al. 1999; Bender et al. 2005). However, we believe that this activation cannot explain our results for the following reasons: (1) The TMS coil was located over the left hemisphere and due to the lateralization of auditory responses (Näätänen and Picton 1987; Ponton et al. 2001), the strongest activation should have been observed over the right hemisphere. Yet our results consistently demonstrated that the strongest activation was over the left hemisphere, indicating that it was not auditory in nature. (2) Using a similar experimental setup and sound dampening methods, we demonstrated in our previous study (Nikulin et al. 2003) that the amplitude of the auditory response due to the click is negligible in comparison to that of the N100 evoked by the magnetic stimulation. (3) In a recent study, Lioumis et al. (2008) showed that the N100 component induced by TMS of the motor cortex was up to five times larger in amplitude than the N100 component induced by the stimulation of the prefrontal cortex. Such modulation of the amplitude of N100 cannot be explained by a change in the acoustic properties of the signal, which were practically identical at both stimulation locations (the TMS intensity was the same, only the coil was moved by approximately 7 cm). Instead, the changes in the amplitude of N100 with different locations of TMS coil most likely reflect the local cyto-architectonic and excitatory configuration of the stimulated cortical areas. (4) In earlier studies, a clear demonstration of a TMS-induced N100 was obtained when the coil click was not heard by the subjects either due to the use of a masking white noise (Paus et al. 2001) or when TMS-evoked EEG responses were recorded in deaf subjects (Kimiskidis et al. 2008).

Attenuation of N100 due to SAI

The present study shows that the attenuation of MEPs due to SAI is associated with the attenuation of the TMS-induced N100 component. Moreover, we showed that the attenuation of MEPs was positively correlated with the amplitude attenuation of the N100 response. These findings are in line with the results of an earlier study (Tokimura et al. 2000) showing that preceding median nerve stimulation attenuated TMS-evoked I-waves, thus supporting the hypothesis that the SAI has a cortical origin. Importantly, while the time courses of epidurally recorded I-waves and EMG responses were similar in a study by Tokimura et al. (2000), there was no correlation between the individual amplitude of I-waves and MEPs. Our study is in fact the first demonstration of a correlation between the EEG and MEP manifestations of SAI, demonstrating that even small individual changes in the amplitude of MEPs parallel changes in cortical excitability. This is an important finding for TMS research in general, providing further grounds for inferring amplitude changes in MEPs from the central neuronal processing.

The synaptic mechanisms responsible for the SAI are complex and are not yet fully understood. Cholinergic neurons are known to be involved in the initiation of SAI (Di Lazzaro et al. 2000). Administration of GABA-A receptor modulators affect SAI (Di Lazzaro et al. 2007), suggesting that these receptors may contribute to its further development. A relevant issue for the present study is that the inhibition that is directed at pyramidal cells should produce hyperpolarization of the neuronal membrane, thus leading to a decrease of the MEP amplitude. The same mechanism can also lead to a decrease of I-waves recorded epidurally (Tokimura et al. 2000). Let us consider the consequences of such hyperpolarization for the TMS-induced N100 response. TMS was applied in the present study 25 ms after the stimulation of the D2, at a time point when SAI was most pronounced. As mentioned earlier, the effect of TMS on a relatively long time scale is likely to be an inhibition

mediated by GABA-B receptors (McDonnell et al. 2006). The activation of these receptors leads to hyperpolarization of the cell through the opening of K^+ channels. However, when TMS-induced GABA-B inhibition starts, the neurons are already hyperpolarized due to SAI. As a consequence, the membrane potential is shifted towards the equilibrium potential for K^+, thus effectively decreasing K^+ outward currents, which in turn results in a smaller amplitude of N100. Naturally, this scenario is only hypothetical, but it is based on known neurophysiological data and provides a sound basis for its testing with pharmacological agents modulating GABA-A and GABA-B receptors.

An alternative scenario could be that the excitatory volley evoked by TMS thereafter generates an inhibitory volley represented by N100, and the latter is reduced when the initial TMS-induced excitation is reduced due to the D2 stimulation. However, the following considerations make this alternative less probable. TMS activates output pyramidal cells indirectly. This is evidenced by the longer latencies of MEP compared to the muscle responses produced by electrical brain stimulation (Hess et al. 1987). Also, the latencies of TMS-evoked responses recorded from axons of corticospinal neurons are longer than the responses evoked by the electrical brain stimulation (Edgley et al. 1997). Moreover, it is reasonable to assume that the initial TMS-EEG response most likely reflects the activity of the cortical cells activated directly by the TMS: these cells are also presumed to be located superficially where the induced electric field is strongest. The fact that the N15 was not affected by the D2 stimulation indicates that the initial recruitment of neurons, directly activated by TMS, was not substantially changed by the preceding peripheral stimulation. Therefore, deeply located pyramidal cells are the most likely candidates to manifest the latest stages of the inhibitory influences of SAI (Tokimura et al. 2000), as demonstrated by TMS and I-waves recordings. Due to the developing inhibition from SAI, the initial excitatory volley from TMS (which is unchanged as evidenced by the stability of N15) reaches pre-inhibited pyramidal cells leading to smaller MEPs. In parallel, pyramidal cells start receiving input from the TMS-activated inhibitory neurons, which in turn would lead to smaller hyperpolarizing currents, as described above.

To conclude, in our study we showed electroencephalographic correlates of SAI thus further proving its cortical origin. Moreover, we found a linear correlation between the attenuation of the cortical responses and MEPs due to SAI, thus directly showing how changes in cortical neuronal activity are related to changes in MEPs. On a more general level, our study provides further grounds for the usefulness of the TMS-EEG approach as a probe of cortical excitability and establishes an additional empirical proof for the possibility to infer changes in cortical activity on the basis of MEPs.

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IV

Publication IV

Lioumis P., Kičić D., Savolainen P., Mäkelä J.P., and Kähkönen S. (2008). Reproducibility of TMS-Evoked EEG responses. Hum Brain Mapp 30(4), 1387-96.

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Reproducibility of TMS—Evoked EEG Responses

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Abstract: Navigated transcranial magnetic stimulation combined with electroencephalography (nTMS-EEG), allows noninvasive studies of cortical excitability and connectivity in humans. We investigated the reproducibility of nTMS-EEG in seven healthy subjects by repeating left motor and prefrontal cortical stimulation with a 1-week interval. TMS was applied at three intensities: 90, 100, and 110% of subjects' motor threshold (MT). The TMS-compatible neuronavigation system guaranteed precise repositioning of the stimulation coil. The responses were recorded by a 60-channel whole head TMS-compatible EEG amplifier. A high overall reproducibility ($r > 0.80$) was evident in nTMS-EEG responses over both hemispheres for both motor and prefrontal cortical stimulation. The results suggest that nTMS-EEG is a reliable tool for studies investigating cortical excitability changes in the test-retest designs. *Hum Brain Mapp 30:1387–1396, 2009.* © 2008 Wiley-Liss, Inc.

Key words: electroencephalography; motor cortex; prefrontal cortex; reproducibility; transcranial magnetic stimulation

INTRODUCTION

Noninvasive transcranial magnetic stimulation (TMS) [Barker et al., 1985] has been used to investigate cortical functions in humans and has become an important tool in the evaluation of central motor pathways [Meyer, 2002; Ziemann, 2002]. Cortical-spinal excitability can be evaluated by recording electromyographic (EMG) responses elicited by TMS pulses from different muscles, and by estimating the pulse strength needed to elicit responses (motor threshold; MT). Both repetitive (rTMS) and single pulse TMS applied to association cortices may disturb [Amassian et al., 1989; Beckers and Homberg, 1991; Grafman et al., 1994; Pascual-Leone et al., 1991, 2000] or enhance [Evers et al., 2001; Klimesch et al., 2003; Kohler et al., 2004; Luber et al., 2007; Topper et al., 1998] performance during cognitive tasks. In addition, rTMS over the dorsolateral prefrontal cortex may ameliorate depression [Fitzgerald et al., 2006; Gross et al., 2007; Klein et al., 1999; Pascual-Leone et al., 1996]. The high reproducibility of TMS motor evoked potentials (MEPs) in single and paired-pulse paradigms is well established [Carroll et al., 2001; Conforto et al., 2004; Corneal et al., 2005; de Carvalho

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et al., 1999; Humm et al., 2004; Kimiskidis et al., 2004; Maeda et al., 2002; Mills and Nithi, 1997; Wolf et al., 2004].

TMS combined with electroencephalography (EEG) enables the noninvasive evaluation of functional connections between brain areas [Ilmoniemi et al., 1997; Massimini et al., 2005; Paus, 1999; Paus et al., 1997, 1998] and provides a tool in investigating cortical excitability [Bailey et al., 2001; Bender et al., 2005; Bonato et al., 2006; Ilmoniemi et al., 1999; Kähkönen et al., 2001, 2003, 2004, 2005; Komssi et al., 2002, 2004; Nikouline et al., 1999; Nikulin et al., 2003; Paus et al., 2001; Schürmann et al., 2001; Tiitinen et al., 1999; Virtanen et al., 1997, 1999].

In navigated TMS (nTMS), the location of the TMS coil is shown over the individual MRI reconstruction of the subject's brain in real time. The locations of the stimulation sites can be saved for repeated measurements. Consequently, nTMS has been suggested as a precise tool for brain mapping studies, particularly for repeated measurements, as it allows reliable coil re-placement [Neggers et al., 2004; Schonfeldt-Lecuona et al., 2005]. nTMS combined with EEG (nTMS-EEG) allows recordings of neuronal responses elicited by stimulation of cortical sites outside the primary motor cortex, e.g., in the prefrontal cortices [Kähkönen et al., 2001, 2003, 2005]. Applying nTMS in prefrontal cortex minimizes the variations in cortical target selection between the subjects.

Reproducibility of the TMS-evoked EEG responses is an essential prerequisite for studies with test-retest design, and it has not yet been reported. We investigated the reproducibility of nTMS-EEG responses elicited by primary motor and dorsolateral prefrontal cortical (M1 and DLPFC respectively) stimulation in healthy subjects. Reproducibility of MT measurements has been investigated both with the "hot spot method" searching the maximum MEP strength [Conforto et al., 2004; Rossini et al., 1994], and the "fixed point" technique using fixed skull landmarks [Kimiskidis et al., 2004]. We have inspected the MT reproducibility by employing the "hot spot" method in nTMS.

METHODS

Seven healthy subjects (age 23–34 years, 4 men and 3 women, all right handed) participated in the study. The ethical committee of the Helsinki University Central Hospital approved the experimental procedures of the study. A written informed consent was obtained from all subjects.

A Magstim-200 stimulator, connected to a co-planar figure-of-eight Magstim-P/N9925 induction coil of 70-mm wing radius (The Magstim Company Ltd., Whitland, UK) was used to produce the TMS pulses. An eXimia NBS navigation system (Nexstim Ltd., Helsinki, Finland) was used for MRI-guided neuronavigation. Our target was to minimize the stimulated cortical area and to ensure that the same cortical location was stimulated in repeated experiments (see Fig. 1).

The individual MRIs required for the 3D reconstruction and navigation were scanned with 1.5 T or 3 T devices (T1 weighted; 0 mm slice gap; 1 mm thickness; sagittal orientation; acquisition matrix 256 × 256; GE Healthcare, UK; Philips Medical Systems, Eindhoven, The Netherlands; Siemens Medical Solutions, Erlangen, Germany).

Two measurements with a 1-week interval were conducted for each subject. The subjects were comfortably sitting in a chair with their eyes open and fixated on a point in the experiment room. The left primary motor cortex stimulation targeted the representation of the right abductor pollicis brevis (APB) muscle in the left hemisphere. The starting target point was located by the Ω-or the reversed ε-shaped structure in precentral gyrus ["motor knob," Yousry et al., 1997] and then optimized by maximizing the MEPs recorded from the APB with a Keypoint electromyograph (Medtronic, Inc., MN). The MT was determined as a TMS intensity evoking contralateral MEPs of minimum 50 μV in resting APB, in 5 out of 10 given stimuli [Rossini et al., 1999, 1994]. The left middle frontal gyrus was located from a 3D MRI reconstruction, based on anatomical sketches [Yousry et al., 1997]. The average Talairach coordinates for DLPFC stimulation sites were $-25 \pm 4, 36 \pm 8, 42 \pm 7$ (x, y, z; mean ± SD). Each site was stimulated at intensities of 90, 100, and 110% of the MT. MT is an appropriate measure for determining the stimulus intensity for targets in the prefrontal cortex [Kahkönen et al., 2004, 2005].

A hundred pulses were applied with each intensity. The interstimulus interval was 3.3 s and the inter-session interval varied between 2 and 5 min. The order of the stimulation sites and stimulus intensities was kept the same for both measurements for each subject, but varied randomly between the subjects. The MR image-guided navigation guaranteed that the coil orientation eliciting maximal MEPs was defined on the first measurement and kept the same in the M1 stimulation. This allowed the measurement of MT from exactly the same point in the second measurement for all subjects. In addition, in the beginning of the second measurement, a possible shift of the site eliciting maximal MTs was inspected before using the stimulation parameters from the first measurement; no such shifts were observed. The navigation tool also allowed the accurate placement of the coil over the selected stimulation site in the DLPFC; the coil was directed to be perpendicular to the middle frontal gyrus with the handle pointing laterally (see Fig. 1). During all sessions, the coil was mounted on a tripod stand with a flexible extension arm (Manfrotto Ltd., Bassano del Grappa, Italy).

