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Spatiotemporal dynamics of brain activation in multiple sclerosis patients and healthy control subjects: A TMS-EEG study

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"Let knowledge grow from more to more and thus be human life enriched."

(Introductory phrase to the Encyclopedia Britannica)

I

1. INTRODUCTION

1.1 Overview and background

Single pulse transcranial magnetic stimulation (TMS) applied on human motor cortex (M1) evokes a time-locked pattern in electroencephalography (EEG) (see figure 1.1). The following study investigates spatiotemporal dynamics of brain activation in patients with multiple sclerosis (MS) and healthy control subjects with TMS-EEG. MS is a demyelinating inflammatory and degenerative disease of the central nervous system. The corpus callosum (CC) is affected by demyelination and axonal loss at early disease stages in MS demonstrated by postmortem analysis, magnetic resonance imaging (MRI) and diffusion-tensor magnetic resonance imaging (DTI) (Wahl, 2011; Bester, 2008; Dietemann, 1988; Evangelou, 2000). While new disease-modifying drugs (DMDs) improve the disease course, there is no finite cure available. For this reason reliable biomarkers for therapy monitoring are highly requested (Ziemann, 2011; Rudick, 2012).

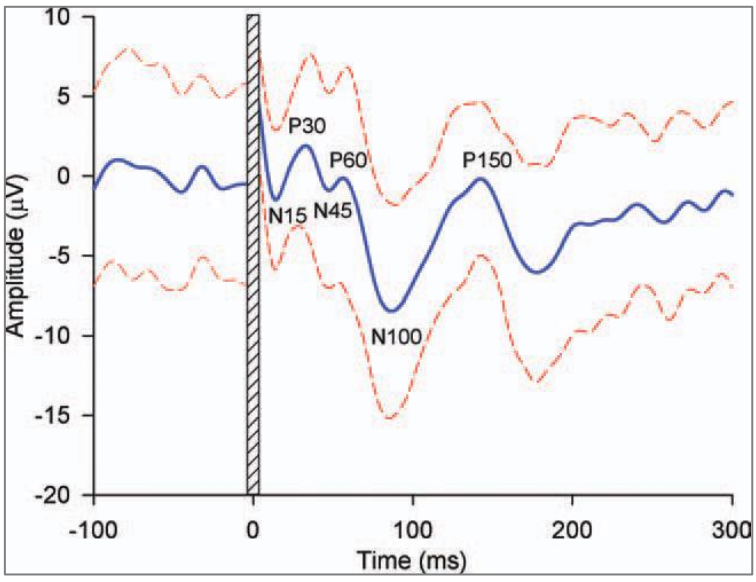


Figure 1.1 M1 TEP response (stimulus application at grey bar = 0 ms; Thick line = mean EEG trace; dashed lines = standard deviation.) [RE-USE PERMISSION by Wiley Periodicals Inc. of Copyright © 2012, (Rogasch & Fitzgerald, 2012)]

We assume that MS may serve as a model disease to test abnormalities of callosal conduction; there is evidence that TMS-evoked EEG responses (TEPs) might propagate from the stimulated to non-stimulated hemisphere via the corpus callosum (Voineskos, 2010). However this has not been tested in MS with CC lesions.

We aim to explore the spatiotemporal dynamics of TEPs to learn more about alterations of information propagation in neural networks modified by multiple sclerosis. By introducing TEP analysis in MS we intend to investigate its potential as a biomarker.

We hypothesize that subclinical callosal lesions might be detected by TEP analysis and herewith provide useful information on cortico-cortical connectivity.

1.2 Transcranial magnetic stimulation (TMS)

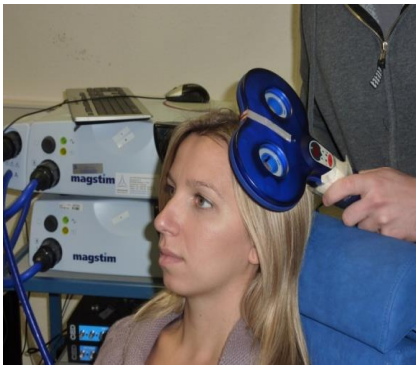


Figure 1.2 TMS stand-alone
(Photography property of the author)

TMS has been introduced by Barker et al. in 1985 and provides a non-invasive, painless and focal method to stimulate the human cortex (Barker, 1985). TMS is therefore a milestone development in cortical stimulation. The magnetic field applied by TMS appears focal when applied by a tangentially oriented figure-eight coil (see figure 1.2) (Mills, 1992).

TMS is inducing an electrical field in the cortex (Ziemann & Siebner, 2007). Depending on the stimulus intensity areas around 1-6 cm below the scalp are reached, and when the motor cortex is stimulated, can be localized by means of electromyography (EMG) and recently by MRI-navigated TMS (Fernandez, 2002; Neggers, 2004; Ziemann & Siebner, 2007).

TMS allows measurement of cortical conduction deficits along the corticospinal tract in MS. It has been demonstrated that motor-evoked potentials (MEPs) in MS are abnormal. Decreased conduction velocity is presumed to reflect spatially disseminated demyelination.

Diminished MEP amplitudes likely represent axonal loss or chronodispersion due to inhomogenous conduction slowing in fibers of the corticospinal tract (Hess, 1987; Hess, 1986).

Conduction velocity is expressed by central motor conduction time (CMCT). CMCT and MEP measurements in MS were confirmed to be of high clinical utility within a report of an IFCN (International Federation of Clinical Neurophysiology) committee (Chen, 2008). This multimodal evoked potential approach allows detection of subclinical lesions, and discrimination of different disease courses of MS.

TMS represents a milestone in the evolution of brain stimulation. In the late 18th century, accompanying the age of enlightenment and dawn of electricity, many scientists started to investigate the significance of electrical currents in human organisms (Wagner 2007). In remembrance of these ancestors we present two quotes:

German multi-genius Alexander von Humboldt (1769-1859), inspired by Luigi Galvani (1737-1798) in Bologna, contributed his treatise 'Über die gereizte Muskel- und Nervenfaser' ('On the stimulated muscle and nerval tissue') Posen, 1797. He clearly concluded in his numerous experiments that human tissue has proper electrical activity:

‚Ich fange von der Erscheinung des Galvanismus an, weil ich durch die Art, wie ich diese Versuche anstellte, unwidersprechlich erweisen zu können glaube, dass der Stimulus in diesem wunderbaren Phänomen größtentheils von den belebten Organen selbst ausgeht und das diese sich dabei keineswegs bloß leidend, …, verhalten.'
- (A. von Humboldt, Über die gereizte Muskel- und Nervenfaser, Band 1, p. 2, 1797)

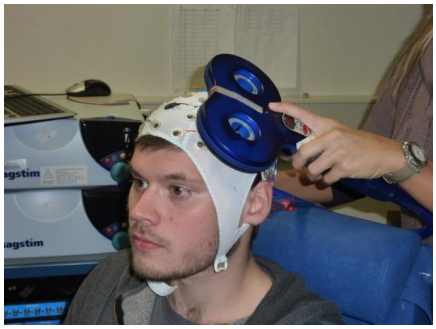
'I start with the epiphenomena of Galvanism, because I found proof, through these specific studies, that this wonderful phenomenon mostly evolves in the living organisms and does not result from external stimuli.' (Translation by the author)

Eduard Hitzig (1838-1907) and Gustav Theodor Fritsch (1838-1927) have finally demonstrated the electrical excitability of the cortex in the late 19th century (Fritsch & Hitzig, 2009). They have furthermore made efforts in linking brain areas to cortical functions:

'Der motorische Theil liegt, allgemein ausgedrückt, mehr nach vorn, der nicht motorische liegt nach hinten. - Durch elektrische Reizung des motorischen Theiles erhält man combinierte Muskelcontractionen der gegenüberliegenden Körperhälfte.' (G.T. Fritsch & E. Hitzig, Über die elektrische Erregbarkeit des Grosshirns, page 311, 1870)

'Generally speaking, the motor area is to be localized more frontal, the non-motor areas more backwards. - Through electrical stimulation of the motor part combined muscle contractions of the contralateral side are evoked.' (Translation by the author)

1.3 TMS-EEG



TMS at the interface with other techniques, such as EEG, has the potential to enhance our knowledge on network properties (Ziemann, 2011; Rogasch & Fitzgerald, 2012). TEP analysis allows assessment of functional interhemispheric and intracortical networks (Ilmoniemi, 1997).

Figure 1.3 TMS-EEG (Photography property of the author)

TMS-EEG is a novel multimodal approach introduced by Cracco and colleagues in 1989 (Cracco, 1989). In this earlier work simultaneous recording of EEG during magnetic stimulation was complicated by the physical (coil-) induced artifact in the EEG traces. Recent advances such as novel EEG amplifiers (sample-and-hold, 16-bit analogue digital converter) and TMS-suitable EEG caps as well as advances in EEG analysis (Ilmoniemi & Kicic, 2010; Sekiguchi, 2011) have largely overcome this problem. Nevertheless, it still is a challenge to obtain an artifact-free EEG recording following TMS.

TMS-EEG is now an approach used by several research groups to analyze network properties in different states of consciousness (Rosanova, 2009; Massimini, 2005; Ferrarelli, 2009). It is employed to investigate underlying patho-physiological mechanisms in neuropsychiatric disorders, such as schizophrenia, attention deficit hyperactivity disorder (ADHD), traumatic brain injury and epilepsy (Ferrarelli, 2008; Bruckmann, 2012; Farzan, 2010; Tallus, 2013; Valentin, 2008).

TEP latencies are composed of a sequence of positive and negative deflections (see figure 1.1). In our experiment the two peaks, in the range around 60 ms and 100 ms (labeled P60 and N100) were investigated. It has been demonstrated that all components, particularly P60 and N100 are well reproducible within subjects and across trials (Komssi, 2004; Bonato, 2006; Lioumis, 2009).