The EEG responses to nTMS were recorded with sixty Ag/AgCl sintered electrodes specially designed for TMS-EEG measurements to avoid overheating by eddy currents induced by TMS (Nexstim Ltd., Helsinki, Finland). The multichannel EEG array was connected to a TMS-compatible EEG amplifier (eXimia, Nexstim Ltd., Helsinki, Finland). EEG sampling rate was 1450 Hz, bandwidth was 0.1–350 Hz, and 16-bit AD conversion resolution was applied. During the magnetic pulse delivery, the EEG

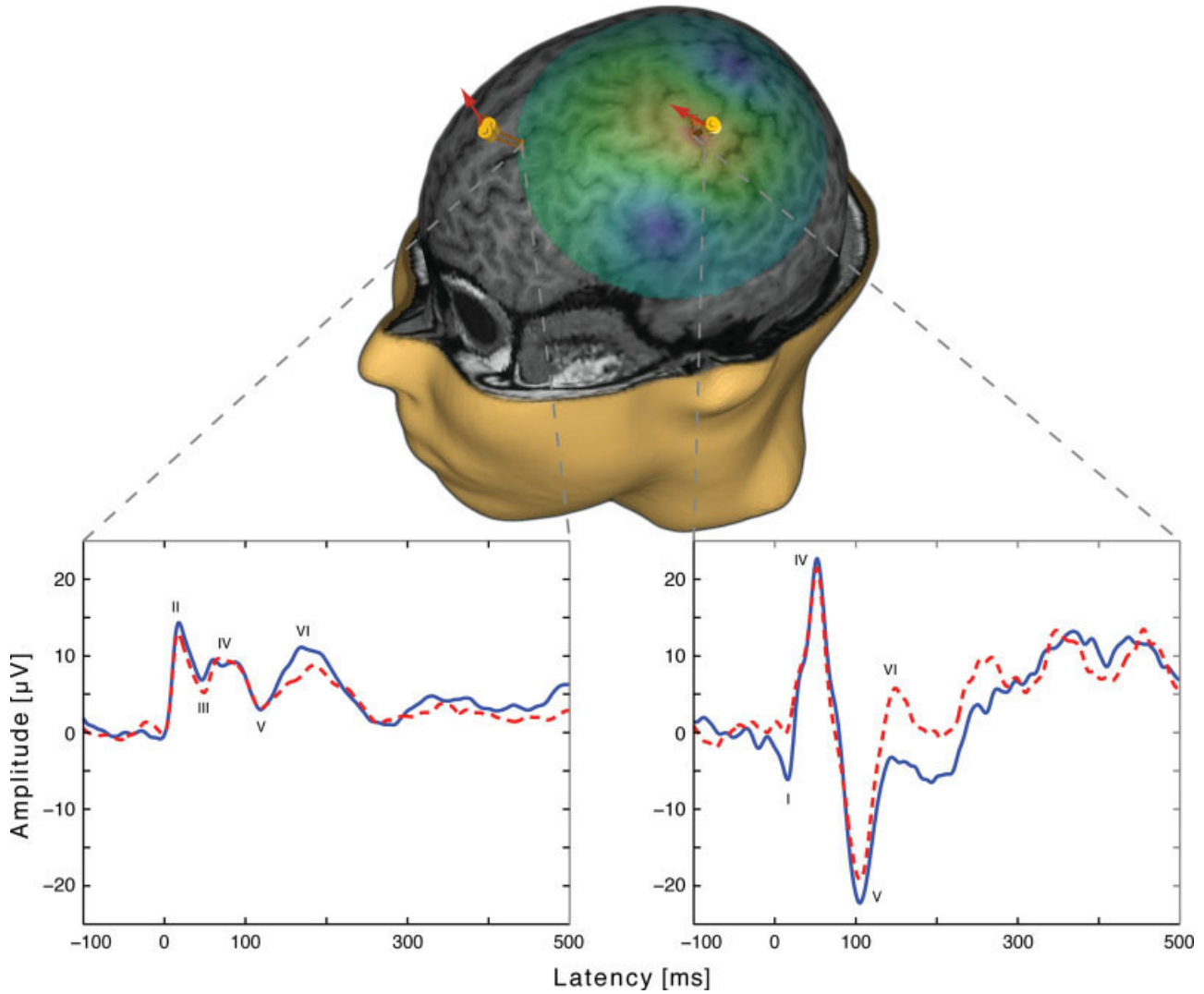

Figure 1.

3D reconstruction of the MRIs from one subject. The two yellow markers over primary motor cortex (M1) and dorsolateral prefrontal cortex (DLPFC) represent the stimulation targets. Each marker shows the site of the delivery of the TMS pulse in two measurements with a 1-week interval. The arrows indicate the coil orientation and induced current direction. The green highlighted area illustrates the estimated induced electric field during M1 stimulation. The two panels display the averaged EEG signals of each measurement from ROI electrodes after M1 and DLPFC nTMS on one subject. The signals were low-pass filtered with a cut-off frequency of 45 Hz. Navigation allows the exact re-positioning of the coil resulting in reproducible TMS-EEG responses.

amplifier was blocked by a sample-and-hold circuitry for 2 ms to remove most of the TMS-induced artefacts. After this "gating period," the EEG signals contained mainly the physiological TMS-evoked responses [Virtanen et al., 1999].

Analysis

Before averaging, the raw EEG was inspected for artifacts caused by eye movements, muscle activity or mechanical disturbances. Epochs with signals exceeding 50 μV were excluded from further analysis. At least 80 epochs per session were eligible for averaging for each subject after removing eye-blinks and residual electrical contamination by TMS. Signals were averaged and low-pass filtered with 45 Hz cut-off frequency. The 600-ms analysis period included a 95-ms prestimulus baseline. Offline data processing was performed with Matlab 6.0 software (The Mathworks, Natick, MA).

To analyze general reproducibility of nTMS-evoked potentials, we selected regions of interest (ROIs) in each hemisphere. Ten electrodes over and around the stimu-

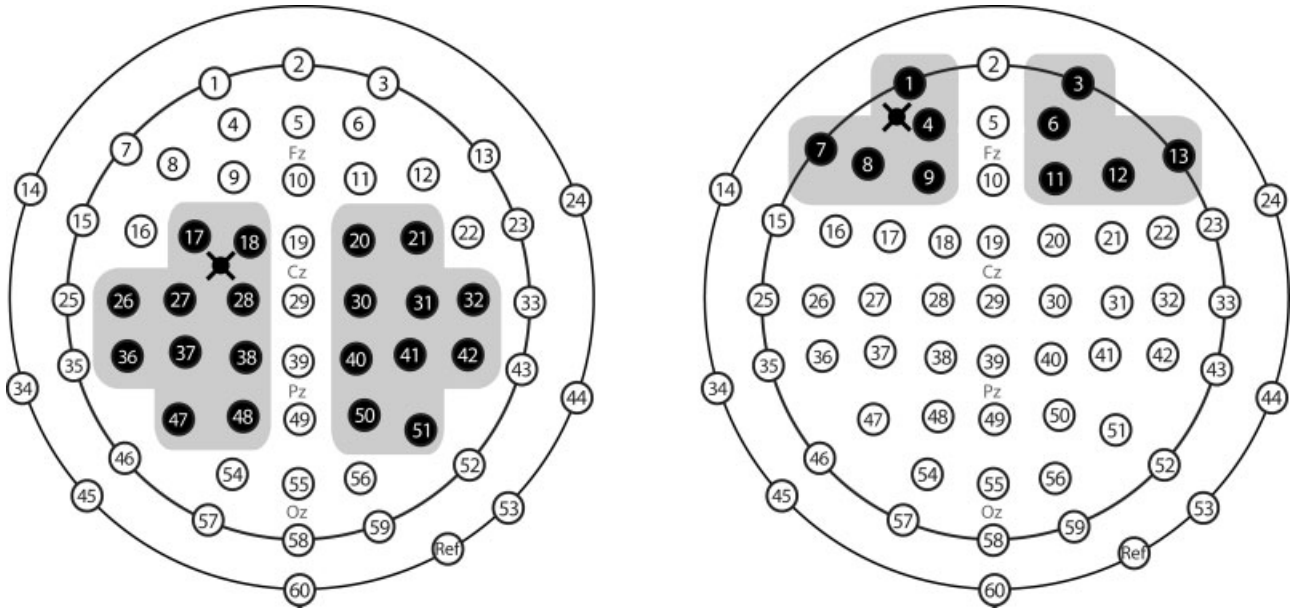

Figure 2.
Schematic illustration of electrode positions in EEG cap. Shaded electrodes represent in the two encircled regions the two ROIs selected for primary motor (left panel) and dorsolateral prefrontal cortex (right panel) stimulation. The cross represents the stimulation site.

lated left M1 and the corresponding electrodes from contralateral hemisphere were selected for comparison of evoked potentials elicited by nTMS to M1. Similarly, bilateral ROIs of five electrodes were used for evaluating responses to prefrontal cortex nTMS (see Fig. 2). The signals from the selected electrodes were averaged for each hemisphere and each stimulation site.

The area bound by the half-maximum of the cortically induced electric field produced by figure-of-eight coil is usually larger than 5 cm^2 [Komssi and Kähkönen, 2006]. Furthermore, in our study stimulation ranged from subthreshold (90% of MT) to suprathreshold (110% of MT) intensities; thus, both depth and size of the stimulated area were different [Heller and van Hulsteyn, 1992; Roth et al., 2002; Ruohonen and Ilmoniemi, 1998; Terao et al., 2000; Zangen et al., 2005]. Consequently, most studies with TMS-evoked EEG responses report average responses from several electrodes in selected ROIs [Kähkönen and Wilenius, 2007; Kičić et al., 2008; Nikulin et al., 2003]. We used similar approach to add compatibility with the results of previous studies.

The peak amplitudes and latencies were calculated. Comparison of the first and second measurement of each session was done by paired two-tailed *t* tests with Bonferroni correction (Table I). In addition, reproducibility was tested by two-tailed Pearson's correlation coefficients with 0.05 level of significance. To increase power of correlation coefficient calculations, data of all intensities were collapsed (Table II). SPSS 14.0 software was employed for statistical analysis (SPSS, Chicago, IL).

RESULTS

Motor Threshold

MT in first and second experiments were highly correlated (43.3% ± 2.5% and 43.1% ± 2.3% of the stimulator's output; $r = 0.99$; $P < 0.001$).

Primary Motor Cortex nTMS

Six peaks from averaged responses were identified after left M1 nTMS at all intensities in electrodes over both hemispheres in each subject. The grand averaged EEG response consisted of six deflections as well (see Fig. 3). The response peak latencies were 13 ± 6 ms, 32 ± 6 ms, 54 ± 11 ms, 66 ± 14 ms, 111 ± 11 ms, and 172 ± 39 ms for the left ROI ipsilateral to the stimulation, and 12 ± 5 ms, 31 ± 7 ms, 50 ± 9 ms, 73 ± 12 ms, 111 ± 11, ms and 176 ± 19 ms for the right ROI. The response peak amplitudes increased with increased TMS intensity (Table I).

The Pearson's correlation coefficients for amplitudes ranged from 0.68 to 0.92 for the left ROI, and from 0.35 to 0.92 for the right ROI. The correlation coefficients for latencies ranged from 0.59 to 0.98 for the left ROI and from 0.75 to 0.95 for the right ROIs (Table II).

TABLE I. Mean Amplitudes (A; μV±SD) and latencies (B; ms±SD) of TMS-evoked EEG deflections in two repeated measurements

	Ipsilateral M1-amplitudes						Contralateral M1-amplitudes					
A	90%MT-1	90%MT-2	100%MT-1	100%MT-2	110%MT-1	110%MT-2	90%MT-1	90%MT-2	100%MT-1	100%MT-2	110%MT-1	110%MT-2
peak I	−6.4 ± 5.1 (5)	−6.8 ± 14.1	−14.9 ± 21.7 (5)	−16.7 ± 27.8	−19.6 ± 26.7 (4)	−32.7 ± 40.8	−3.6 ± 2.9 (3)	−3.6 ± 3.8	−5.0 ± 6.3 (4)	−4.9 ± 6.9	−9.8 ± 3.9 (2)	−12.6 ± 7.7
peak II	2.0 ± 3.3 (4)	3.9 ± 4.0	4.2 ± 4.6 (5)	5.6 ± 4.2	6.1 ± 4.8 (4)	6.6 ± 4.5	0.2 ± 3.1 (4)	1.3±1.1	2.0 ± 2.5 (5)	3.3 ± 2.6	4.4 ± 3.3 (5)	7.0 ± 7.6
peak III	−2.3 ± 6.3 (6)	−1.4 ± 8.2	−3.4 ± 7.5 (5)	−2.3 ± 9.1	−0.7 ± 9.0 (4)	−0.8 ± 8.0	−3.5 ± 2.5 (7)	−1.6±2.3	−2.8 ± 2.3 (7)	−2.5 ± 2.9	−1.2 ± 1.7 (7)	−0.2 ± 4.7
peak IV	4.7 ± 5.6 (7)	6.9 ± 3.0	5.4 ± 8.7 (7)	6.9 ± 8.5	13.7 ± 9.3 (6)	14.2 ± 12.5	1.7 ± 1.7 (7)	3.9±2.7	1.9 ± 2.3 (7)	2.1 ± 1.9	4.2 ± 3.5 (7)	6.7 ± 5.0
peak V	−4.9 ± 4.8 (7)	−2.6 ± 4.7	−7.8 ± 8.0 (7)	−8.1 ± 7.2	−7.4 ± 8.7 (7)	−9.8 ± 11.4	−5.7 ± 3.2 (7)	−3.5±3.7	−5.6 ± 3.9 (7)	−4.9 ± 3.3	−5.6 ± 5.6 (7)	−5.9 ± 5.6
peak VI	8.9 ± 3.5 (7)	8.4 ± 5.3	8.5 ± 5.0 (7)	6.7 ± 6.9	6.0 ± 9.0 (6)	4.4 ± 15.6	8.2 ± 3.0 (7)	8.8±4.1	9.2 ± 3.6 (7)	8.4 ± 4.5	9.5 ± 5.8 (6)	10.6 ± 5.9
	Ipsilateral DLPFC-amplitudes						Contralateral DLPFC-amplitudes					
	90%MT-1	90%MT-2	100%MT-1	100%MT-2	110%MT-1	110%MT-2	90%MT-1	90%MT-2	100%MT-1	100%MT-2	110%MT-1	110%MT-2
peak I	— (—)	—	— (—)	—	— (—)	—	0.1 ± 1.2 (4)	−0.1 ± 1.3	−1.5 ± 1.9 (3)	−2.1 ± 1.2	−1.2 ± 1.9 (3)	−2.3 ± 1.8
peak II	8.4 ± 7.3 (7)	7.9 ± 6.9	9.9 ± 9.4 (5)	9.5 ± 11.1	10.6 ± 13 (5)	13.2 ± 14.1	2 ± 2.1 (6)	2.6 ± 2.3	1.4 ± 1.8 (5)	3.4 ± 3.9	2.5 ± 4.5 (5)	4.1 ± 5.0
peak III	2.1 ± 3.6 (6)	3.2 ± 2.3	1.5 ± 4.1 (5)	1.7 ± 3.5	1.6 ± 4.6 (5)	3.9 ± 3	−0.9 ± 1.3 (5)	0.1 ± 1	−1.6 ± 1.8 (6)	−0.8 ± 1.9	−1.9±3.2 (6)	−0.2 ± 2.5
peak IV	4.1 ± 3.8 (7)	6.6 ± 3.2*	4.2 ± 5.1 (6)	5.8 ± 6.6	7.0 ± 5.6 (7)	9.3 ± 6.4	1.8 ± 2.4 (6)	3.2±3.0*	1.6 ± 4.4 (6)	4.3 ± 5.4	4.5 ± 6.0 (7)	5.5 ± 5.0
peak V	−0.5 ± 4.4 (7)	0.2 ± 4.6	−1.8 ± 3.8 (7)	0.8 ± 5.2	−0.8 ± 6.1 (7)	1.4 ± 5.9	−1.2 ± 4.5 (7)	−1.1 ± 4.6	−2.5 ± 3.8 (7)	−0.8 ± 4.8	−1.6 ± 5.9 (7)	−0.9 ± 1.8
peak VI	8.9 ± 3.0 (7)	9.6 ± 2.3	8.0 ± 2.9 (7)	10.3 ± 3.3	9.4 ± 2.9 (7)	9.7 ± 2.3	8.3 ± 3.3 (7)	9.1 ± 2.9	7.5 ± 3.2 (7)	10.3 ± 3.6	9.5 ± 3.7 (7)	8.9 ± 1.5
	Ipsilateral M1-latencies						Contralateral M1-latencies					
B	90%MT-1	90%MT-2	100%MT-1	100%MT-2	110%MT-1	110%MT-2	90%MT-1	90%MT-2	100%MT-1	100%MT-2	110%MT-1	110%MT-2
peak I	12 ± 8	9 ± 8	14 ± 6	13 ± 6	16 ± 2	15 ± 1	13 ± 5	10 ± 7	12 ± 5	12 ± 7	15 ± 3	14 ± 3
peak II	29 ± 7	33 ± 5	31 ± 5	35 ± 9	32 ± 6	34 ± 7*	33 ± 8	34 ± 15	30 ± 3	30 ± 3	31 ± 5	31 ± 7
peak III	49 ± 6	50 ± 11	60 ± 11	61 ± 10	53 ± 15	56 ± 16	47 ± 8	51 ± 9	50 ± 9	54 ± 9	48 ± 11	50 ± 10
peak IV	67 ± 15	67 ± 18	71 ± 15	71 ± 15	60 ± 11	60 ± 10	72 ± 12	75 ± 11	74 ± 13	73 ± 14	72 ± 14	73 ± 15
peak V	110 ± 18	110 ± 12	108 ± 9	109 ± 9	111 ± 8	115 ± 11	113 ± 14	113 ± 11	108 ± 12	109 ± 12	110 ± 11	113 ± 10
peak VI	170 ± 19	169 ± 24	174 ± 37	191 ± 44	172 ± 26	151 ± 69	178 ± 16	182 ± 19	176 ± 19	175 ± 25	170 ± 20	174 ± 22
	Ipsilateral DLPFC-latencies						Contralateral DLPFC-latencies					
	90%MT-1	90%MT-2	100%MT-1	100%MT-2	110%MT-1	110%MT-2	90%MT-1	90%MT-2	100%MT-1	100%MT-2	110%MT-1	110%MT-2
peak I	—	—	—	—	—	—	23 ± 6	23 ± 7	22 ± 6	20 ± 7	20 ± 8	22 ± 5
peak II	27 ± 16	26 ± 11	24 ± 16	29 ± 19	22 ± 6	22 ± 6	32 ± 7	34 ± 10	31 ± 9	31 ± 9	32 ± 11	33 ± 10
peak III	49 ± 10	48 ± 9	54 ± 15	54 ± 16	46 ± 11	47 ± 11	50 ± 9	52 ± 6	47 ± 10	46 ± 9	46 ± 11	46 ± 11
peak IV	60 ± 14	60 ± 13	71 ± 20	69 ± 19	62 ± 17	62 ± 18	63 ± 13	62 ± 12	66 ± 20	66 ± 18	63 ± 19	61 ± 20
peak V	113 ± 19	113 ± 17	111 ± 20	115 ± 16	112 ± 19	113 ± 16	112 ± 19	112 ± 18	112 ± 20	114 ± 16	113±20	116 ± 18
peak VI	174 ± 15	172 ± 18	174 ± 19	169 ± 16	171 ± 18	164 ± 13	175 ± 15	173 ± 18	180 ± 18	177 ± 19	175±16	168 ± 13

Number of subjects in which particular peak was detected is indicated in brackets.
*Significant differnces ($P < 0.0166$) for the paired t-tests between repetitions with Bonferroni correction.

TABLE II. Amplitude (A) and latency (B) peak correlations

	Amplitude peak correlation			
	M1		DLPFC	
A	IPSI	CONTRA	IPSI	CONTRA
Peak I	0.918***	0.919***	—	0.806**
Peak II	0.683**	0.703**	0.965***	0.918***
Peak III	0.916***	0.349	0.88***	0.982***
Peak IV	0.862***	0.478*	0.922***	0.883***
Peak V	0.83***	0.677***	0.867***	0.887***
Peak VI	0.85***	0.816***	0.644**	0.527*
	Latency peak correlation			
	M1		DLPFC	
B	IPSI	CONTRA	IPSI	CONTRA
Peak I	0.929***	0.754**	—	0.947***
Peak II	0.802***	0.946***	0.900***	0.939***
Peak III	0.940***	0.876***	0.967***	0.974***
Peak IV	0.975***	0.914***	0.986***	0.982***
Peak V	0.810***	0.903***	0.947***	0.924***
Peak VI	0.594**	0.851***	0.841***	0.829***

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (2-tailed).

Prefrontal Cortex nTMS

Prefrontal nTMS-evoked EEG responses consisted of six peaks. The peak latencies were 25 ± 12 ms, 49 ± 11 ms, 64 ± 16 ms, 113 ± 17 ms, and 170 ± 16 ms for responses in the left ROI (the first peak was masked by TMS artefact) and 21 ± 6 ms, 32 ± 9 ms, 48 ± 9 ms, 63 ± 16 ms, 113 ± 17 ms, and 174 ± 16 ms for the responses in the right ROI (for grand averages, see Fig. 3; for mean amplitudes and latencies see Table I). The correlation coefficients for peak amplitudes ranged from 0.64 to 0.97 for the responses in the left ROI and from 0.53 to 0.98 for the right ROI. The correlation values for peak latencies ranged from 0.84 to 0.99 for the responses in the left ROI and contralateral between from 0.83 to 0.98 for the right ROI (Table II).

DISCUSSION

Our results display a high reproducibility of nTMS-evoked EEG responses over M1 and DLPFC. This result was achieved by using exactly the same stimulation parameters for each subject in both measurements. Additionally, in line with previous studies [Conforto et al., 2004; Kimiskidis et al., 2004; Mills and Nithi, 1997], we observed no changes of MT.

Reproducibility of EEG Deflections

The test–retest correlation of all peak amplitudes ipsilateral to nTMS for both M1 and DLPFC stimulation generally exceeded 0.83, and was highly significant. The correlation for the peak amplitudes (Table II) in contralateral hemisphere was lower for the M1 stimulation. This might be caused by signal fluctuations originating from callosal transfer of the activity to the opposite hemisphere. Peak amplitude correlation for prefrontal nTMS in the ROI contralateral to the stimulation was generally stronger than for M1 (peak VI excluded); this suggests more robust interhemispheric connections in the prefrontal than primary motor regions in line with anatomic evidence [Boussaoud et al., 2005; Innocenti et al., 1995; Rouiller et al., 1994; Zarei et al., 2006]. Regardless, a smaller electrode number in ROIs of DLPFC than of M1 may decrease the variation in the average responses as well.

The amplitudes of peak II elicited by M1 nTMS and peak VI elicited by prefrontal nTMS were clearly less replicable than the other deflections. The sources of peak II (positivity at around 30 ms) are not clearly defined in dipole modelling of the TMS-evoked responses [Paus et al., 2001] indicating a complex source structure; this may render it more vulnerable to changes e.g. in alertness. The generators of prefrontal peak VI (positivity at about 170 ms) have not been studied by source modeling.

Test–retest correlations of response peak latencies were generally high and similar ($r > 0.8$) for motor and prefrontal ROIs (Table II). The only exceptions were peak III for contralateral ROI and peak VI in ipsilateral ROI to primary motor cortex nTMS. Peak I (negativity at 15 ms) is considered to reflect excitatory events [Komssi et al., 2004] because of its sharp waveform and its high dependence on nTMS intensity; however, contribution of the remaining stimulus artefact may be considerable at this early stage of responses.

Paired two-tailed t test differences for amplitude and latency between the two measurements were not significant. Only exceptions were the latency of ipsilateral peak II after M1 stimulation at 110% MT ($P < 0.0166$) and the amplitudes of ipsilateral and contralateral peak IV after DLPFC stimulation at 90% MT ($P < 0.0166$; shown with asterisks in Table I). The absence of significant differences for the vast majority of the peaks (135 out of 138) supports high test-retest reproducibility of nTMS-evoked EEG responses.

The origins of deflections N15 (peak I), P35 (peak II), N45 (peak III), P55 (peak IV) and P180 (peak VI) are not yet well understood [Bonato et al., 2006; Komssi et al., 2002, 2004; Paus et al., 2001]. A dipolar source in the M1 was found for N45 whereas no such dipoles were found for P30 (peak II) and N100 (peak V), suggesting different generator mechanisms of the latter deflections [Paus et al., 2001]. Later studies suggest that N45 depends on circuits intrinsic to M1 [Van Der Werf and Paus, 2006]. Ipsilateral peak II was more reproducible in responses to the prefrontal nTMS than for the primary motor nTMS, whereas ipsilateral peak III is reproduced better in nTMS to the primary motor cortex (Table II), also supporting different generator mechanisms of the two deflections. The replicability of peak V was good. N100, which is the dominant

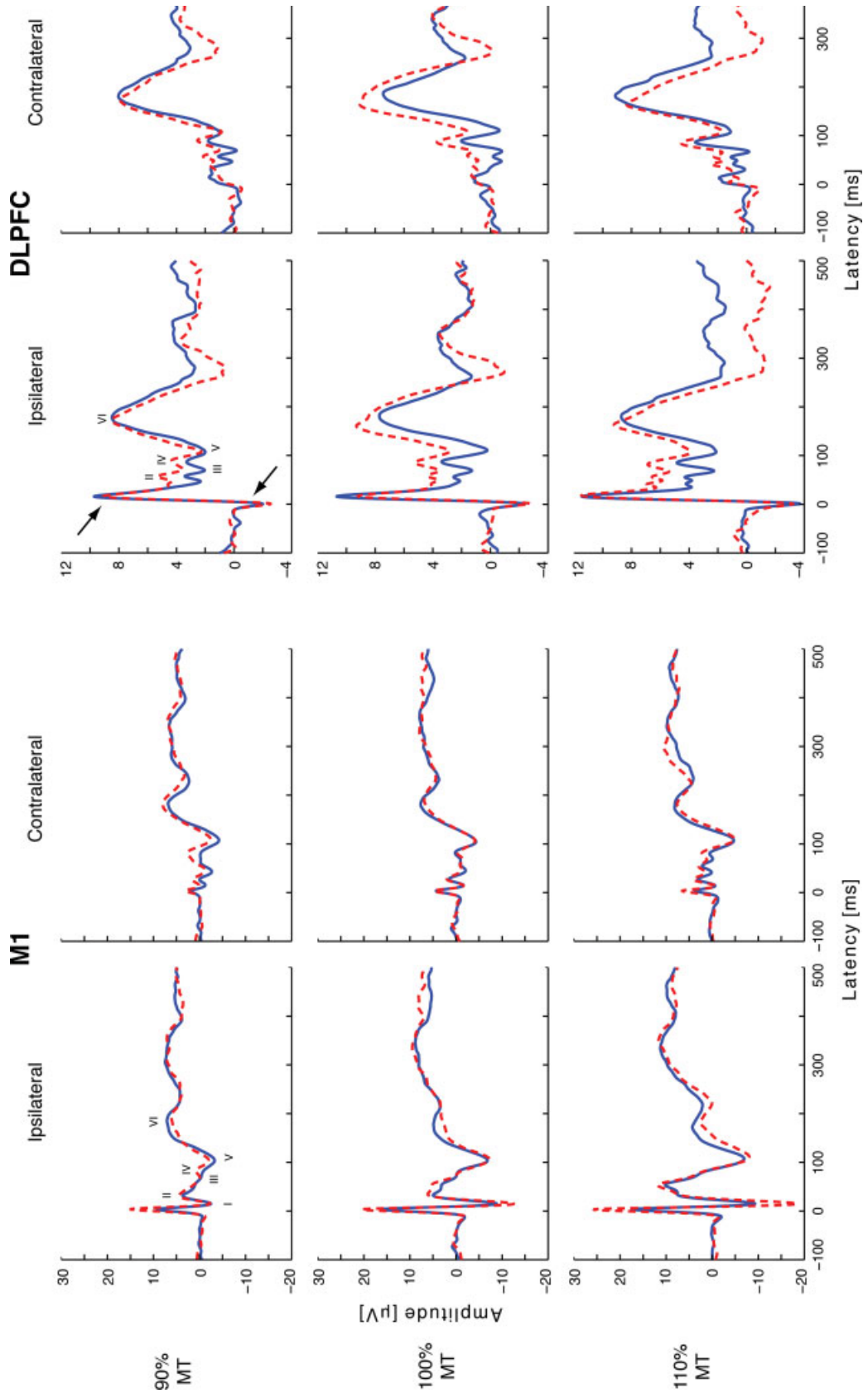

Figure 3.

Grand average waveforms of the two measurements from ROI electrodes after primary motor (M1) and dorsolateral prefrontal cortex (DLPFC) stimulation. Dashed line illustrates the first and the solid line the second recording. The arrows indicate the residual TMS artefact. The signals were low-pass filtered with a cut-off frequency of 45 Hz. Note different amplitude scales for M1 and DLPFC responses. Variability of response peaks is larger than the baseline noise. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

peak in TMS-evoked EEG, is very sensitive to small changes in cortical excitability. It may represent cortical inhibition elicited by TMS [Bender et al., 2005; Kähkönen and Wilenius, 2007; Kičić et al., 2008; Nikulin et al., 2003]. The N100-P180 complex may contain an auditory response to the TMS coil click; part of this response is due to bone-conducted sound [Nikouline et al., 1999]. However, peak V is evoked primarily by TMS [Komssi et al., 2004; Nikulin et al., 2003; Paus et al., 2001]. High reproducibility of peak V (Table II) enhances its value as a marker of cortical processing for basic and clinical research studies.

Prefrontal TMS-evoked responses in ipsilateral ROI contained five peaks, in line with previous results [Kähkönen et al., 2003, 2004, 2005]. However, in the contralateral ROI, an early additional (sixth) deflection was detected. The responses had the same latencies as those elicited by the primary motor cortex TMS [Kähkönen et al., 2005]. However, the response amplitudes were smaller for prefrontal than primary motor cortex nTMS, indicating different reactivity of the two regions [Kähkönen et al., 2003, 2004]. Our study presents the first evidence that nTMS produces reproducible EEG responses with 1-week interval after stimulation of cortical sites where no behavioral or motor responses can be measured.