There is no direct evidence about the exact origin, function and trans-cortical pathways of TEP components. Up to date the N100 is the most extensively investigated TEP latency. It is believed to represent inhibitory mechanisms, as the N100 decreases during movement execution and motor preparation, that require an increase of excitability, and it increases during situations where cortical inhibition increases (Nikulin, 2003; Kähkonen and Wilenius, 2007; Bonnard, 2009). This possible mechanism is strongly supported by a study which has shown a decrease of the N100 amplitude by application of alcohol to healthy subjects. Alcohol acts as positive modulator at GABA-A receptors and hereby constrains glutamatergic neurotransmission through NMDA receptors, so it bears an inhibitory effect. The diminution of the N100 by alcohol provides evidence that it plays a role in cortical inhibitory circuits. There is also a large body of evidence supporting a relationship between the N100 and GABA-B receptor mediated inhibitory neurotransmission (Rogasch & Fitzgerald, 2012). Furthermore, Daskalakis and colleagues combined TMS and EEG to demonstrate that the suppressed mean cortical evoked activity on EEG elicited by paired-pulse TMS reflects inhibition as tested by a paired-pulse TMS-EMG protocol (LICI, long-interval cortical inhibition) (Daskalakis, 2008). According to Bender et al., the N100 could be interpreted as a “wave” response to an externally generated spike. It might therefore provide an in vivo model to assess thalamocortical inhibitory processes in human subjects (Bender, 2005).

Lately the frequency of updated reviews on TMS-EEG applications reflects its highly dynamic development and significance as a useful tool for providing in-depth information on brain properties (Rogasch & Fitzgerald, 2012; Daskalakis, 2012; Taylor & Thut, 2012).

1.4 Electroencephalography (EEG)

Originally, human EEG was introduced by the German neurologist Hans Berger in the 1920s (see figure 1.4). His work was preceded by Richard Caton who was able to record electrical activity in exposed animal brains in the 1870s (Collura,1993). EEG allows recording of electric activity of the brain with high resolution in time (real time) but low spatial resolution. This drawback goes back to the so called „inverse problem“, which means that signals recorded from single electrodes cannot be attributed to specific brain areas.

Figure 1.4 Birth of human EEG: Dr. Berger, ~1924
[PHOTO COURTESY of the California State University]

The physiological correlates for amplitudes in EEG-recording are neuronal dipoles (Wellach, 2011). On the cellular level a single neuron has a stable membrane potential until there are more excitatory than inhibitory potentials inducing a discharge. Basically neurons are firing all the time. The discharge of a single cortical neuron is too weak to be registered in surface EEG so the recorded potentials result from a mass of firing neurons (compound-potential). In other words, EEG records the spatial and temporal summation of post-synaptic potentials which can be either excitatory or inhibitory. The more neurons are firing at the same time, the stronger the field becomes, resulting in higher amplitudes. Secondly the more synchronous neurons are firing as an ensemble, the steeper the potentials become. Thirdly, with the sources lying deeper in the cortex, the signals get weaker. In practical regards, EEG is a basic powerful clinical diagnostic tool in epilepsy, sleep-disorders and intra-operative monitoring among others. Facing the advancement in EEG data analysis procedures and the trend towards fusion methods, it is also an indispensable scientific tool.

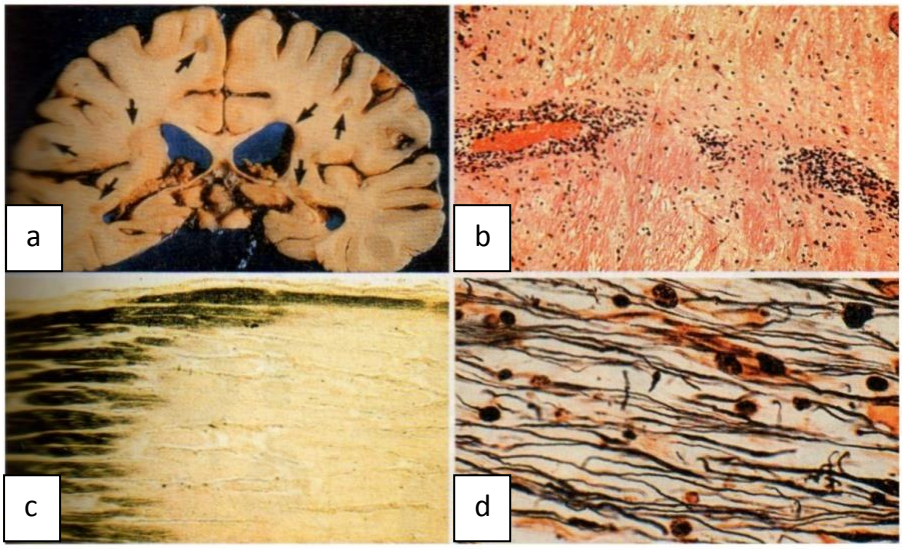


Figure 1.5 MS-pathology (from left to right and top to bottom):

a) Macroscopic MS-lesions in anatomical sample, indicated by arrows
b) Histological proof of lymphoid infiltrates (HE stain)
c) Pathological (white) and intact (blue-green) myelin tissue (Luxol fast blue stain)
d) Cellular swelling indicating axonal degeneration (silver stain)
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1.5 Multiple Sclerosis (MS)

In this study we have investigated functional properties in patients with early-stage relapsing-remitting multiple sclerosis (RRMS). Initially described by Jean-Martin Charcot in 1869, as „Sclérose en Plaques“ (Charcot, 1869), the exact patho-physiological mechanism and etiology still remain unclear. Latest data documented that 149 in 100,000 people in Germany are affected, which leads to 122.000 patients in total (Hein, 2000). Onset age is typically between twenty and forty years, most often with trouble of vision (optic neuritis) and/or sensory-motor impairment. Women are at least twice as often affected as men (Wiendl & Kieseier, 2010). MS still is a rare disease but a major topic in clinical neurology. There is no cure available for the patients. The etiology of MS still is controversially debated, varying from autoimmune and viral origin to genetic factors and geographically determined environmental factors and socioeconomic theories (Pugliatti, 2006; Koch-Henriksen & Sorensen, 2011; Gilden, 2005; DeLorenze, 2006; Ascherio & Munger, 2007; Tzartos, 2012).

It is agreed that an autoimmune component plays a decisive role in the development of MS. Complex interactions in the immune-system with activated T-cells crossing the blood-brain barrier and initiating an inflammatory cascade, which leads to demyelination and axonal degeneration are discussed (Hohlfeld & Wekerle, 2001; Sospedra & Martin, 2005).

RRMS is characterized by its heterogeneous clinical course, usually marked by acute-attacks with varying symptoms and periods of remission. In the course of the disease, symptoms tend to persist because of axonal and neuronal injury. These injuries account for permanent disability (Lassmann, 2010). For this reason, current guidelines emphasize the importance of early diagnosis and treatment. MRI is an essential tool in the early diagnosis of MS (McDonald, 2001; Polman, 2011; Dalton, 2002) but it is not sensitive enough to detect micro-structural changes during the disease course (Ziemann, 2011). Acute attacks are treated by the immunosuppressive properties of intravenous high-dose corticosteroids (Gold, 2001). Immunomodulating drugs, also termed disease-modifying drugs (DMDs), have revolutionized MS-therapy in the last two decades, intending to halt disease progress and reduce frequency of acute attacks. For instance, at the time of our study, the European Medicines Agency (EMA) approved dimethyl fumarate, a long-standing pharmacological agent for the treatment of psoriasis, for RRMS treatment. Notably administered orally, in placebo-controlled phase 3 studies and in comparison to one of the current gold-standard DMDs, glatiramer acetate, dimethyl fumarate has been shown to be efficacious within the CONFIRM and DEFINE trial (Gold, 2012; Fox, 2012; Ropper, 2012). Main criteria for therapeutic success were reduction of acute attacks and the safety profile. There is no data on the long-term outcome available yet, which underlines the need for therapy-monitoring biomarkers.

1.6 Diffusion-tensor imaging (DTI)

In addition to functional investigation of the corpus callosum with TMS-EEG we have obtained structural diffusion-tensor imaging (DTI-) data, which could not be used for analysis because of a technical problem – a homogeneous artifact in CC-segments 1 and 2 occurred across all trials. Section 9 of this thesis will provide a detailed overview of methods and artifact analysis.

1.7 General aims and hypotheses

In summary, we investigated functional properties of the corpus callosum in early MS-patients by evaluating the N100 and P60 TEP components. We expected a delayed interhemispheric propagation of the N100, as prior studies demonstrated diminished structural integrity of the corpus callosum at an early disease stage (Bammer, 2000; Wahl, 2011; Dietemann, 1988; Evangelou, 2000). Given that the N100 in the non-stimulated hemisphere is an easy-to obtain, and well reproducible measure of transcallosal conduction, we aimed to explore its potential to detect alterations in interhemispheric connectivity. We hypothesized that the interhemispheric propagation of the N100 would be significantly delayed in MS patients compared to healthy controls.

2. MATERIAL AND METHODS

2.1 Participants

Sixteen healthy volunteers (mean age, 30 ± 8 years, range 23 to 49 years, 8 females) and twelve age- and gender matched early RRMS patients (mean age 36 ± 9 years, range 22 to 48 years, 8 females) participated in the study after having given their written informed consent (see appendix); (for an overview see table 2.1). The RRMS patients were diagnosed according to the revised McDonald criteria (Polman, 2011). Only patients with a score lower than 3 on the expanded disability status scale (EDSS) (Kurtzke, 1983) were included in the study. Median EDSS was 1.0 (range 0 to 2.5) and median disease duration was 16 months (range 4 to 144). All of the subjects were right-handed according to the Edinburgh Handedness Inventory (laterality score ≥ 75%) (Oldfield, 1971), as varying network properties from left-handers are reported (De Gennaro, 2004). None of the participants had contra-indication to TMS, or any neurological, psychiatric or relevant medical problems apart from MS (Groppa, 2012; Rossi, 2011). Further exclusion criteria were recent intake of CNS active drugs and pregnancy. The study protocol conformed to the latest revision of the Declaration of Helsinki and was approved by the Ethics Committee of the Medical Faculty of the Goethe-University of Frankfurt/Main (Geschäftsnummer: 21/12).