Reproducibility of MT

The "fixed-point" stimulation is adequate for accurate determination of MT [Kimiskidis et al., 2004]. However, the accuracy can be enhanced by applying the "hot spot" method [Conforto et al., 2004]. Our results indicate that utilization of nTMS and "hot spot" approach of MT measurements provides accurate results. In addition, we found that when MT is measured with a 1-week interval, the "hot spot" remains stable and provides a highly replicable MT.

CONCLUSION

Reproducible TMS-evoked EEG responses are a valuable tool for investigating changes of cortical excitability in healthy subjects and in patients with cortical pathologies. In nTMS, stimuli can be delivered over the same site repeatedly. Thus, response changes elicited by e.g., rTMS over the dorsolateral prefrontal gyrus in healthy subjects or patients with depression, as well as changes elicited by M1 TMS in patients with movement and degenerative disorders, can be tracked precisely to get information about the pathophysiological mechanisms in test re–test designs. In addition, reproducibility of the EEG responses contralateral to the stimulated site may provide a supplementary tool in clinical paradigms requiring high-intensity TMS, which may produce stimulus and muscle artifacts and contaminate EEG responses from the ipsilateral hemisphere.

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V

Publication V

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Parallel input makes the brain run faster

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In serial sensory processing, information flows from the thalamus via primary sensory cortices to higher-order association areas. However, association cortices also receive, albeit weak, direct thalamocortical sensory inputs of unknown function. For example, while information proceeds from primary (SI) to secondary (SII) somatosensory cortex in a serial fashion, both areas are known to receive direct thalamocortical sensory input. The present study examines the potential roles of such parallel input arrangements. The subjects were presented with median nerve somatosensory stimuli with the instruction to respond with the contralateral hand. The locations and time courses of the activated brain areas were first identified with magnetoencephalography (MEG). In a subsequent session, these brain areas were modulated with single-pulse transcranial magnetic stimulation (TMS) at 15–210 ms after the somatosensory stimulus while electroencephalography (EEG) was recorded. TMS pulses at 15–40 ms post-stimulus significantly speeded up reaction times and somatosensory-evoked responses, with largest facilitatory effects when the TMS pulse was given to contralateral SII at about 20 ms. To explain the results, we propose that the early somatosensory-evoked physiological SII activation exerts an SII→SI influence that facilitates the reciprocal SI→SII pathway – with TMS to SII we apparently amplified this mechanism. The results suggest that the human brain may utilize parallel inputs to facilitate long-distance cortico-cortical connections, resulting in accelerated processing and speeded reaction times. This arrangement could also allow very early top–down modulation of the bottom–up stream of sensory information.

Keywords: Brain; Human; Somatosensory; Parallel processing; Bottom–up; Top–down

Introduction

Serial bottom–up flow of information from sensory thalamic nuclei via primary sensory cortices to higher-order association areas has been well-established (Pons et al., 1987). However, direct thalamocortical inputs bypassing the primary sensory cortices also exist. In non-human primates, direct input to the secondary somatosensory cortex SII (Kaas and Garraghty, 1991; Zhang et al., 2001, 1996) and crossmodal inputs to islets in sensory association cortices (Schroeder et al., 2001) have been reported. In humans, higher-order cortices may become activated even earlier than primary sensory cortices (Barba et al., 2002; ffytche et al., 1995; Karhu and Tesche, 1999), which suggests parallel pathway arrangements. However, the functional roles of parallel sensory inputs to association cortices are unknown. The current study examines the possible advantages of such inputs. Specifically, inspired by the "counter streams" theory of visual processing (Ullman, 1995, 1996), we hypothesized that they facilitate cortico-cortical communications between primary sensory cortex and the higher-order cortical areas that receive parallel inputs directly from the thalamus.

To this aim, we first presented somatosensory median nerve stimuli with a reaction time (RT) task while measuring the brain activations with magnetoencephalography (MEG). This provided the locations and timings of the activated somatomotor network. In a subsequent session, the identified brain areas were then modulated with a transcranial magnetic stimulation (TMS) pulse at different latencies after the somatosensory stimulus. The resulting modulations were detected with simultaneous RT and electroencephalographic (EEG) recordings. We hypothesized that a TMS pulse given immediately after the somatosensory stimulus would speed up brain processing and RTs. Moreover, we anticipated that the RT advantage would be greatest when higher-order cortical

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areas, rather than the primary somatosensory cortex, were stimulated with TMS.

Materials and methods

Subjects, stimuli, and task

The subjects were three healthy human males (age 26–41 years, one left-handed). The somatosensory stimuli were 0.2-ms electrical impulses to the dominant hand median nerve, generating a visible thumb twitch. To preclude anticipatory effects, the interstimulus interval was variable (mean 2.3 s, range 1.5–21 s). The experiment was conducted in 4-min runs, each containing 40 stimuli/responses. The task was to respond to each stimulus with the index finger of the non-dominant hand (contralateral to the somatosensory stimulus) as quickly as possible while RT was measured. Outlier RTs (4.3%) were removed based on falling outside mean ± 2 SD across all runs.

Magnetic resonance imaging (MRI)

Structural T1-weighted images were obtained with a 1.5-T Siemens Allegra (Siemens, Germany) scanner and segmented with the FreeSurfer (Fischl et al., 2002) software (http://surfer.nmr.mgh.harvard.edu).

Experiment 1: MEG

Whole-head 306-channel MEG was recorded with a VectorView neuromagnetometer (Elekta Neuromag, Finland) at 0.01–330 Hz and sampled at 1 kHz. Responses from 120 trials were averaged with respect to the somatosensory stimuli to reveal event-related fields (ERFs); epochs containing electro-oculogram (EOG) signals exceeding ±150 μV were discarded. The generators of the ERFs were located using dipole modeling. The dipole amplitudes were then allowed to vary in a multidipole model as a function of time while keeping their locations and orientations fixed. This resulted in

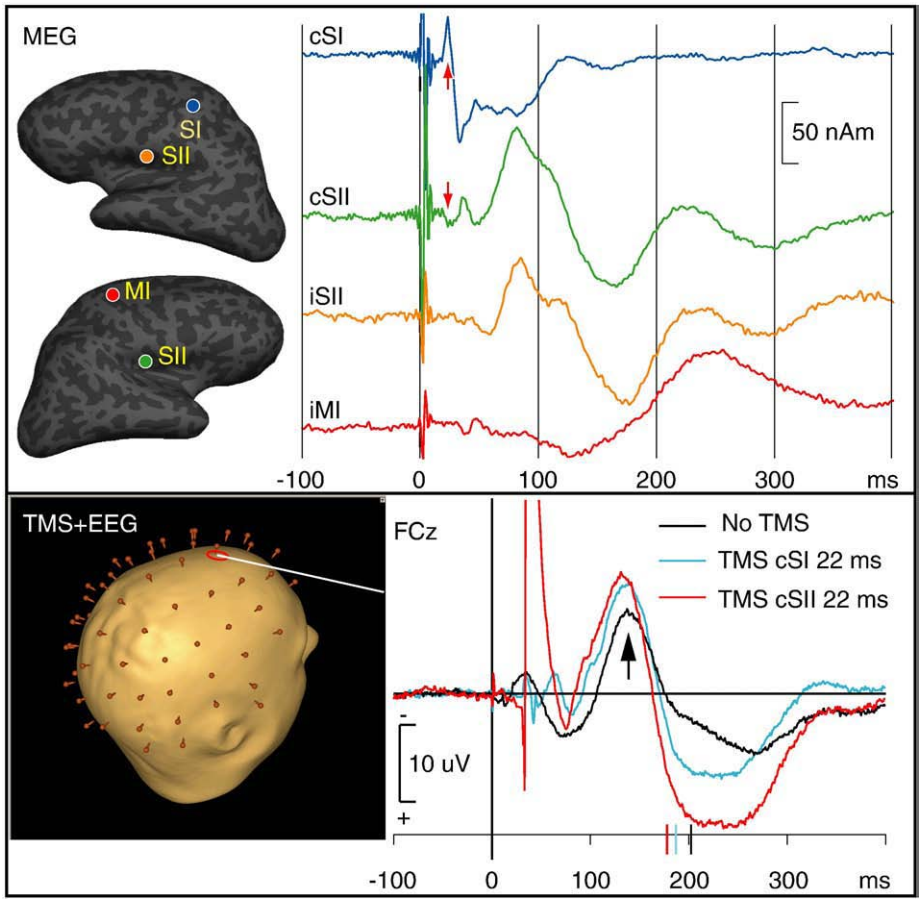

Fig. 1. Upper panel: MEG experiment. MEG source locations, shown on inflated cortex, and time courses from a typical subject. The subject responded to right median nerve stimuli with the left index finger. The evoked MEG responses were generated by four sources: the primary somatosensory cortex in the hemisphere contralateral to the median nerve stimulus (cSI), the secondary somatosensory cortices bilaterally (cSII, iSII), and the primary motor cortex contralateral to the motor response (but ipsilateral to the median never stimulus, iMI). In the time courses, a somatosensory stimulator artifact is observed at time 0. The cSI waveform showed maxima at 23 ms (upper red arrow)/33 ms, while cSII showed early activity already at about 20–35 ms (lower red arrow) and major peaks at 90/170 ms. The iSII exhibited similar 90/170 ms deflections as cSII. The iMI showed typical motor-evoked activity with a maximum slightly after the movement onset at 210 ms. Lower panel: TMS + EEG experiment. Somatosensory ERPs (unfiltered grand average waveforms) to identical stimuli and task as above. Responses recorded from the midline frontal location FCz were selected for display; according to our simulations these mainly reflect bilateral SII activity. Compared to the condition without TMS (black line), the two shown TMS conditions (blue and red lines) reveal earlier and stronger SII activity already at ~140 ms (black arrow). The ERP time shifts appear to correspond to the speeded RTs (ticks on the ERP time scales).

millisecond-accuracy time courses of the activated brain areas (Hämäläinen and Hari, 2002).

Experiment 2: Navigated TMS and EEG

Single-pulse TMS (Ilmoniemi et al., 1999) was delivered with a Magstim Rapid stimulator (Magstim Company, UK) and figure-of-eight coil (Magstim 9925) navigated with eXimia NBS™ (Nexstim Ltd., Finland) to target the brain areas identified with MEG. TMS intensity was 120% of the subject-specific motor threshold. A total of 25–31 runs were recorded per subject (including three without TMS pulses) resulting in 1040–1240 trials per subject. To probe different stages of processing, the TMS pulse latency across runs was varied 15–210 ms after the somatosensory stimulus, with the TMS latencies tailored for each subject individually based on their MEG responses. The order of TMS latencies in each brain location was randomized. Simultaneous EEG was recorded using a 60-channel TMS-compatible eXimia EEG system (Nexstim), band-pass filtered at 0.1–350 Hz, and sampled at 1.45 kHz at 16-bit depth (mean reference). The EEG amplifiers were decoupled from the electrodes for 9 ms during delivery of the TMS pulse. The EEG responses were averaged with respect to the somatosensory stimuli to reveal event-related potentials (ERPs) separately for each TMS location and latency. Epochs contaminated by eye blinks were discarded using ±100 μV threshold. EEG sensor locations that best reflected the activity of cSI, cSII, iSII, and iMI were determined by forward modeling the MEG data to simulate corresponding ERPs. Peak ERP latencies were then identified for somatosensory-evoked N20/P45/P75/N140 components separately for each TMS location and latency (provided that the strong TMS-evoked response did not distort the somatosensory-evoked component beyond recognition).

Results

First, MEG (Fig. 1, upper panel) revealed expected (Hari and Forss, 1999) sources and their activation time courses in the contralateral primary somatosensory cortex (cSI), SIIs bilaterally (cSII, iSII), and ipsilateral motor cortex (iMI). Simulations suggested that the observed cSII activity at about 20–35 ms could not be explained by volume conduction from unaccounted cSI sources.

Second, in a subsequent session, processing in these four brain areas was modulated with a single TMS pulse at 15–210 ms after the somatosensory stimulus while EEG and RT were recorded (Fig. 1, lower panel). Without TMS pulses, RT was 203±29 ms (mean±SD, collapsed across subjects; means of individual subjects had a range of 197–209 ms).

Fig. 2 shows that the TMS pulse after the somatosensory stimulus clearly effected RTs. In each of the four targeted brain areas, TMS pulse latency was positively correlated with RT. The linear correlation was strongest in cSII (Pearson's correlation $r=0.83$), somewhat weaker in iSII ($r=0.74$) and cSI ($r=0.74$), and weakest in iMI cortex ($r=0.54$). Lack of correlation between run order and RT suggested that fatigue did not play a role (for individual subjects, Pearson's r ranged from −0.17 to +0.13).