Group	Age	Gender	EDSS	MDD*
Control subjects [N=16]	**30±8 (23-49)**	**8F, 8M**		
RRMS-patients [N=12]	**36±9 (22-48)**	**8F, 4M**	**1±1 (0-2.5)**	**16 (4-144)**

Table 2.1 Demographic and clinical measures of control subjects and patients with relapsing-remitting multiple sclerosis: Mean ± SD; *median disease duration in months

2.2 TMS-EEG

2.2.1 Experimental Setup

Participants were seated on a comfortable reclining chair, with eyes open. TMS pulses were delivered using a Magstim-200 stimulator connected to a figure-of-eight coil with external loop diameters of 90 mm through a BiStim module (all devices from Magstim Co, Whitland, Dyfed, UK). The coil was held tangentially to the skull with the handle pointing backward and laterally at a 45° angle to the sagittal plane. EMG signal was recorded through D360 amplifier (Digitimer Ltd.,UK), digitalized via CED 1401 (CED Products, UK) and displayed online. MEPs were recorded from the abductor pollicis brevis muscle (APB) on the corresponding contralateral side to the M1 stimulation with Ag/AgCl surface cup electrodes in a belly-tendon montage. TMS-compatible EEG equipment (BrainAmp DC and BrainCap Fast'n'Easy, BrainProducts GmbH, Munich, Germany) allowed EEG online recording with a coil-induced artifact lasting not longer than 15ms. The EEG was acquired from 64-channels, labeled according to the extended international 10-20 system. In addition, two sensors registered vertical and horizontal eye movements.

The scalp of each participant was prepared by cleaning with alcohol, specific abrasive gel (Nuprep, Aurora, USA) and EEG-suitable electrode cream (GE GmbH, Freiburg, Germany) to keep optimal skin/electrode impedance below 5kOhm. Throughout the trial impedances were checked regularly. During measurement participants wore earphones with masking sound to avoid possible contaminations in the EEG recordings related to the coil click (Nikouline, 1999). Participants were required to stay awake and to focus on a fixation cross.

2.2.2 Data acquisition

Magnetic stimulator and EEG amplifier were linked to allow online recording and offline analysis. The TMS-EEG setup was established taking into account recent technical guidelines (Ilmoniemi, 2010). The EEG-cap was TMS-suitable due to flat Ag/AgCl pellet pin electrodes. In order to minimize the TMS induced artifact lead wires were rearranged radially away from the fixed coil position (Sekiguchi, 2011).

Single pulse magnetic stimulation on resting motor threshold (RMT) intensity was performed on the left and right motor cortex, in randomized order, in all participants. The RMT, defined as the minimum intensity able to elicit reproducible MEPs of at least 50 μV in peak-to-peak amplitude in the resting APB muscle in at least 5 out of 10 consecutive stimuli (Rossini, 1994), was identified separately on each hemisphere. 150 stimuli were applied, randomly starting either on right or left M1, with an inter-trial interval (ITI) of on average 5 seconds and inter-trial variability (ITI) of 25% (Fig. 2.1). EEG signals were recorded with at a sampling rate of 5000 Hz without preset filters. The EMG raw signal was amplified and low- / high-band filtered (0.02- 20 kHz).

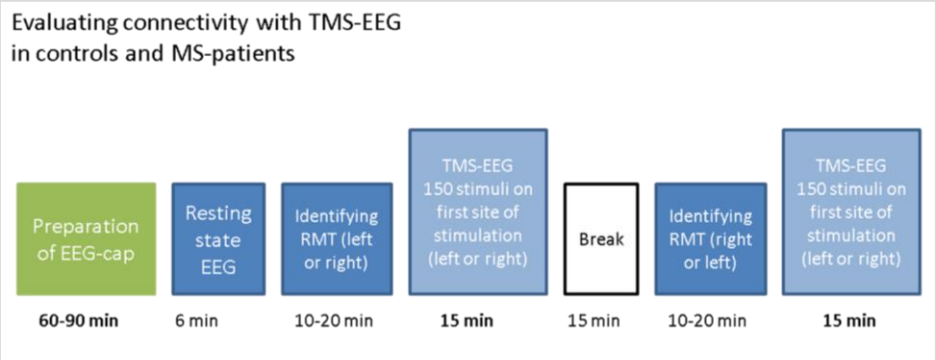


Figure 2.1 TMS-EEG experimental design

2.3 Data analysis

2.3.1 Preprocessing of EEG data

The EEG raw data were first analyzed using BrainVision Analyzer 2.0 (BrainProducts GmbH, Munich, Germany). Data were re-referenced to the linked mastoids (TP9/TP10) and downsampled to 1000 Hz. TMS trials containing large artifacts, eye movements, or muscle activation were visually detected and discarded from further analysis. In the healthy controls, on average 18 ± 11 stimuli in the left-hemisphere stimulation condition and 14 ± 10 in the right-hemispheric stimulation condition out of 150 stimuli were removed prior to further analysis because of artifacts described above. In the RRMS patients, an average of 19 ± 10 stimuli with left-hemispheric stimulation and 19 ± 11 with right-hemispheric stimulation were removed (Table 2.2). The surviving trials underwent linear interpolation from -10 to 10 ms (see paragraph 8), and were exported to Matlab 2008b version 7.7 (MathWorks, Natick, Mass), a technical computing language interface.

Site of stimulation / Group	Left-hemispheric stimulation	Right-hemispheric stimulation
Control subjects	**18 ± 11**	**14 ± 10**
RRMS-patients	**19 ± 10**	**19 ± 11**

Table 2.2 Excluded trials (out of 150) because of artifacts in left- and right-hemispheric stimulation in control subjects and RRMS-patients reported in mean ± standard error of the mean (SEM)

Analysis of TMS-evoked potentials was performed using the open code Matlab toolbox Fieldtrip (version 2012, www.ru.nl/fcdonders/fieldtrip/) and own scripts developed in the group. Ms. Nazareth Castellanos made essential contributions and supervised the process of programming. TMS-EEG trials were defined from continuously recorded EEG time series from -500 ms to 500 ms considering the TMS pulse as 0 ms. Every trial was detrended and band pass filtered between 1 and 45 Hz.

A notch filter was applied to reduce the line noise contamination. Artifact-free EEG trials were then baseline corrected by subtracting the mean amplitude during an interval between 500 and 100 ms before the TMS onset. To avoid technical problems with the artifact produced by the TMS onset we did not consider the signal during and interval between -12 ms to 12 ms, with respect to the TMS stimulus. In order to keep the temporal information of the trials we replaced the time-window by a non-numerical number (a Matlab-based strategy to obviate the analysis in this interval, allowing continuity of the data). Analysis of event-related potentials (ERPs) was conducted on two different regions of interest (ROIs) comprised of 10 channels including and surrounding the area of stimulation for the left and right hemisphere, respectively (see figure 2.2) (Lioumis, 2009; Kähkonen & Wilenius, 2007). ERP was calculated by means of a robust mean of the trials for every ROI. In order to smoothen the ERP, data were filtered between 1 and 45 Hz. Amplitude and latency of early TEPs were evaluated in each time window of interest.

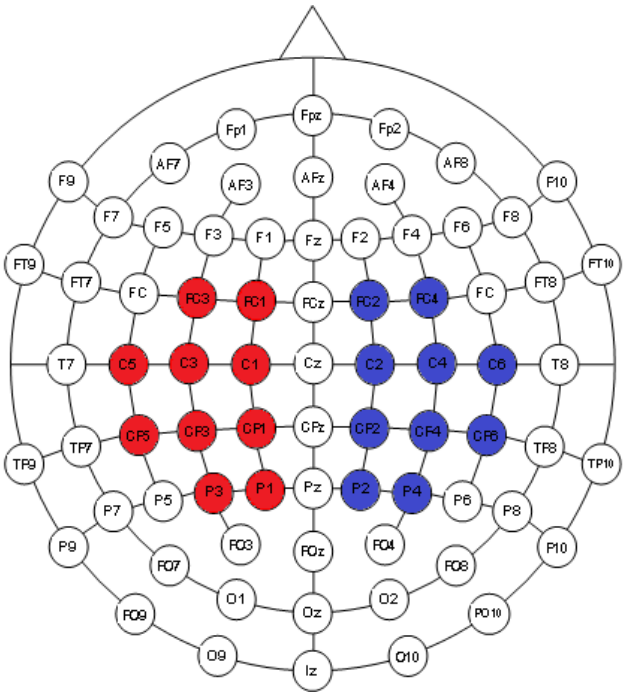


Figure 2.2 Regions of interest (ROI) for event-related potential analysis. The red channels correspond to the LEFT-ROI while the blue channels correspond to the RIGHT-ROI. Latencies were evaluated on the signal averaged over the two different ROIs.

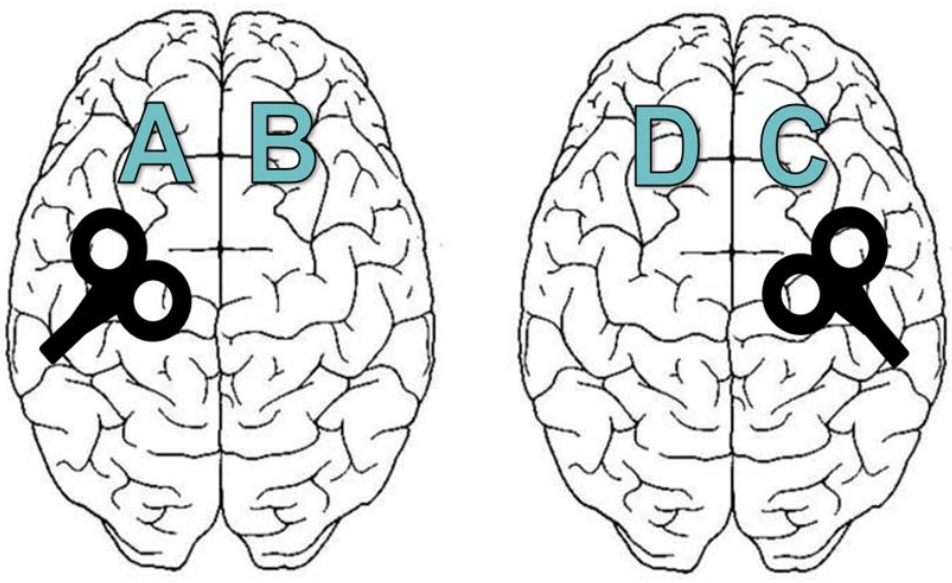


Figure 2.3 Scheme for evaluating event-related potentials in both trial conditions; L (left) and R (right) M1-stimulation. Trial conditions are described as following:

A = Left-hemispheric stimulation, left-hemispheric ROI
B = Left-hemispheric stimulation, right-hemispheric ROI
C = Right-hemispheric stimulation, right-hemispheric ROI
D = Right-hemispheric stimulation, left-hemispheric ROI

2.3.2 Latency evaluation

The N100 latency was the time of the maximal negative amplitude (μV) in the time window between 80 and 120ms (Ferreri, 2011; Lioumis, 2009; Bonato, 2006). The P60 latency was the maximum positive amplitude (μV) in the time window between 45 and 80 ms (Ferreri, 2011; Lioumis, 2009; Bonato, 2006). Latencies were detected in Matlab using the data cursor tool and transferred into SPSS (IBM Corp. Released 2010. IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp.) for statistical analysis. The parameters for each trial were left-hemispheric stimulation with left- (A) and right-hemispheric ROI (B); respectively right-hemispheric stimulation with right- (C) and left-hemispheric ROI (D) - (see Figure 2.3).