Prolonged RTs demonstrated that the TMS pulse interfered with the neuronal processes. As hypothesized, early TMS pulses (15–40 ms after the somatosensory stimulus) were associated with significantly faster RTs than without TMS (Student's 2-tailed heterosce-

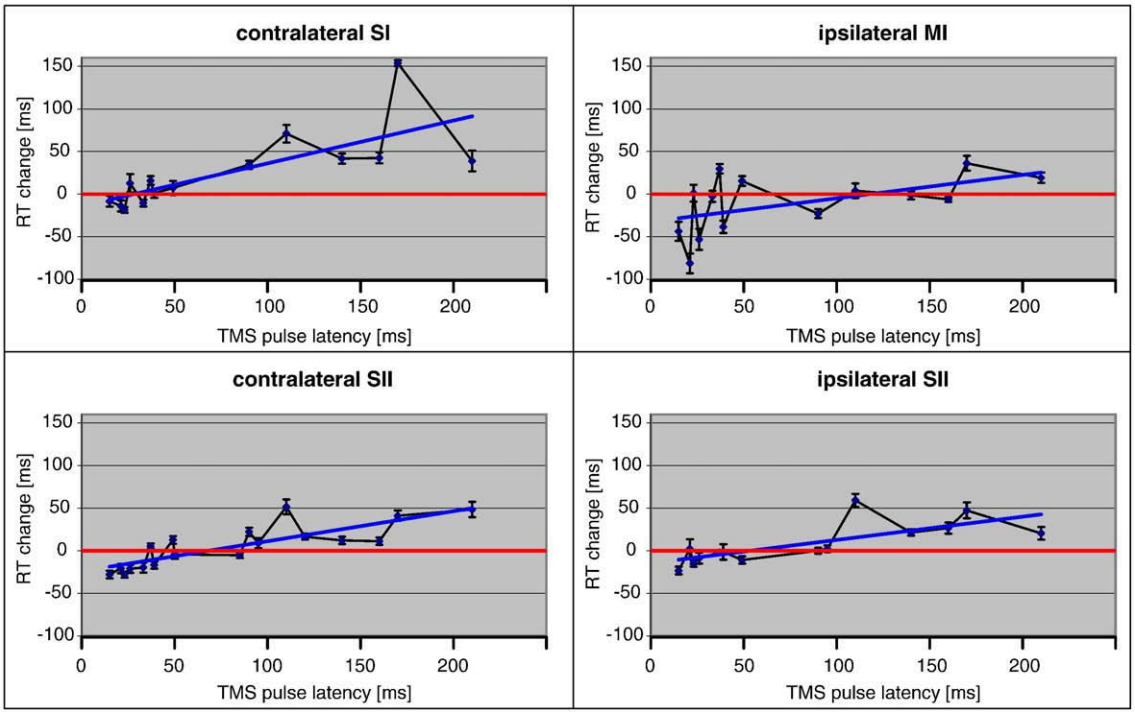

Fig. 2. Reaction time change (0 ms=RT without TMS) as a function of TMS pulse latency (0 ms=Median nerve somatosensory stimulus). The panels show the effects separately for the four brain areas that were targeted with TMS. All brain areas showed prolonged RTs with increasing TMS pulse latency (blue trend lines), with the strongest linear correlation with TMS of the contralateral SII (lower left panel). The earliest TMS pulse latencies were associated with significantly faster RTs than without TMS. While this effect was observed in each of the four brain areas, it was clearest for ipsilateral MI (contralateral with respect to the reaction key hand) and for contralateral SII. Data collapsed across subjects, mean±SEM error bars. For details, see text.

dastic *t*-test $p<0.001$; collapsed across all four brain areas from all subjects at TMS latencies 15–40 ms; for each TMS target area individually $p<0.05$). The motor cortex (iMI; Fig. 2, upper right panel) showed the largest RT speeding effects, but this may have occurred simply because TMS activated the motor system regardless of the somatosensory stimulus. However, the earliest (15–23 ms) TMS pulses to cSI (RT = 189 ± 35 ms), cSII (RT = 176 ± 27 ms), or iSII (RT = 187 ± 42 ms) also significantly speeded up the RTs ($p<0.001$ for each area separately collapsed across subjects; for cSII $p<0.001$ for each subject individually). This effect was TMS location-specific: supporting our hypothesis, RT was significantly faster when TMS was given to cSII than to cSI ($p<0.001$; collapsed across subjects at TMS latencies 15–23 ms) or to iSII ($p<0.01$).

We then analyzed the ERP data to understand at which level of processing the RT speeding effect occurred. Fig. 1, lower panel, shows that the latency of the SII-generated ~140 ms component was shifted earlier with a TMS pulse at ~20 ms after the somatosensory stimulus. Peak latency analyses revealed that TMS pulses at 15–40 ms speeded the 140-ms ERP component by 8 ± 8 ms compared with the no-TMS condition. This effect was statistically significant ($p<0.05$; collapsed across brain areas and subjects). The ERP data were thus consistent with the idea that the brain activations were speeded already at the SII level. The ERP waveforms selected for display in Fig. 1, lower panel, further suggests that, similar to the RT data, the largest latency shifts were observed when TMS was targeted at cSII; however, in the ERP data, this trend did not reach statistical significance.

Compared with the time window when TMS pulses speeded RTs (15–40 ms), the observed ERP latency shift at ~140 ms appears a relatively late phenomenon. However, the strong TMS-evoked ERPs, maximal under the TMS target location and lasting ~100 ms after the TMS pulse, resulted in that, of the somatosensory-evoked components, only the 140-ms deflection could be reliably identified across experimental conditions. It is thus possible that the somatosensory system latency shifts started before 140 ms but our paradigm could not detect them. Future studies may benefit from a subtraction technique that allows separation of the sensory- and TMS-evoked components (Thut et al., 2003, 2005).

Discussion

We observed speeded RTs and somatosensory-evoked responses when a TMS pulse was delivered to the somatomotor network 15–40 ms after a median nerve stimulus. Largest facilitatory effects were observed when the TMS pulse was targeted at the contralateral SII at about 20 ms post-stimulus.

Previous studies utilizing human intracranial recordings have shown SII activity beginning already at 15–27 ms post-stimulus, which is simultaneous or earlier than onset of the SI activity (Barba et al., 2002). Therefore, SII must receive direct early parallel sensory input independent of the pathway via SI, consistent with the current and earlier (Karhu and Tesche, 1999) MEG observations.

Speeded RTs for TMS at early post-stimulus latencies have been described before (Gregori et al., 2005), thus supporting our behavioral results. However, these effects have been attributed to multisensory redundancy caused by the auditory click from the stimulator coil, largely because in these studies the RT effect has been similar regardless of the TMS target area (Gregori et al., 2005; Walsh and Pascual-Leone, 2003). Our results show TMS site specificity and therefore are not compatible with this interpretation. To examine this further, we made control measurements in one subject where the auditory click from the TMS coil was identical but the TMS-evoked currents were reduced by over 50% (sham coil). The TMS pulse was given 21 ms after the somatosensory stimulus. The subject did not know when real vs. sham TMS was used. RTs were significantly faster for real vs. sham stimulation over cSII ($p<0.05$) and iMI ($p<0.001$) but not over cSI ($p=0.15$); iSII was not tested. Both TMS site specificity and the control measurement therefore support the idea that the speeded RTs were caused by TMS-evoked neuronal currents.

We propose that the speeded RTs can be best explained if the somatosensory-evoked physiological SII activation at about 20 ms normally exerts a top–down SII→SI influence that facilitates the reciprocal SI→SII pathway. With TMS to SII at ~20 ms, it appears that we amplified a brain-speeding mechanism already in place. This interpretation is supported with the current findings of site specificity of TMS and ERP latency shifts already at the SII level.

More generally, fast thalamocortical parallel sensory inputs to multiple cortical sites could drop the activation thresholds of the cortico-cortical connections between the areas (Ullman, 1996). This mechanism could almost immediately after a stimulus establish a widespread network where the nodes receiving parallel input would be likely to communicate with each other.

Theoretical and physiological studies have suggested that top–down effects may facilitate and guide the reciprocal bottom–up flow, even though the cellular-level mechanisms are still poorly known (Siegel et al., 2000; Ullman, 1995, 1996). However, in order to be effective, top–down processes should be running already when the bottom–up stream is finding its way towards higher levels of cortical hierarchy (Ullman, 1996). This is obviously difficult to achieve with serial processing. One possibility is that the brain utilizes serial pathways specialized for very fast information transfer to initiate early activity in high-order association cortices. For example, visual recognition has been shown to utilize early top–down influences from orbitofrontal cortex initiated by fast serial (via V1) magnocellular pathways (Bar, 2003; Bar et al., 2006; Kveraga et al., 2007). The somatosensory data are inconsistent with serial processing models because activations start earlier in SII than SI. The current study therefore offers an appealing alternative mechanism: association cortices could receive direct thalamocortical sensory input, allowing simultaneous top–down and bottom–up processing. Both mechanisms may well coexist.

The idea of parallel thalamocortical sensory inputs to multiple cortical areas may appear inconsistent with the view that transmission of sensory information is hard wired from the thalamic sensory nucleus to the corresponding sensory projection cortex. However, first-order thalamic nuclei receiving driving input from sensory organs are reciprocally connected with and heavily modulated by both higher-order thalamic nuclei (e.g., pulvinar) and cortex, reflecting attentional and other task-related demands (Guillery and Sherman, 2002; O'Connor et al., 2002; Sherman, 2007; Sherman and Guillery, 1996, 2002; see Bender and Youakim, 2001; Briggs and Usrey, 2007; Zikopoulos and Barbas, 2007 for recent related work in primates). Pulvinar, on the other hand, has massive reciprocal connections throughout the neocortex (e.g., Adams et al., 2000; Buchsbaum et al., 2006; see Shipp, 2003 for a review). The cortex can thus receive fast sensory input from the thalamus directly from the first-order thalamic nucleus (when such pathways exist) or through a higher-order thalamic area such as the pulvinar. Modulating inputs to thalamic nuclei could in a dynamic manner adjust which cortical areas receive parallel sensory input.

Given that both association and low-level sensory cortices appear to receive very early parallel crossmodal inputs (Fort et al., 2002, 2000; Foxe and Schroeder, 2005; Giard and Peronnet, 1999; Molholm et al., 2002; Murray et al., 2005; Schroeder and Foxe, 2002, 2005; Schroeder et al., 2003), some via the pulvinar (Budinger et al., 2006; Hackett et al., 2007), a similar mechanism as suggested in the current study could also explain why reaction times to multisensory stimuli are faster than to unisensory stimuli (Raab, 1962; Schröger and Widmann, 1998). Early physiological SII activations may also serve a protective function due to the roles of SII in pain processing (Timmermann et al., 2001) and sensorimotor integration (Forss and Jousmäki, 1998; Huttunen et al., 1996).

It has been suggested that serial processing is more prevalent in higher primates, and there seems to be an evolutionary shift in mammals where humans have the least amount of parallel sensory inputs to higher-order areas (Coleman et al., 1999; Kaas and Garraghty, 1991; Zhang et al., 2001, 1996) and therefore increased serial processing of sensory input. Thus, it appears that in the course of evolution humans may have traded some processing speed for better cognitive control.

From the large number of trials and consistent results across subjects, it follows that the current results are reliable within the studied population, but due to limited access (the instruments were located on different continents), our number of subjects was small. Hence, more studies with larger subject populations are needed to estimate how abundant this mechanism is.

Concluding remarks

The cerebral cortex receives sensory input from the thalamus not only to primary projection areas but also directly to hierarchically higher-order cortices in a parallel fashion. The current results suggest that this facilitates cortico-cortical communication between the areas that receive parallel input, thus making the brain faster. This also allows very early top–down modulation of the bottom–up stream of sensory input. The same mechanism could drop the activation thresholds between the participating cortical nodes, therefore establishing a distributed neuronal network almost immediately after a stimulus. Further studies are needed.

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VI

Publication VI

Kičić D., Bikmullina R., Lioumis P., Nurminen J., Kaakkola S., Mäkelä J.P., and Pekkonen E. (2007). Effects of 10 Hz rTMS on spontaneous brain oscillations in non-demented Parkinson's patients: Preliminary results of combined MEG-rTMS study. International Congress Series 1300, 1717-20.

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Effects of 10 Hz rTMS on spontaneous brain oscillations in non-demented Parkinson's patients: Preliminary results of combined MEG-rTMS study

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Abstract. Therapeutic effects of repetitive transcranial magnetic stimulation (rTMS) are extensively studied in patients with Parkinson's disease (PD). rTMS to primary motor cortex (M1) induces dopamine release in the putamen: consequently M1 is an interesting target for rTMS in PD. The rTMS over M1 in PD patients reduces the reaction time, improves the Unified Parkinson's Disease Rating Scale (UPDRS) scores and alleviates bradykinesia and hypokinesia. However, the effects of rTMS on spontaneous brain activity are not known. We investigated whether subthreshold rTMS to M1 modulated spontaneous oscillations recorded with magnetoencephalography (MEG). MEG from nine medicated, non-demented PD patients displayed a significant increase in beta oscillations after rTMS in the stimulated hemisphere. Minimum current estimate calculations revealed an increase of beta oscillatory activity after the rTMS treatment over the Rolandic regions. The rTMS in PD patients alters spontaneous brain activity as seen with MEG, probably by modulating cortico-thalamo-basal ganglia networks.

Keywords: Magnetoencephalography; Transcranial magnetic stimulation; Spontaneous oscillations; Parkinson's disease

1. Introduction

Parkinson's disease (PD) is caused by a progressive loss of dopaminergic neurons. However, its detailed pathophysiology is still poorly understood. According to the basal

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ganglia–thalamocortical circuitry model [1], degeneration of dopaminergic nigrostriatal pathways results in functional deafferentation of the primary motor cortex (M1).

In PD, repetitive transcranial magnetic stimulation (rTMS) to prefrontal cortex induces dopamine release in the caudate nucleus [3] and rTMS to M1 [4] releases dopamine in the ipsilateral putamen. Thus, M1 is an appealing target for neuromodulation therapy in PD.

Several MEG studies demonstrated that M1 is involved in a tremor-generating network in PD [6,7], that rhythmic cortical activity is transmitted through a network consisting of the subthalamic nucleus (STN) and globus pallidus (GP, [2]) and that cortical oscillations are coherent with oscillations in the thalamus, basal ganglia and the cerebellum [6].

The short-term effects of rTMS on spontaneous oscillatory MEG activity are unknown. We investigated whether subthreshold rTMS to M1 affects the spontaneous cortical oscillations in PD patients and whether these changes are correlated with eventual improvements of motor symptoms of PD.

2. Patients and methods

Nine non-demented Parkinsonian patients (age 49–74 years; 5 females) with mild bilateral symptoms though more affected unilaterally (modified Hoehn and Yahr, stages 2–2.5/5) participated in the study. Written informed consent was obtained from all patients before the study. Duration of the Parkinson's disease ranged from four to nine years. Seven out of nine patients were on levodopa therapy, with the dose ranging from 300 to 650 mg/day. In addition, seven patients used selegiline (5–10 mg/day), and eight patients had dopamine agonists (7 patients pramipexole, 0.75–3 mg/day; one patient ropinirole 6 mg/day). Mini Mental Status Examination (MMSE) was performed prior the study to exclude dementia; the mean MMSE score was 29/30. MRI scans excluded brain atrophy and brain tumors in all patients. The Beck depression inventory suggested mild depression in three patients. Six patients had no signs of depression. None of the patients used antidepressant or neuroleptic medication. In the morning prior to the measurements the patients did not take antiparkinsonian medication.

Unified Parkinson's Disease Rating Scale (UPDRS) motor scores were measured before and after the measurements. The TMS coil was navigated using Nexstim eXimia (Nexstim Ltd., Finland) navigated brain stimulation (NBS) system that utilizes individual MRI scans to target the cortical site of interest. In two experiments, performed in two consecutive days, the hemisphere contralateral to the more affected limb was stimulated with rTMS intensity at 80% of patient's motor threshold (MT). Twenty trains consisting of 100 pulses at 10 Hz were delivered with 1 min inter-train interval. A coplanar figure-of-eight coil was used to deliver trains of rTMS pulses produced by Magstim Rapid Stimulator (Magstim Co., UK). One rTMS session took approximately 20 min. Sham TMS experiment was run in one subject.