The P60 latency was detected in 97% of all trial conditions in controls, and in 94% in the MS-group. The N100 latency was detected in 100% of all trial conditions in controls, and in 98% of the MS-group.

2.3.3 Statistical analysis

Statistics were done using SPSS (v.19). For the P60 and N100 latencies analysis we adopted two independent repeated measures ANOVAs (one for the right-hemispheric and one for the left-hemispheric stimulation) with group (controls vs. MS) as between-subject factor and detection side (left vs. right) as within-subject factor. The peak latencies of the P60 and N100 in the stimulated hemisphere were subtracted from those of the non-stimulated hemisphere and healthy controls and RRMS patients were compared by an independent measures t-test.

2.3.4 Amplitude evaluation

TEPs were calculated by a robust mean of the trials for every electrode channel. In order to smoothen the TEP, we used data filtered between 1 and 45 Hz. We considered two TEP components that appeared to be the most reproducible: P60 (45 – 80 ms), and the N100 (80-120 ms). These two peaks have been previously reported in the literature (Rogasch & Fitzgerald, 2012). The amplitude of each TEP component was evaluated for left- and right-hemispheric stimulation in controls and MS patients in the time windows of interest. For amplitude analysis we chose a time window of interest based upon visual inspection of both MS- and control group. To avoid bias by high inter-subject variability, we also employed an analysis in which we compared each time point in a chosen time-window in both groups.

Additionally we created topographical plots using the Matlab toolbox Fieldtrip (version 2012, www.ru.nl/fcdonders/fieldtrip/). Because we did not state any a-priori assumption about the involved brain area we adopted a spatial cluster-based permutation test, a type of analysis which is considering every channel and also avoiding the multiple comparison problem (Maris & Oosterveld, 2007). A paired t-test was conducted at each electrode, at each time point of the 2 different time windows of interest, and t-values exceeding a threshold of $p < 0.05$, were clustered based on adjacent time bins and neighboring electrodes. Cluster-based corrected p-values were produced by randomly permuting the assignment of individual subjects' values 1500 times, and counting the number permutations in which larger clusters appear (defined by the total t-values of all time points).

3. RESULTS

3.1 Resting motor threshold

There was no statistically significant difference in RMT ± SEM [%maximum stimulus output (MSO)] between groups with right-hemispheric stimulation (Controls: 52.12 ± 7.69; MS: 55.84 ± 7.74; p = .20) and a non-significant trend with left-hemispheric stimulation (Controls: 50.88 ± 5.67; MS: 55.61 ± 7.28; p = .055) (see Table 3.1).

Site of stimulation / Group	Left-hemispheric stimulation [%MSO]	Right-hemispheric stimulation [%MSO]
Control subjects	**51 ± 6**	**52 ± 8**
RRMS-patients	**56 ± 7**	**56 ± 8**

Table 3.1 Resting motor threshold (range: 0- 100% Maximum stimulator output = %MSO) in left- and right-hemispheric stimulation in control subjects and RRMS-patients reported in mean ± standard error of the mean (SEM); %MSO = %Maximum stimulator output

3.2 P60 latency

3.2.1) The P60 latency analysis of the *left-hemispheric* TMS (L-P60) showed a main effect of side ($F(1, 26) = 24.40$, $p < .001$), with the P60 peaking earlier in the stimulated left (59.9 ms ± 2.1) than in the non-stimulated right (73.7 ms ± 2.2) hemisphere. There were no statistically significant main effects of group ($F(1, 26) = 0.17$, $p = .68$) and side by group interaction ($F(1, 26) = 0.95$, $p = .34$) (see tables 3.2 and 3.3).

3.2.2) The P60 latency analysis of the *right-hemispheric* TMS (R-P60) showed a main effect of side ($F(1, 26) = 9.43$, $p = .005$), with the P60 peaking earlier in the stimulated right (61.4 ms ± 2.3) than in the non-stimulated left (69.1 ms ± 2.5) hemisphere. There were no statistically significant main effect of group ($F(1, 26) = 0.05$, $p = .83$) and side by group interaction ($F(1, 26) = 0.003$, $p = .96$) (see tables 3.2 and 3.3).

Grand average (average of all electrodes in selected ROI) for demonstration of interhemispheric propagation - for P60 and N100 - is presented for the control group in figures 3.2 a, b and for the MS group in figures 3.3 a, b. The whole (P60-) range of topographical plots is presented in this section (figures 3.4 - 3.7).

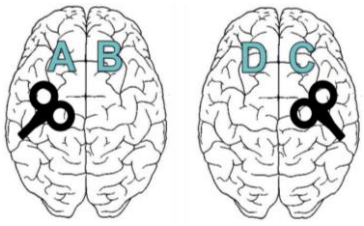


Figure 3.1 Scheme for evaluating ERPs in both trial conditions; L (left) and R (right) M1-stimulation

Group/Latency	P60 (A)	P60 (B)	P60 (C)	P60 (D)
Control subjects	62 ± 12 (42-90)	73 ± 11 (45-84)	61 ± 11 (51-83)	69 ± 11 (51-87)
RRMS-patients	58 ± 8 (47-76)	74 ± 13 (59-93)	62 ± 13 (51-88)	70 ± 15 (48-96)

Table 3.2 Mean P60 latencies ± SEM and range in brackets, reported in ms according to the scheme in figure 3.1; in left- and right hemispheric stimulation between control subjects and RRMS-patients there was no significant side- by group interaction (p-values for L-P60: p=.34 and for R-P60: p=.96) and no significant group effect (p-values for L-P60: p=.68 and for R-P60: p=.83). There was a main effect of detection-site between groups, indicating that P60 was peaking earlier in the stimulated hemisphere during left- (p=<.001) and also during right-hemispheric stimulation (p=.005); level of significance = <0.05

Group/Latency	P60 ([B-A])	P60 ([D-C])
Control subjects	**11 ± 12**	**8 ± 11**
RRMS-patients	**16 ± 11**	**8 ± 14**

Table 3.3 Mean P60 interhemispheric propagation time reported in ms ± SEM; the values of every individual subject were used to determine group differences

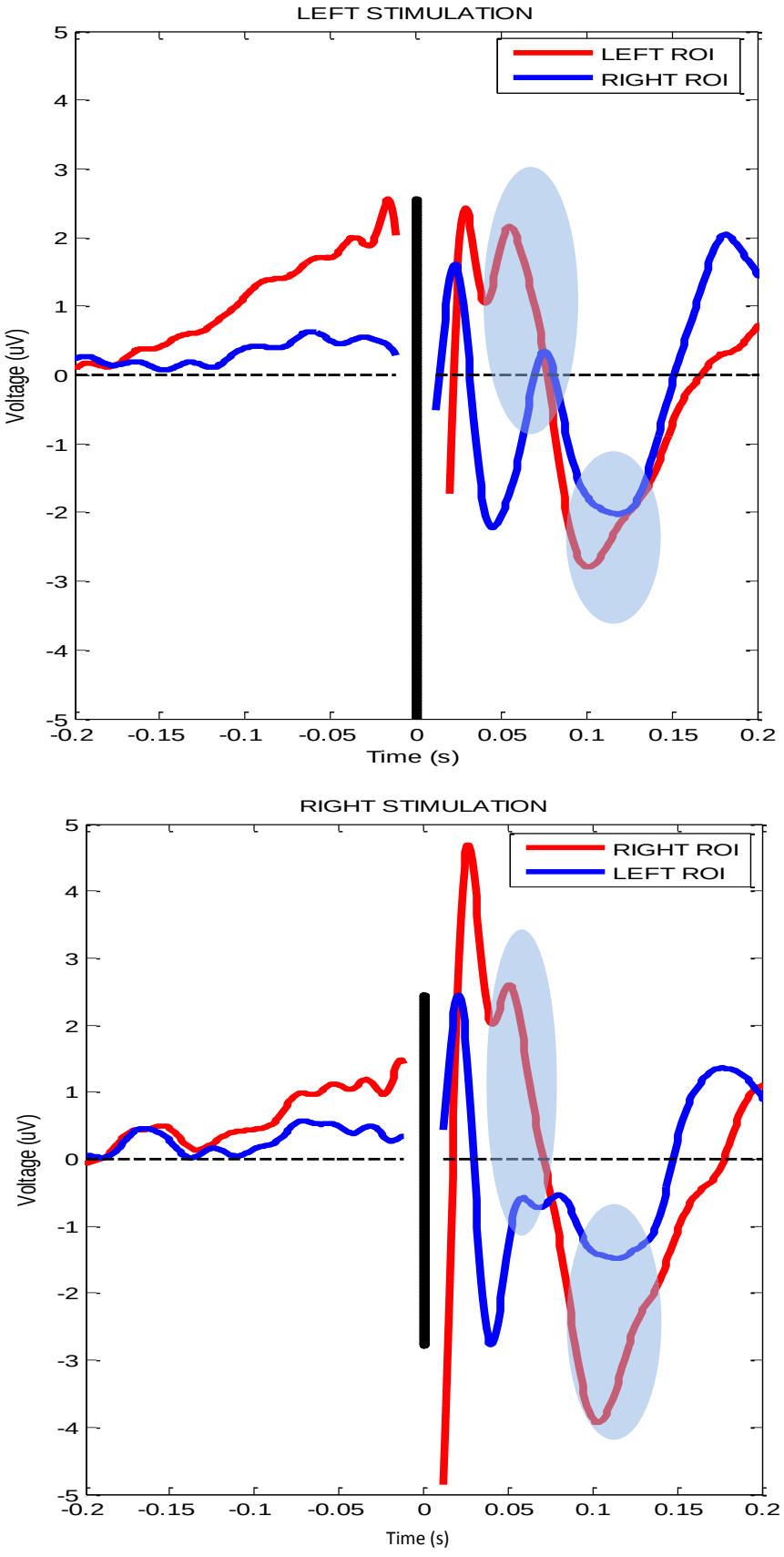


Figure 3.2 a, b Grand average in <u>controls</u> for left- (top) and right-hemispheric stimulation (bottom); light blue ellipsoids label the P60 and N100; thick line = stimulus application; y-axis: amplitude in µV, x-axis: time in seconds