On each experimental session, spontaneous brain activity in eyes open/closed conditions was recorded over the whole head with a 306-channel Elekta Neuromag MEG instrument (Elekta Neuromag OY, Finland) before and about 5 min after the rTMS treatment. Signal space separation (SSS, [5]) was applied on all collected data to suppress noise from sources outside the brain (e.g. heart artifacts). Spectral power (SP) was calculated in beta band before and after the rTMS treatment. Each patient's data were individually inspected for SP changes before vs. after rTMS treatment and eight gradiometer channels bilaterally over

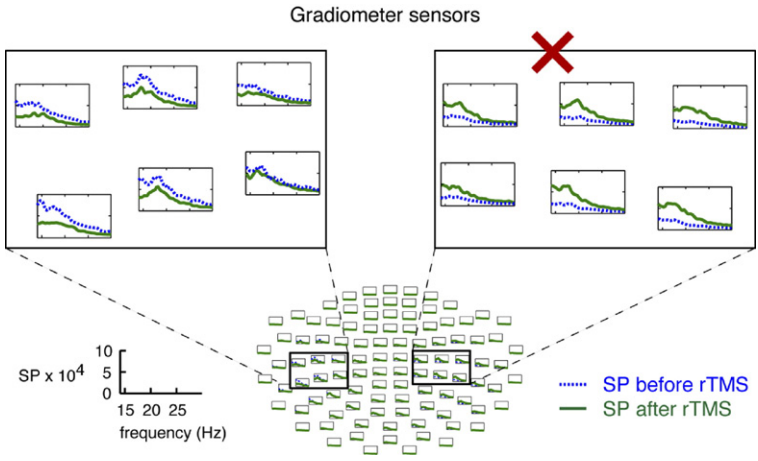

Fig. 1. Bilateral beta SP changes over the Rolandic regions (eyes open) in one PD patient. The red cross marks the center of the stimulating coil. After rTMS, the SP increase occurs in the right (stimulated) hemisphere and SP decrease in the left (non-stimulated) hemisphere.

Rolandic regions were selected for further comparison. For selected channels an average trapezoidal numerical integration was calculated. L1 norm minimum current estimate (MCE) was calculated on 2-min data segments using a discrete Fourier transform (DFT) size of 1024 samples for the frequencies of interest to illustrate the spectral distribution over the hemispheres. Paired *t*-tests were used for statistical analysis.

3. Results

The rTMS treatment was effective for at least 24 h according to the UPDRS motor scores, which were improved significantly only the first day ($p<0.01$). In seven out of nine patients, the beta power (14–30 Hz) was increased after the first rTMS treatment. Activity

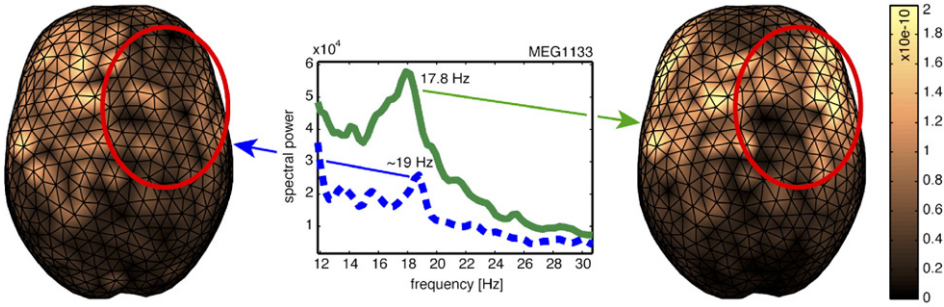

Fig. 2. Visualization of MCE in one patient before (left) and after rTMS (right) shows distribution of the increase of beta SP in Rolandic regions after rTMS to the right hemisphere. The head is seen from above. The spectrum from one sensor is illustrated in the middle panel. Dotted line = SP before rTMS; full line = SP after rTMS. Data set used for this calculation is the same as for Fig. 1.

in beta range changed consistently and significantly ($p=0.03$) bilaterally over the Rolandic regions. In all patients the second rTMS treatment produced less pronounced increase or even no increase of beta SP (compared with second-day SP before rTMS). Sham rTMS in one patient did not change SP significantly.

MCE calculations showed an increase of beta oscillatory activity in the stimulated hemisphere (Fig. 2). The patients reported decreased rigidity but no changes in resting tremor.

4. Discussion

Beta SP changes may reflect positive alterations in the abnormal synchronization of spontaneous activity generated by the thalamocortical–basal ganglia circuitry in Parkinson´s disease. Relief of rigidity in patients suggests that beta oscillations may be related to akinetic features of PD. Because of short duration of the measurement sessions (total 2 h), it is unlikely that medication withdrawal effects caused the observed changes. Beta oscillations are related to a resting or an idling state of the motor cortex [9,10]. MCE calculations showed decreased beta activity in the more affected hemisphere of PD patients (Left panel in Fig.2). Effectiveness of rTMS to excite thalamocortical circuits is demonstrated by elevation of this activity after rTMS treatment (right panel in Fig.2). Opposite direction of change in beta SP in stimulated and non-stimulated hemispheres (Fig.1) suggests unaffected interhemispheric conductivity in PD patients.

MEG results correspond with total UPDRS motor scores, which improved only after the first treatment. This is contrary to the reports about placebo effects of rTMS [8]. Future work will include analysis of other brain oscillations (theta, delta, alpha). Additional sham measurements will address issues such as contribution of arousal fluctuations and eventual effect of fatigue during MEG measurements.

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VII

Publication VII

Vitikainen A.M., Lioumis P., Paetau R., Salli E., Komssi S., Metsähonkala L., Paetau A., Kičić D., Blomstedt G., Valanne L., Mäkelä J.P., and Gaily E. (2008). Combined use of non-invasive techniques for improved functional localization for a selected group of epilepsy surgery candidates. Neuroimage 45(2), 342-8.

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Combined use of non-invasive techniques for improved functional localization for a selected group of epilepsy surgery candidates

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ABSTRACT

Invasive cortical mapping is conventionally required for preoperative identification of epileptogenic and eloquent cortical regions before epilepsy surgery. The decision on the extent and exact location of the resection is always demanding and multimodal approach is desired for added certainty. The present study describes two non-invasive preoperative protocols, used in addition to the normal preoperative work-up for localization of the epileptogenic and sensorimotor cortical regions, in two young patients with epilepsy. Magnetoencephalography (MEG) was used to determine the primary somatosensory cortex (S1) and the ictal onset zones. Navigated transcranial magnetic stimulation (nTMS) was used to determine the location and the extent of the primary motor representation areas. The localization results from these non-invasive methods were used for guiding the subdural grid deployment and later compared with the results from electrical cortical stimulation (ECS) via subdural grids, and validated by surgery outcome. The results from MEG and nTMS localizations were consistent with the ECS results and provided improved spatial precision. Consistent results of our study suggest that these non-invasive methods can be added to the standard preoperative work-up and may even hold a potential to replace the ECS in a subgroup of patients with epilepsy who have the suspected epileptogenic zone near the sensorimotor cortex and seizures frequent enough for ictal MEG.

Introduction

Epilepsy surgery candidates whose epileptic focus is close to eloquent cortical areas need accurate identification of the epileptogenic zone and the irretrievable cortex. This is usually done with intracranial recordings and electrical cortical stimulation (ECS), presently the standard technique for preoperative localization. However, subdural investigations require diagnostic surgery, associated with significant risk of complications (Hamer et al., 2002). Therefore accurate non-invasive methods for localizations with added precision and reliability would be highly appreciated. In two patients who subsequently underwent a weeklong intracranial recording via subdural electrodes and resective surgery, we applied two non-invasive methods; magnetoencephalography (MEG) to identify the ictal onset zone and primary somatosensory cortex (S1) (Mäkelä et al., 2006) and navigated transcranial magnetic stimulation (nTMS) to determine the boundaries of the primary motor cortical representation areas of selected muscles (Hannula et al., 2005; Krings et al., 1997a; Wilson et al., 1993).

Methods and patients

Mapping of the primary motor cortex with nTMS

Single-pulse nTMS delivered by a figure-of-eight coil, with concurrent electroencephalography (EEG) (eXimia NBS and EEG,

Abbreviations: ADM, Adbuctor digiti minimi; AEM, Antiepileptic medication; AH, Abductor hallucis; APB, Abductor pollicis brevis; BB, Biceps brachii; CT, Computed tomography; ECD, Equivalent current dipole; ECS, Electrical cortical stimulation; EDC, Extensor digitorum communis; FCR, Flexor carpi radialis; FDI, First dorsal interosseus; MEG, Magnetoencephalography; MEP, Motor evoked potential; MRI, fMRI, Magnetic resonance imaging, Functional magnetic resonance imaging; MT, Motor threshold; nTMS, TMS, Navigated transcranial magnetic stimulation, Transcranial magnetic stimulation; PET, Positron emission tomography; RF, Rectus femoris; S1, Primary somatosensory cortex; SEF, Somatosensory evoked field; TA, Tibialis anterior.

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Nexstim Ltd., Helsinki, Finland) was used to map the primary motor cortical representation areas of selected upper and lower extremity muscles. The EEG amplifier keeps the signal constant from 100 μs before the pulse to 2 ms after the TMS pulse by a sample-and-hold-circuitry (Virtanen et al., 1999). The motor area of the abductor pollicis brevis (APB) was first selected in magnetic resonance images (MRI) on the basis of the "hand knob" (Yousry et al., 1997). The resting motor threshold (MT) was defined as the lowest stimulation intensity at which 5 out of 10 pulses evoked a motor potential (MEP) of 50 μVp-p (peak-to-peak amplitude) or greater (Rossini et al., 1994; Rossini et al., 1999) recorded with a Keypoint EMG device (Keypoint, Medtronic, Minneapolis, USA). The localized motor cortex was stimulated at an intensity of 105–110% MT (Macdonell et al., 1991) with the coil held tangentially to the scalp with handle pointing backward and laterally; this induced a posterior-to-anterior current flow in the cortex, perpendicular to the central sulcus (Brasil-Neto et al., 1992). The area where nTMS evoked MEPs of 50 μVp-p or larger, or a clear silent period (Tataroglu et al., 2004) within the pre-activated target muscle, was determined as the primary motor representation area of the target muscle. Possible enhancement of epileptiform activity was constantly monitored with a 60-channel EEG; no such increase or seizures were induced during nTMS.

Localization of the epileptogenic and somatosensory cortices with MEG

Spontaneous interictal and ictal brain activity and somatosensory evoked fields (SEFs) to median and tibial nerve stimulation were recorded with a 306-channel magnetoencephalograph (Elekta, Helsinki, Finland) in a magnetically shielded room (Euroshield, ETS-Lindgren, Eura, Finland). Head movements were continuously monitored by four coils on the scalp activated at 154–166 Hz, enabling correction of the head movements (Medvedovsky et al., 2007; Uutela et al., 2001) and accurate ictal recordings. Due to frequent seizures, MEG recordings didn't require withdrawal of medication, and one or more habitual seizures were recorded in both patients within 1 h. Recording frequency band was 0.03–172 Hz and sampling frequency 600 Hz. SEFs were elicited by 100 constant current pulses at 0.5 Hz to wrist, and 500 to ankle, using stimulus intensity above the motor threshold. Data was band-pass filtered at 0.3–90 Hz in off-line analysis. All individual MEG traces were screened visually for epileptiform signal morphology according to traditional EEG criteria, and for corresponding dipolar magnetic field patterns, both during and between clinical seizures.

Single equivalent current dipoles (ECDs) were fitted to the magnetic field pattern of interest by the least-square search. The center of this pattern was focused on the largest gradiometer signal of interest, and a sufficient number (range 36–40) of sensor locations were selected to cover both magnetic field extremes. Single ECDs were first computed for the separate dipolar fields at different time points during the signal. Subsequently, these dipoles were used as initial guesses for a single- or multi-dipole fit for all 306 channels. Finally, the analysis period was extended to cover the entire signal of interest, and the optimal dipole strengths were computed assuming fixed dipoles at the locations and orientations given by the initial least-squares search (Scherg, 1990). Such an ECD (or set of ECDs) typically explained over 80% of the selected field. When testing the dipole with the measured data, we also accepted lower goodness-of-fit values, but required a good visual congruity between the measured signal and the waveform predicted from the estimated dipole. The dipole had to explain the signal of interest (e.g. a spike), but not other MEG signals (e.g. posterior alpha activity).

The nTMS and MEG recordings were analyzed by different experimenters, blinded to the results obtained with the other method. However, the results were used in guiding the subdural electrode grid deployment and were later combined and compared with the results from the ECS. All available expertise was used in the decision making process before the resection.

Electrical cortical stimulation

A subdural grid (AD-TECH, Racine, Wisconsin, USA) of 8, 32, or 64 platinum plate electrodes with 4.0/2.3 mm (overall/exposed) diameter and 10 mm center-to-center distance was inserted over the affected cortex. Five days later, ECS was done with a Grass S-12 biphasic stimulator (Grass Instrument Co., Quincy, MA, USA) using 5-s trains of repetitive square pulses (duration 0.3 ms/phase, pulse interval 50 Hz) of alternating polarity. The intensity was gradually increased to the level of a functional response or after-discharges induction in EEG; predetermined maximum current level was 13.5 mA (Lesser et al., 1984, 1987). A distance reference technique (Lesser et al., 1987) was used first to find a reference electrode with a high after-discharge threshold, located preferably at the periphery of the grid, and subsequently to determine the relative topography of the eloquent areas. When the known or suspected epileptogenic area intervened the line between the target and the reference electrode or when a spatially more focused stimulation was required in the region of interest, a bipolar stimulation arrangement was used between adjacent electrodes (Lesser et al., 1987). This forces the peak current density into the region immediately beneath the electrodes (Nathan et al., 1993). In the results, the number of active and reference electrodes are notated as G20–G9, G9 being the reference.

Image registration, fusion and 3-D visualizations

For visualization of all results relative to the patient's cerebral anatomy, nTMS, MEG, brain computed tomography (CT) data showing subdural cortical stimulation electrodes, and head MRI data sets were transformed to a common coordinate system. CT of the subdural grid electrode positions was acquired on the first postoperative day after the grid implantation. The T1-weighted contrast-agent enhanced MRI visualizing the superficial cortical veins, T1-weighted MRI for the general anatomy, CT and T2-weighted 3-T MRI emphasizing the lesion area were rigidly co-registered by maximizing mutual information metrics (Maes et al., 1997) with a medical image processing software (Van Leemput and Hämäläinen, 2004) utilizing the open-source Insight Segmentation and Registration Toolkit (Ibáñez et al., 2005). For Patient 2, a neuroradiologist defined the lesion area from T2-weighted MRI; for Patient 1, no lesion was detected. Subsequently, nTMS and MEG localizations were co-registered with MRI on the basis of two pre-auricular points and nasion. The brain area (including superficial cortical veins in contrast-agent enhanced MRI) was extracted from the T1-weighted MRIs, and the locations of the cortical electrodes were determined by intensity thresholding the CT data. The cortical grids, the lesion, and localizations from the nTMS and MEG were fused with the T1-weighted MRIs. The nTMS and MEG localization results were presented as small spheres in the combined data. The volume renderings (3-D visualizations) of the combined data were created using the medical image processing software utilizing the open-source VTK toolkit (Schroeder et al., 2003). The 3-D MRI reconstructions were available during resection. For Patient 2, the lesion outlines were uploaded to the neuronavigator.