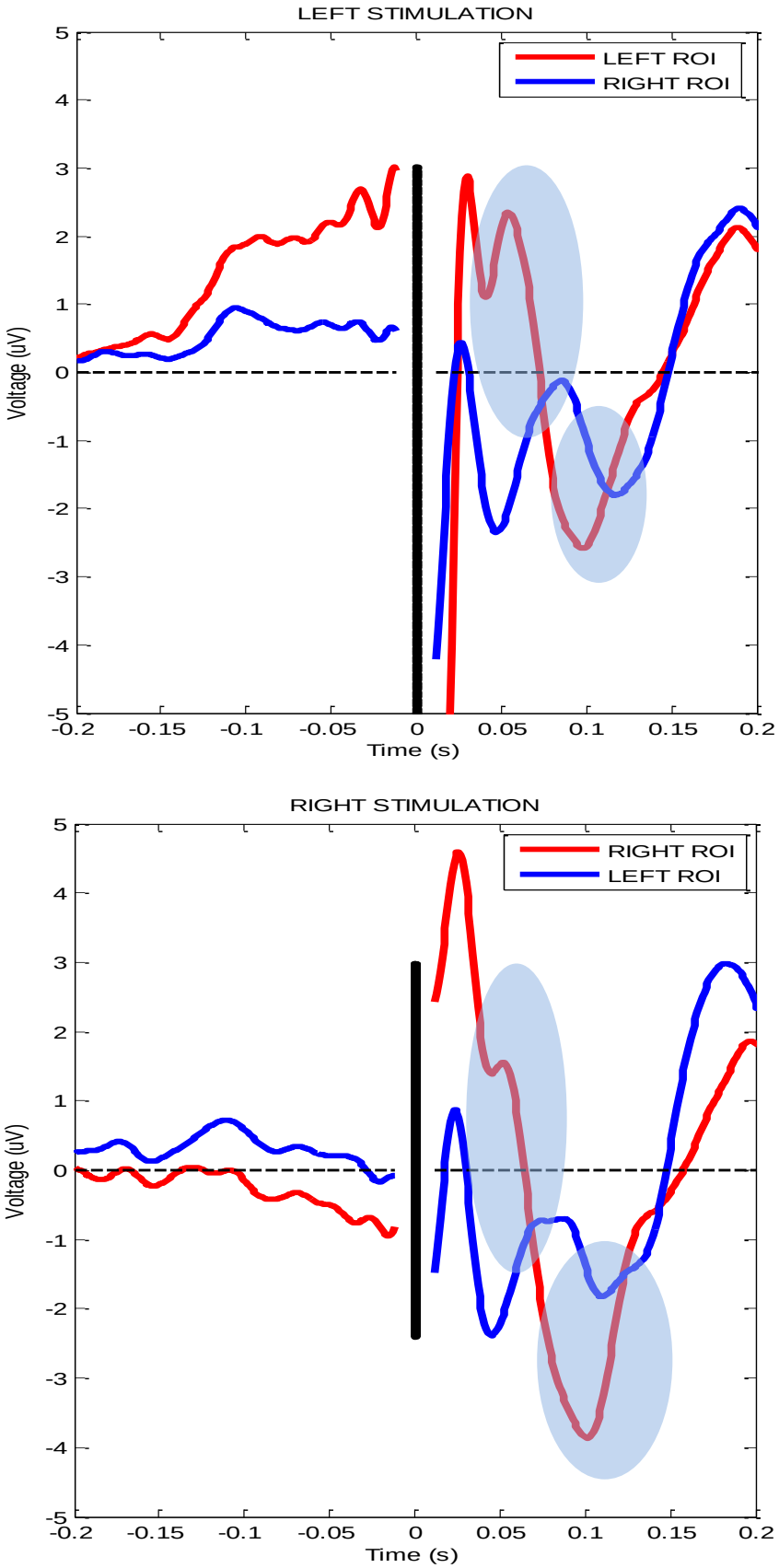


Figure 3.3 a, b Grand average in MS for left- (top) and right-hemispheric stimulation (bottom); light blue ellipsoids label P60 and N100; thick line = stimulus application; y-axis: amplitude in µV, x-axis: time in seconds

3.3 P60 Topographical plots

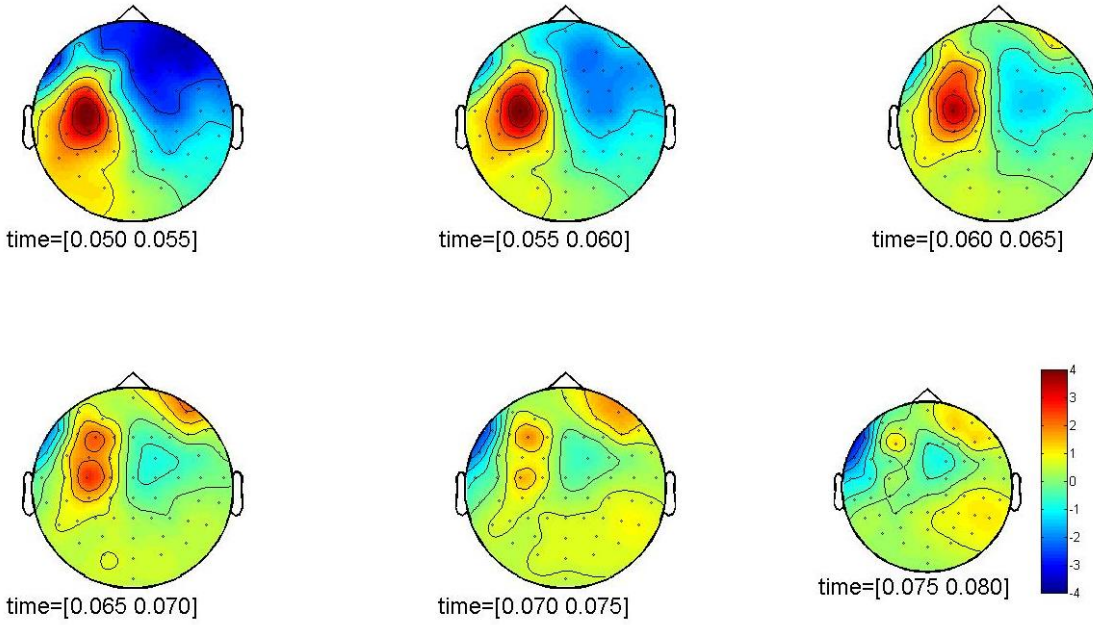


Figure 3.4 P60 controls; left-hemispheric stimulation, time window 50-80ms in steps of 5 ms; topographic distribution of amplitudes in -4 to 4 μV.

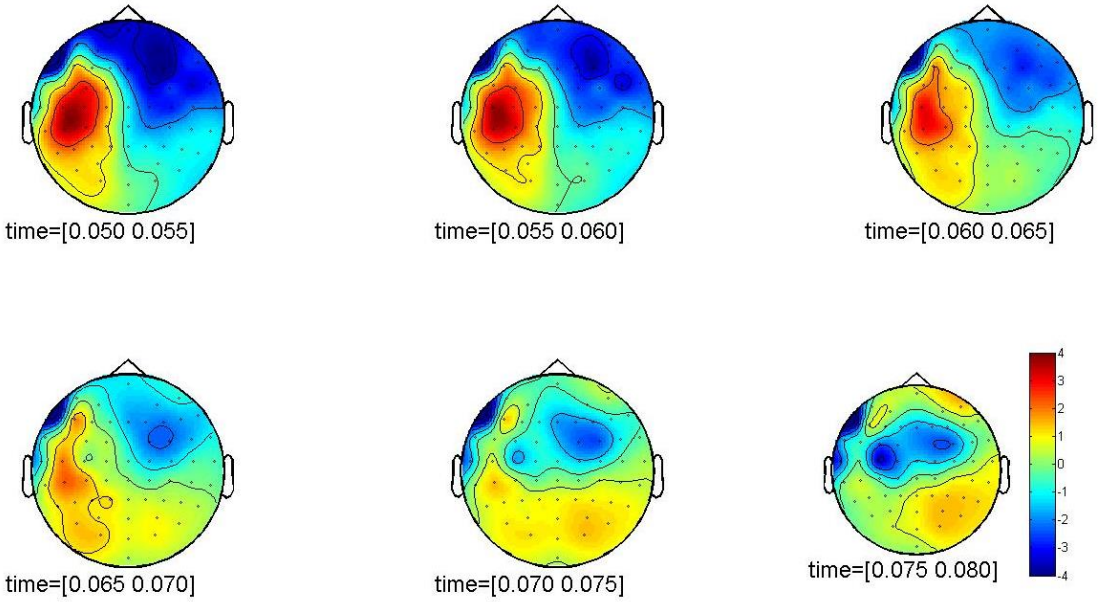


Figure 3.5 P60 MS; left-hemispheric stimulation, time window 50-80ms in steps of 5 ms; topographic distribution of amplitudes in -4 to 4 μV.

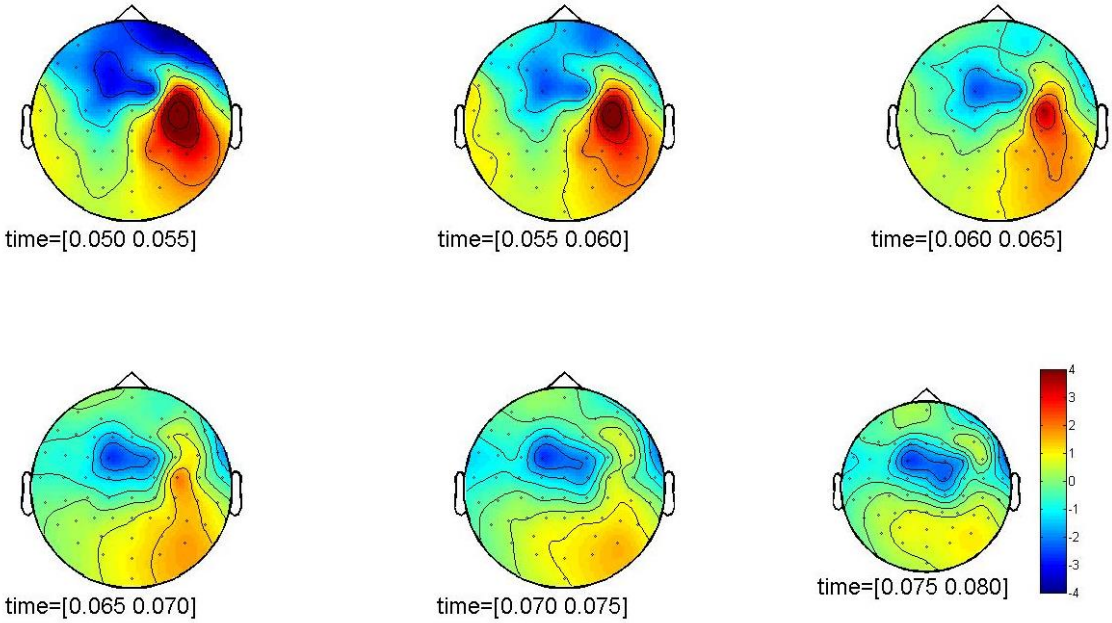


Figure 3.6 P60 controls; right-hemispheric stimulation, time window 50-80ms in steps of 5 ms; topographic distribution of amplitudes in -4 to 4 µV.

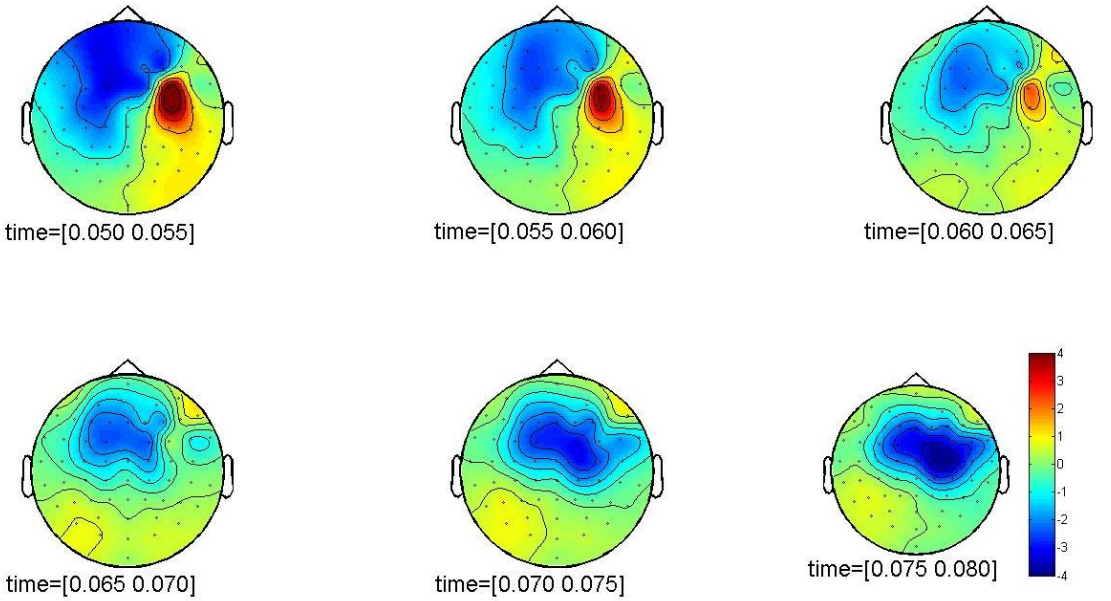


Figure 3.7 P60 MS; right-hemispheric stimulation, time window 50-80ms in steps of 5 ms; topographic distribution of amplitudes in -4 to 4 µV.

3.4 P60 amplitude

There were no significant differences between control subjects and MS-patients in amplitudes on stimulated left-hemisphere [A] in the chosen time-windows of 40-65 / 75, 45-60 / 70 / 75 ms and 75-85 ms on a *p*-level of .05; all *p*-values were >.05. Neither were there any significant clusters on the non-stimulated right hemisphere [B] in the chosen time-windows of 60-80 ms, 70-90 ms and 75-85 ms employing the same limit. For right-hemispheric stimulation, *R-P60*, at the site of stimulation [C] we ran the analysis for significant clusters in TOI 45 - 65 / 75 / 80 ms and 50-70 / 75 / 80 ms without any significant result. On the non-stimulated left hemisphere [D] we did not find either any significant result in a time-window of 45 - 60 / 70 / 80 ms and 50 -70 / 80 ms (for an overview see table 3.4).

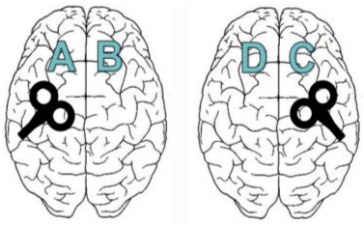


Figure 3.8 Scheme for evaluating ERPs in both trial conditions; L (left) and R (right) M1-stimulation

	TOI* A	TOI B	TOI C	TOI D
L-P60 [ms]	*~ 40 - 70*	*~ 60 - 85*		
R-P60 [ms]			*~ 45 - 80*	*~ 45 - 80*

Table 3.4 Time windows of interest (*TOI in ms) were the same for control subjects and RRMS-patients. In terms of the amplitudes there were no significant differences between groups in the selected time windows (all *p*>.05) in left-hemispheric and right-hemispheric stimulation in the regions of interest. Results are presented following the scheme in figure 3.8.

3.5 N100 latency

3.5.1) The N100 latency analysis of *left-hemispheric* TMS (L-N100) showed a main effect of side ($F(1, 26) = 8.298$, $p = .008$), with the N100 peaking earlier in the stimulated left (100.2 ms ± 13.2) than in the non-stimulated right (108.5 ms ± 15.3) hemisphere. There was no statistically significant main effect of group ($F(1, 26) = 0.208$, $p = .65$). However, there was a statistically significant side by group interaction ($F(1, 26) = 5.21$, $p = .002$) showing that the propagation time was higher in MS (16.8 ms) than in controls (1.9 ms) (see tables 3.5 and 3.6 and figure 3.9).

3.5.2) The N100 latency analysis of the *right-hemispheric* TMS (R-N100) showed no main effect of side ($F(1, 26) = 0.64$, $p = .43$), no main effect of group ($F(1, 26) = 0.288$, $p = .60$), and no side by group interaction ($F(1, 26) = 2.368$, $p = .14$). (see tables 3.5 and 3.6).

The finding of delayed interhemispheric propagation of N100 in MS-patients is displayed in figure 3.9. Furthermore the results are illustrated on the following pages by a grand average ERP of a single (representative) MS-subject in two separate figures (figure 3.10) and in direct comparison of ERPs in the stimulated vs. non-stimulated hemisphere in one representative MS-subject versus one single representative control subject (figure 3.11). On a descriptive single subject level, we found that in 5 out of 12 MS-subjects the N100 latency in stimulated vs. non-stimulated hemisphere was > 20ms whereas there was only 1 control of 16 who was showing this phenomenon.

The whole (N100-) range of topographical plots is presented in this section (figure 3.13 - 3.16); the possible significance of these data will be discussed in part 4.3.

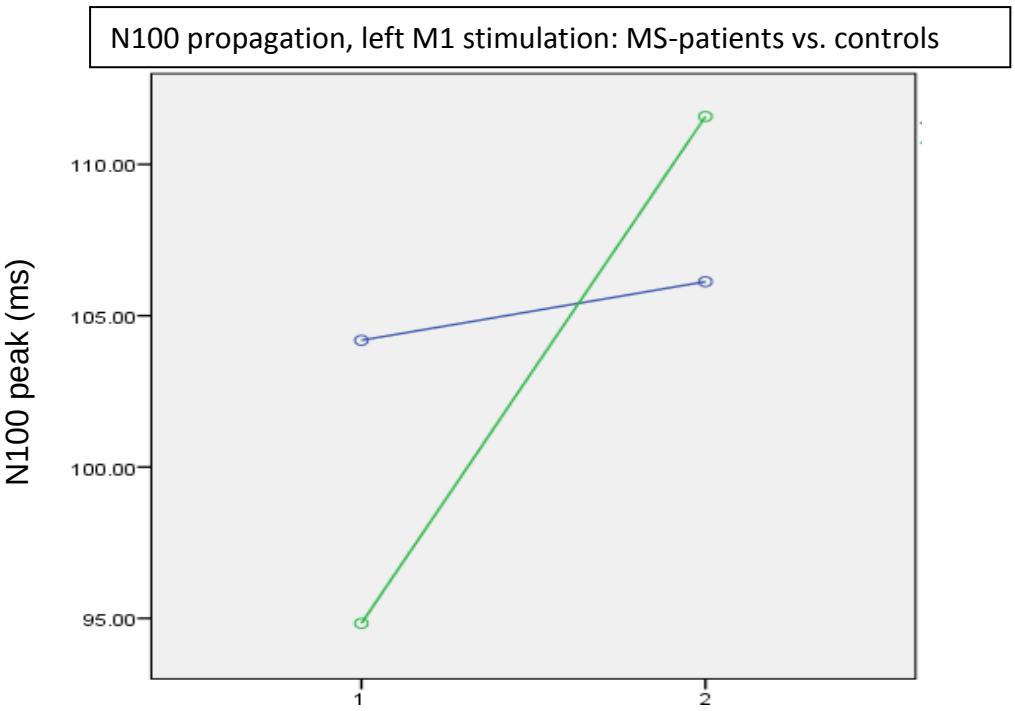


Figure 3.9 Line diagram displaying interhemispheric propagation of L-N100 in MS-patients (green line) and healthy controls (blue line). X-axis indicates N100 peak on stimulated left hemisphere (1) and non-stimulated right hemisphere (2). Note the delayed interhemispheric propagation of the N100 in MS-patients.