Patients

A 22-year old woman (Patient 1) had drug-resistant epilepsy from the age of twelve. Typical seizures started with paraesthesia in the left arm and progressed to motor seizures, which rarely generalized. An MEG recording at age 14 revealed rare spikes 1 cm posterior to the S1 localized by the sources of 20 and 35 ms responses to left median and ulnar nerve stimulation. 3-T MRI carried out at age 22 was normal. Long-term video EEG recording done preoperatively with 39 scalp electrodes showed localized ictal epileptiform activity in electrodes C4 and P4 (International 10–20 system). Because weeklong periods of almost continuous drug-resistant seizures hampered the use of the

left hand, surgery was considered as a relevant treatment option. The median nerve SEF sources were defined and ictal onset zones were determined during seizures in whole-head MEG, recorded before surgery. nTMS representation areas were obtained for APB, abductor digiti minimi (ADM), flexor carpi radialis (FCR), extensor digitorum communis (EDC) and biceps brachii (BB). Subsequently, a subdural 64-electrode grid was placed over the right frontoparietal cortex and ECS was applied during the week of subdural video EEG recording. Only a partial medication withdrawal was necessary; oxcarbazepine was replaced by intravenous phosphenytoin, and sulthiame was stopped before the grid placement, the dose of clonazepam was halved from 2 mg to 1 mg/day and the dose of levetiracetam was reduced from 3 g to 1.5 g/day.

Seizures of Patient 2, a 16-year old girl, started at age five, and drug-resistance developed at age ten. Surgery was considered due to her frequent, disabling seizures (5–20/day). A right-sided sensory aura in the leg, back, or the whole right side of the body progressed to a motor seizure with most marked tonic–clonic activity in the right foot and leg. 3-T MRI revealed a small lesion in the left medial parietal lobe, close to the left lower limb S1. Preoperative video EEG with 39 scalp electrodes showed beta-frequency ictal activity with maximum amplitude in electrodes Pz and Cz. An ictal MEG and SEFs to median and tibial nerve stimulation were recorded three months before surgery. nTMS was used to localize the motor representations of the APB, ADM, BB, tibialis anterior (TA), rectus femoris (RF), and abductor hallucis (AH) in the left hemisphere. A 32-electrode grid was then placed over the area of the lesion and the left central sulcus, and two 2×4 electrode strips (SA1-8 and SP1-8) were inserted into the interhemispheric fissure. Electrodes SP7 and SP8 were not used, because they overlapped the electrodes SA1 and SA2. A partial medication withdrawal was carried out after the grid insertion: oxcarbazepine was replaced by intravenous phosphenytoin for the first two postoperative days, and then by oral phenytoin for the rest of the recording to reduce the risk of hyponatremia. Topiramate was stopped for two days starting with the implantation and continued at a reduced dose from the evening of the second postoperative day. The ECS was carried out 5 days after the grid deployment while being monitored in video EEG. Informed consent was obtained for both patients for all the applied recordings.

Results and outcome

Patient 1

The results from nTMS for Patient 1 match with the common motorotopy of the pre-central gyrus (Fig. 1A). The analysis of the 3-D MRI reconstruction containing the results from different modalities (Fig. 1B) revealed excellent match between preoperative and subdural recordings. The preoperative SEF source for left median nerve stimulation and the subdural cortical stimulation site (electrode G30 referred to G9, G30–G9) producing left hand sensation were within 1 cm distance. The sources of ictal MEG activity in the post-central sulcus were close to or partly overlapped the cortical stimulation sites in the post-central gyrus (G21–G9, G29–G9 and G30–G9) triggering typical seizures.

nTMS elicited localized movements of the BB, EDC, and FCR muscles pinpointing the medial limit of upper arm area. Accordingly, ECS of electrodes G14–G9 produced motor responses mainly from the left shoulder and upper arm, whereas G15–G33 produced motor responses from the lower arm. ECS of electrodes G38–G9 elicited movements in left APB, correlating well with nTMS palm representations, which were located within 1 cm distance. ECS of G7–G9 and G6–G9 also evoked trunk and leg movements, not directly related to nTMS representations. ECS of electrode G31–G9 produced both the motor phenomena typical of seizures and a motor response from the whole hand.

During subdural EEG recording altogether 46 seizures were captured, 4 of which proceeded to motor seizures. MEG, scalp, and subdural EEG showed bursts of polyspikes; ictal and interictal signals were similar in morphology. The MEG polyspikes were generated in the wall of the postcentral sulcus. Nearly continuous interictal polyspike and gamma activity was observed in electrodes G21–23, G29–31 and G38–39, agreeing well with interictal findings in MEG and scalp EEG. The ictal findings were very stereotypic starting with an intensive polyspike in electrodes G21–23, G29–31, and G38, continuing with gamma activity in electrodes G21–23, G29–30, and G38. Thereafter, the ictal polyspike activity spread to electrodes G20, G28, G36–37, G45–46, and G11–16. Typical seizures were elicited by stimulation

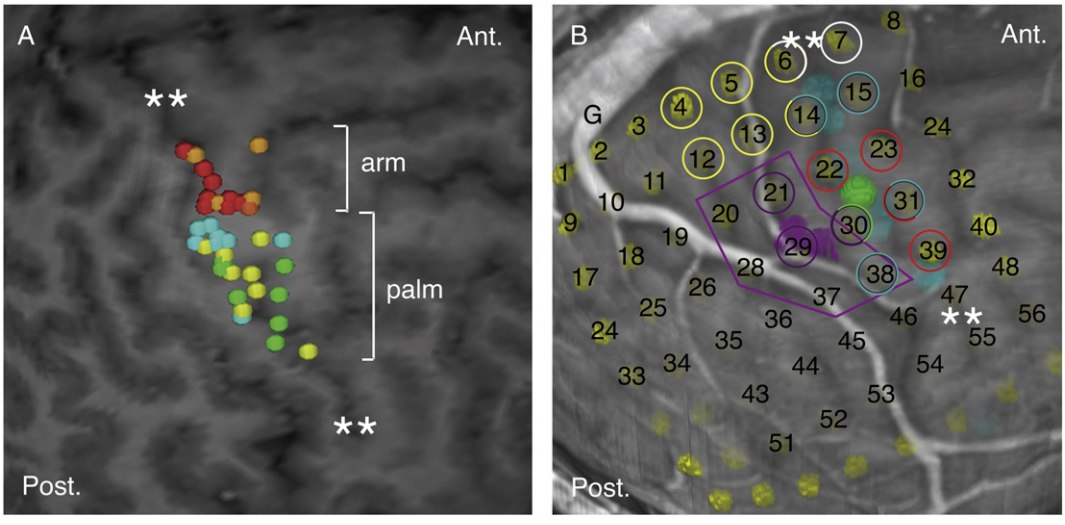

Fig. 1. The results for Patient 1. (A) nTMS results, the dots representing the stimulation sites. Red indicates MEPs from BB and EDC, orange only from BB, turquoise only from ADM, green from ADM and APB, and yellow only from APB [figure from eXimia NBS]. (B) A 3-D MRI reconstruction showing the results: MEG ictal and SEF ECD-source areas (purple = epileptic; green = left median nerve representation area) and nTMS representation areas (all areas marked with turquoise) are shown on top of the ECS-grid (yellow, partly numbered, center-to-center inter-electrode distance 10 mm). Responses to ECS are shown with colored circles. Sensory responses from the left hand are color coded with green (G30–G9) and from the left leg, shoulder and abdomen with yellow. Motor responses from the arm and hand are coded with turquoise and from abdomen and shoulder with white. Habitual seizures elicited by stimulation are coded with purple and non-habitual, elicited motor seizures with red. Circles with several colors represent several types of responses provoked by the ECS. The electrode locations included in the resection are encircled by a purple line. Double asterisks (**) in both figures mark the central sulcus at both ends of the stimulation area, Ant. indicates anterior and Post. posterior direction.

of electrodes G21, G29, G30 (ref G9) and untypical clonic seizures by stimulation of electrodes G22, G23, G31, and G39 (ref G9) with widespread after-discharges. However, based on the complementary preoperative results, a more limited cortical resection was carried out to preserve as much motor and sensory functionality as possible. The resection included the areas covered by electrodes G20–21, G28–29, G37–38, and the sulcal part of the cortex under the electrode G30, containing some sensory cortex but no motor cortex. Histological study of the resection revealed a focal microscopic cortical dysplasia type 2b (Taylor type), not detected preoperatively from the 1.5-T or 3-T MRI. Eighteen months after surgery the patient is seizure-free and has a mild sensory defect in the left hand, as expected.

Patient 2

For Patient 2, the cortical representations for arm, palm, leg, and foot were located by using nTMS (Fig. 2A). ECS of grid electrodes G12, G20, and G28 (ref G9) elicited motor responses from the arm and hand, coinciding accurately with the locations defined by nTMS. Stimulation of strip electrodes SA8, SA4 (ref G9), SA7, SA6, and SA5 (ref G24) elicited motor responses from the foot, with excellent correspondence to the preoperative nTMS localizations of the foot representations (Fig. 2C).

Seventeen habitual clinical seizures were captured during the subdural recording. The patient showed repetitive interictal polyspikes and ictal gamma activity in electrodes SA1–5 (maximum SA3), SP5, G5–7, G13–14 (Fig. 3), overlapping the MEG source cluster. Habitual seizures were provoked by ECS from SA3–G9/G24, SA4–G9 and G7–SP2. In the 3-D MRI reconstruction combining the non-invasive and invasive data (Figs. 2B and C), SEF sources matched closely with the ECS stimulations: median nerve SEF source with electrodes G20–G9 eliciting sensations from the right hand and G12–G9 from lower arm, and tibial nerve SEF source with electrodes G4–G9 and G5–G9/G13, eliciting sensory responses from the right leg and

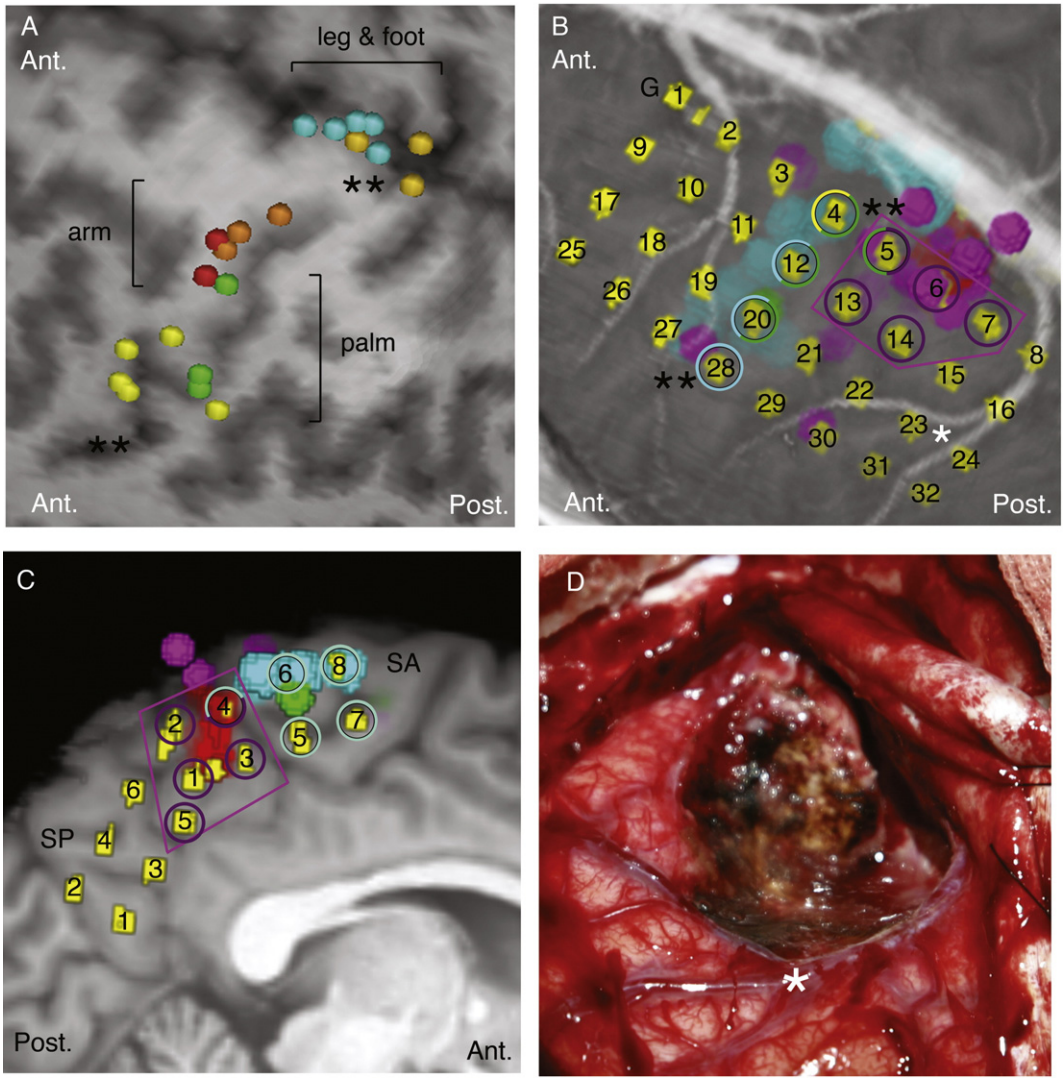

Fig. 2. The results for Patient 2. (A) nTMS results, the dots representing the stimulation sites. For hand and palm area, red indicates MEPs from BB and EDC, orange only from BB, green from ADM and APB, and yellow only from APB, responses from the leg area are represented with turquoise (RF muscle) and from foot area (TA and AH muscles) with light orange [figure from eXimia NBS]. (B and C) 3-D MRI reconstructions in lateral (B) and cross sectional view (C) MEG ictal and SEF ECD-source areas (purple = epileptic; green = right median and tibial nerve representation area) and nTMS representation areas (all areas marked with turquoise) are shown on top of the ECS-grid and strips (yellow, numbered). The lesion area is depicted in red. Responses to ECS are shown with colored circles. Sensory responses from the right hand and lower arm (G20–G9 and G12–G9) as well as from the right leg and foot (G4–G9 and G5–G9) are color coded with green in panel B. Sensory responses from the right shoulder are represented with yellow. Motor responses from the arm and hand are coded with turquoise circles in panel B and from leg and foot in panel C. Habitual seizures elicited by stimulation are coded with purple. Circles with several colors represent several types of responses provoked by the ECS. The electrode locations included in the resection are encircled by a purple line. Double asterisks (**) mark the central sulcus at both ends, also depicted in panel A. (D) A photograph after the resection showing the removed area anterior to the cortical vein, marked with a white asterisk, also in the (B). Ant. indicates anterior and Post. posterior direction.