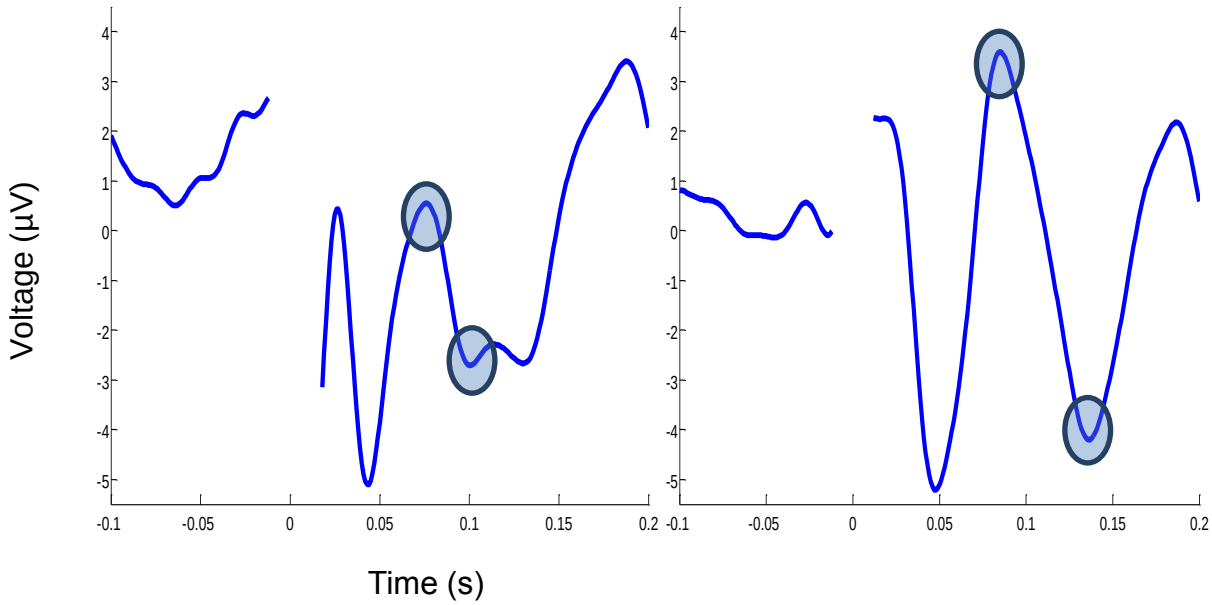


Figure 3.10 Representative ERP of single MS-subject S20; 0 sec -> Stimulus application; LEFT GRAPH - grand average left-hemispheric stimulation, latencies identified: P76 and N101; RIGHT GRAPH - grand average non-stimulated right-hemisphere, latencies identified: P85 and N136; y-axis: µV, x-axis: time in seconds

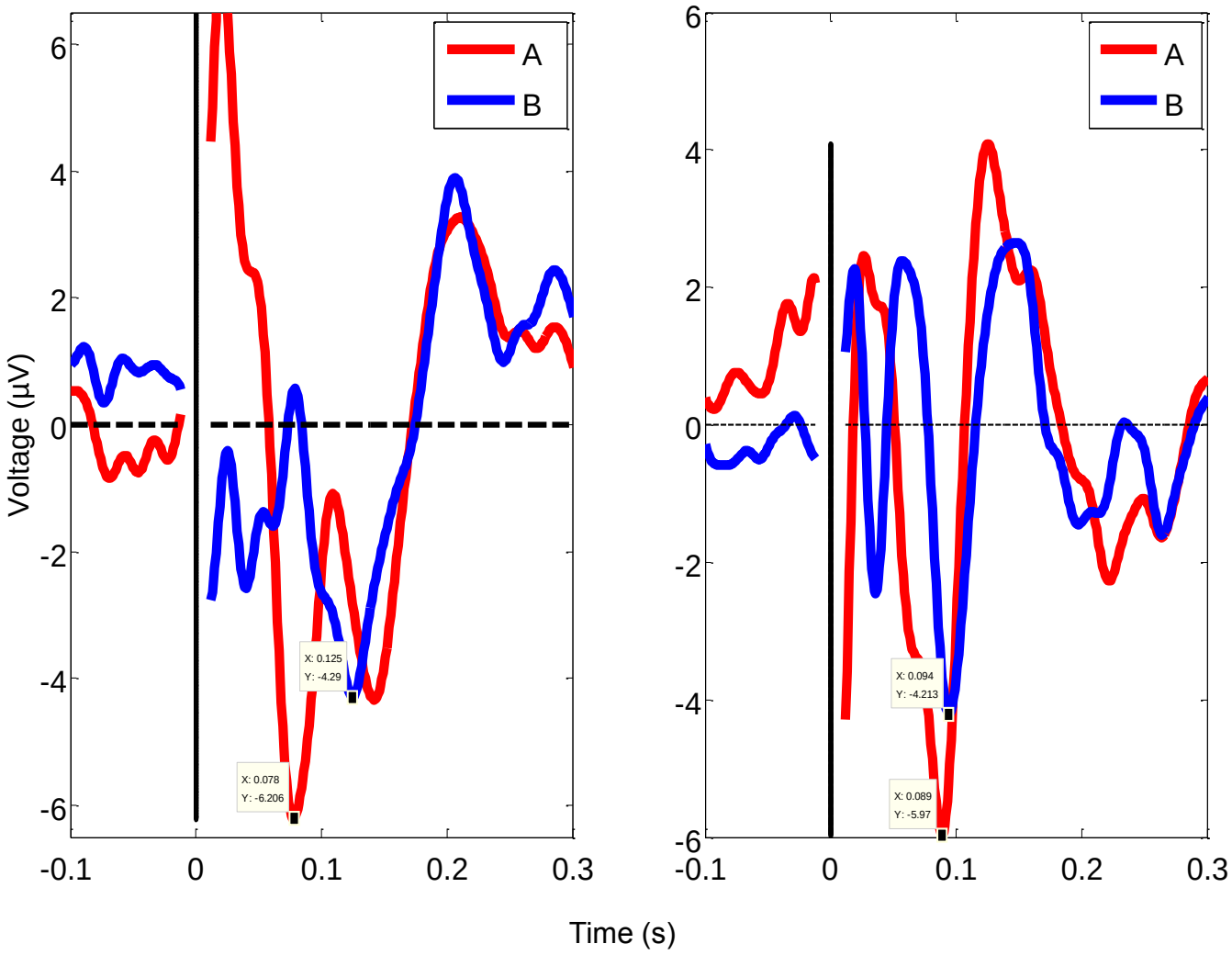


Figure 3.11 Representative ERP of single MS-subject S21 (left figure) and single control subject S7 (right figure) showing the stimulated left hemisphere (A – red graph) vs. non-stimulated right hemisphere (B – blue graph); 0 sec -> Stimulus application; y-axis: µV, x-axis: time in seconds

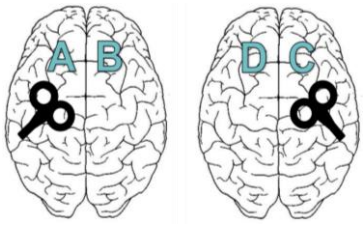


Figure 3.12 Scheme for evaluating ERPs in both trial conditions; L (left) and R (right) M1-stimulation

Group	N100 (A)	N100 (B)	N100 (C)	N100 (D)
Control subjects	104 ± 13 (86-134)	106 ± 12 (89-123)	102 ± 12 (89-127)	99 ± 16 (83-136)
RRMS-patients	95* ± 11 (79-108)	112* ± 19 (77-137)	99 ± 13 (73-123)	107 ± 19 (78-138)

Table 3.5 Mean N100 latencies ± SEM and range in brackets, reported in ms according to the scheme in figure 3.12; in left-hemispheric stimulation there was a significant side- by group interaction (p=.002), indicating that the propagation time from (A) to (B) was higher in RRMS-patients - highlighted by the asterisk in the table. There was no significant side- by group interaction (p=.14) in right-hemispheric stimulation. There was no significant group effect in left- (p=.65) and right-hemispheric stimulation (p=.60). In left-hemispheric stimulation there was a main effect of detection-site (p=.008), indicating that N100 was peaking earlier in stimulated- than in the non-stimulated hemisphere. In right-hemispheric stimulation there was no significant main effect of detection site (p=.43).

Group/Latency	N100 ([B-A])	N100 ([D-C])
Control subjects	2 ± 13	-3 ± 14
RRMS-patients	17* ± 15	8 ± 16

Table 3.6 Mean N100 interhemispheric propagation time reported in ms ± SEM; the values of every individual subject were used to determine group differences. The asterisk highlights the statistically significant delayed interhemispheric propagation time in RRMS-patients compared to control subjects.