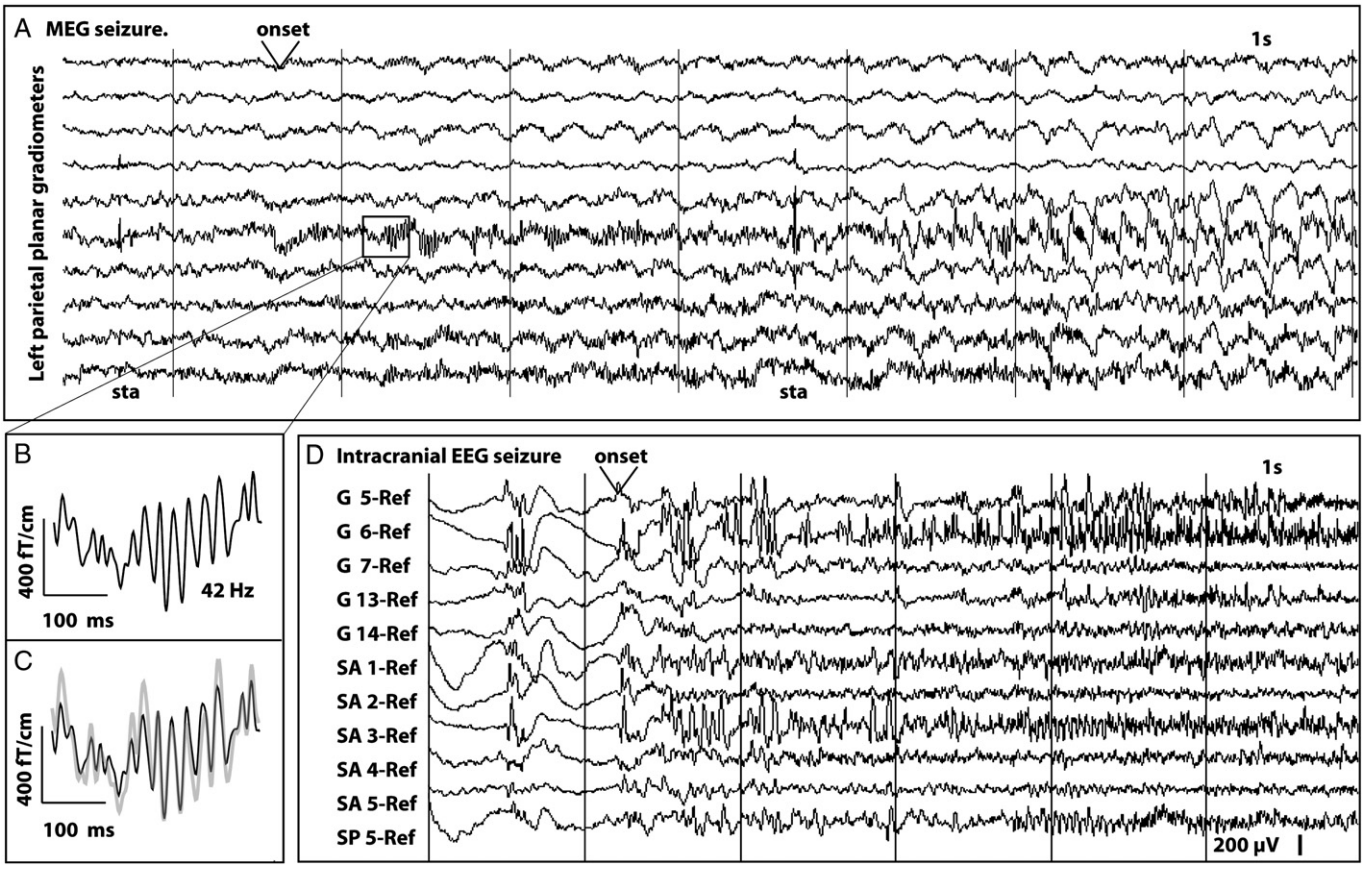

Fig. 3. (A) MEG-seizure onset and ictal onset beta and gamma activity. The most prominent oscillations were seen over the left medial fronto-parietal area. Artefacts from electric stimulation of the left tibial nerve are annotated with "sta". The electric stimulation was aborted shortly after seizure onset. (B) Enlarged MEG-signal. (C) Original MEG-signal (black), forward calculated signal from the ECD (grey). (D) Intracranial EEG-seizure onset in subdural grid and strip electrodes (for electrode locations see text and Fig. 2). Reference (Ref) in the subdural recording was a silver wire-electrode placed in the scalp.

foot. Both SEF source locations were within 1 cm distance of the corresponding ECS electrodes (Figs. 2B and C). The ECDs explaining the interictal MEG spikes and ictal onset 10–20 Hz MEG rhythms were clustered in the lesion area (Fig. 3).

A cortical resection including the cortical areas covered by electrodes G5–7, G13–14, SA1–4 and SP5 was carried out (Fig 2D). Histology revealed focal cortical dysplasia, type 2b (Taylor type). Mild right-sided motor dysfunction was observed immediately after surgery. Two months after surgery motor function was fully recovered, but mild sensory dysfunction was still present in the right leg. The patient remains seizure-free after 16 months, but has experienced a few sensory auras.

Discussion

The presented data illustrate the accuracy of preoperative functional mapping by nTMS and of MEG dipoles in epilepsy surgery. The noninvasively defined functional organization and the localization of the epileptogenic area were confirmed by ECS and postoperative seizure freedom. For these patients, the non-invasive localization of the sensorimotor cortex and of ictal discharging areas were useful in planning the placement of subdural electrodes and subsequent stimulation sequence. Complementary results provided by multimodal approach add reliability and may serve as a salient source of information in case of complications during the subdural recording or difficulties in interpreting ECS due to after-discharge production or inclination to seizure induction. Detailed knowledge of the sensorimotor functional locations as well as the locations and extent of the epileptogenic cortex is essential for informed decision making, even when lesional substrates can be demonstrated.

Single-pulse TMS is a safe technique, both for healthy subjects and neurological patients, as long as the general safety recommendations (Wassermann, 1998) are followed. Only a few reports exist on accidental seizures induced during TMS experiments in neurological patients (Schrader et al., 2004; Tassinari et al., 2003). The risk of seizure induction ranged from 0.4% in patients whose anti-epileptic medication (AEM) was not changed to 2.8% in those whose AEM was tapered before TMS. However, in most patients, the causal relation between the seizure and TMS remained questionable (Schrader et al., 2004). In contrast, there are no reports of seizures during TMS or immediately afterwards in well-controlled studies with epilepsy patients. Neither are there reported seizures related to single-pulse TMS in persons younger than 18 years (Quintana, 2005); the review included also patients with epilepsy. No epileptic seizures occurred during the presented TMS studies, whereas several seizures were elicited during ECS.

The reliability of TMS localization of the motor cortex has been evaluated by different methods. Correspondence between the projected locations of the center of gravity of averaged motor responses evoked by TMS and of the increased brain activity due to voluntary motor activation detected with positron emission tomography (PET) was good in four healthy volunteers (Wassermann et al., 1996). The match between motor responses evoked by TMS to the nodes of a 1×1 cm grid drawn on the scalp and peak activation elicited by hand clenching in functional MRI (fMRI) has been compared by using frameless stereotaxy in three healthy subjects and two tumor patients (Krings et al., 1997a). The sites of TMS eliciting compound muscle action potentials from first dorsal interosseus (FDI) and FCR muscles and the fMRI activation over the motor cortex were separated by less

than 1 cm. Comparison of motor cortex representations of individual hand and arm muscles obtained with 2-D TMS and intraoperative ECS in two tumor patients (Krings et al., 1997b) showed that the maximum of the compound muscle action potential for FDI was within 1 cm of the electrode locations producing movements of fingers 3–5 for the first patient and almost overlapped the electrode locations producing the index finger movements in the second one. In addition, fMRI results for voluntary thumb tapping has been validated with TMS representation of APB muscle in 10 patients with a lesion near the central sulcus (Krings et al., 2001). Separation between the activations was less than 1 cm in seven and less than 2 cm in three patients. The peak fMRI activation was located in cortical depth, whereas TMS sites were projected on the cortical surface. The difference may be due in part to different motor activations, as the fMRI task activated also other muscles than APB. In these studies (Krings et al., 1997a,b), the peak MEPs were elicited from sites overlaying the central region, including pre- and postcentral sulcus, not over the crown of the central sulcus as in our study, probably because TMS was delivered to predefined grid and anatomical knowledge was combined to these locations later on. With our method the MRI-based anatomical data is in view during the stimulation, enabling more precise selection of target sites. TMS had been advocated also for precentral gyrus localization during anaesthetic surgery by repetitive high frequency stimulation (Krings et al., 2001). On-line use of anatomical data will improve the accuracy of this approach as well. In our study, single-pulse TMS intensities close to the MT did not elicit epileptiform or ictal EEG activity, although the stimulated sites occasionally overlapped the MEG estimated localizations of the epileptogenic cortical region or lesion. If preoperative elicitation of ictal EEG changes is considered necessary, rapid-rate TMS rather than single pulse TMS may be a more adequate technique.

MEG is superior to EEG in locating interictal epileptic discharges, when a priori knowledge of the epileptogenic area is available, through e.g. ictal video EEG recordings (Shibasaki et al., 2007). The localizations of ECD sources of SEFs have earlier been shown to be in line with direct ECS stimulations (Mäkelä et al., 2001); in general, MEG localization of the S1 is in satisfactory concordance with intraoperative localization (for references see (Mäkelä et al., 2006)). A comparison between MEG, fMRI, and direct ECS displayed a close concordance between ECD sources of SEFs to median nerve stimulation and fMRI primary activation area for self-paced palmar flexion contralateral to the lesion, both coinciding with central sulcus as verified by ECS; MEG localized the central sulcus more reliably than fMRI (Korvenoja et al., 2006). Motor cortex can be localized directly by motor evoked fields in about 50–70% of patients (Lin et al., 2006). In addition, corticomuscular MEG–EMG coherence pinpoints motor cortex in about 70% of the patients (Mäkelä et al., 2001); with proper signal processing this percentage approaches 100% (Kim and Chung, 2007). However, neither MEG nor fMRI can reliably detect the extent of the motor representation. nTMS mapping provides this information and adds a new dimension to preoperative planning of surgery.

Application of 2-D TMS to map cortical motor hand areas has been previously described in case reports of two patients with epilepsy. In a patient with cortical dysplasia at the right central sulcus (Macdonell et al., 1999), the motor cortex mapping was successful on the healthy hemisphere whereas no detectable activity was elicited in the affected hemisphere by TMS or ECS. However, the patient's fMRI identified several cortical areas activated by motor task in both hemispheres. The removal of the dysplastic area caused transient dyspraxia. In another patient with epilepsy, the site of TMS eliciting APB activation was consistent with fMRI peak activation to voluntary hand clenching, as well as with the SEF source locations (Morioka et al., 1995). The co-registration of the TMS-localized APB representation and MRI was done with external markers, reducing the accuracy compared with our on-line navigation. We are not aware of studies combining information from TMS, MEG, and ECS for mapping of the functional motor cortex and epileptogenic areas in patients with epilepsy.

Comparison of pre- and intraoperative localizations is affected by methodological factors. For example, the grid implementation deforms the underlying cortical geometry. This may lead to some displacement of the grid in 3-D reconstruction images, as the rigid CT—MRI registration cannot account for the deformation. Therefore, some electrodes may be depicted below the cortex (Fig. 1B). High-quality post-implementation images revealing the structure of the cortex might allow quantification of the displacement error. Moreover, the brain shifts after dural opening. The main shift occurs in the direction of gravity, and is sensitive to the head posture during operation (Roberts et al., 1998). However, the relative orientations of the cortical veins and the underlying cortical volume do not change significantly. Depicting surface veins in combination with 3-D brain structures and functional landmarks, as we have done, provides visual guidance for intraoperative orientation (Mäkelä et al., 2001). This may alleviate problems in neuronavigation caused by brain tissue movement during surgery. In the future, detecting cortical surface during operation by e.g. laser range scanner, and using this data to warp the preoperative MRI (Liu and Song, 2008) may alleviate these problems further and add the usefulness of preoperative landmarks.

nTMS provides information on the extent of the motor representation. The need for detailed nTMS mapping is well founded when the epileptogenic focus is located near the sensorimotor cortex, where malformation might alter the anatomical organization of the motor representation. Such a detailed map is necessary, particularly when the functional and surgical margins overlap in the sensorimotor cortex. MEG recording is useful when the sources of interictal activity or of seizure onset area need to be located in 3-D more precisely than what is possible with normal scalp EEG. The combined use of nTMS and MEG recordings is useful when representation of the sensorimotor areas and epileptogenic onset zones are suspected to be close or overlapping. Our two patients were exceptional in having frequent daily seizures, which could be easily recorded during a 1 h MEG recording session. Using recent software developments (Uutela et al., 2001; Taulu and Simola, 2006), head movement and movement-related artifacts can be efficiently removed within reasonable spatial and temporal limits, enabling long ictal MEG recordings. In our experience 25 out of 37 patients referred for ictal MEG have successful seizure recording within 1–40 h (mean 7.8 h, median 6.3 h).

In our study, nTMS produced spatially more precise mapping of the motor cortical representations than ECS, having spatial separation limited by 1-cm inter-electrode distance. The nTMS representation areas were in line with the ECD sources of SEFs, adding reliability to the preoperative localization of the primary motor and somatosensory cortices. Based on the two patient cases these methods seem to provide valuable complementary information on sensorimotor localizations and epileptogenic cortical areas. Future studies with larger patient populations will show if the combination of nTMS and MEG could replace subdural grid recordings in patients whose seizures originate from the vicinity of the sensorimotor areas and are frequent enough to allow ictal MEG recordings.

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