3.6 N100 Topographical plots

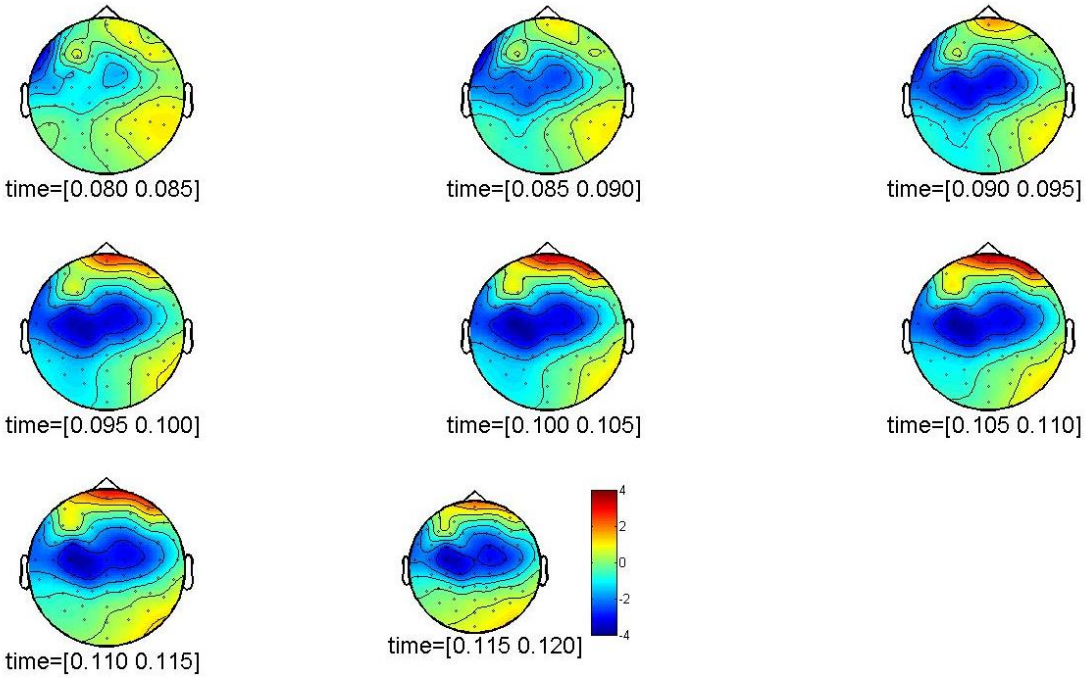


Figure 3.13 N100 controls; left-hemispheric stimulation, time window 80-120ms in steps of 5 ms; topographic distribution of amplitudes in -4 to 4 μV.

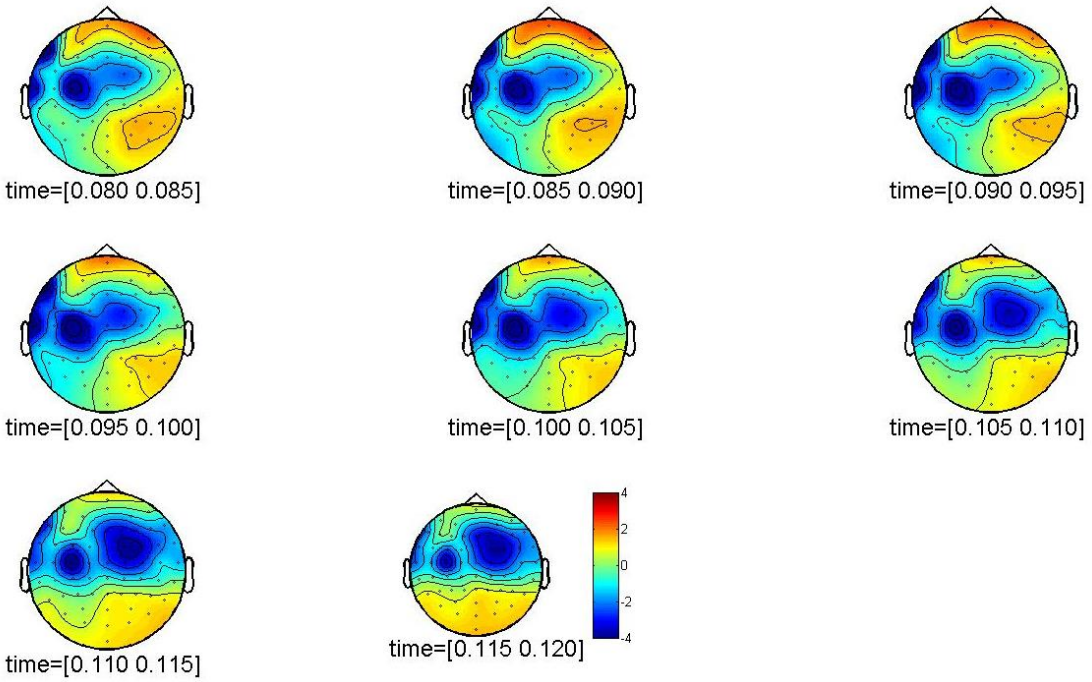


Figure 3.14 N100 MS; left-hemispheric stimulation, time window 80-120ms in steps of 5 ms; topographic distribution of amplitudes in -4 to 4 μV.

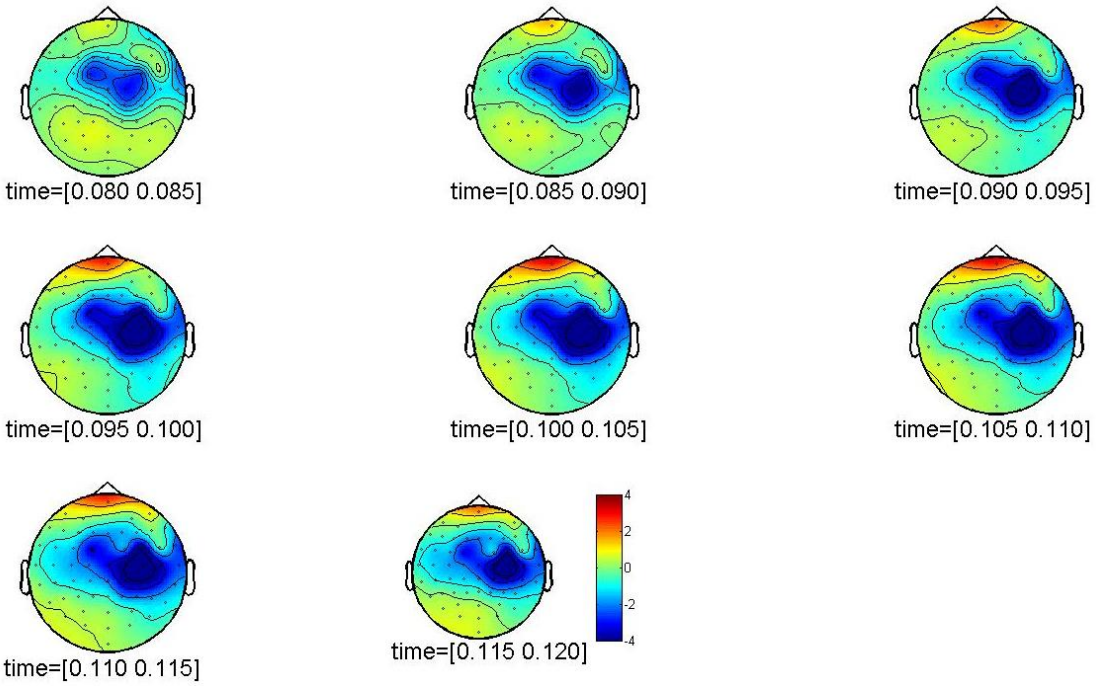


Figure 3.15 N100 controls; right-hemispheric stimulation, time window 80-120ms in steps of 5 ms; topographic distribution of amplitudes in -4 to 4 μV.

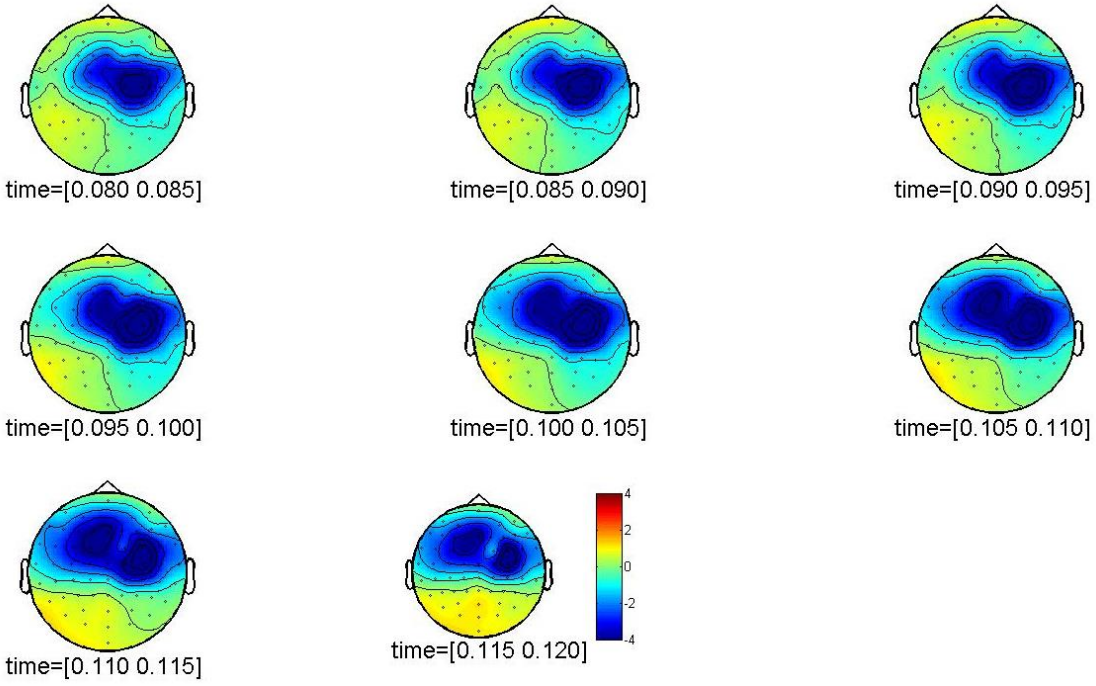


Figure 3.16 N100 MS; right-hemispheric stimulation, time window 80-120ms in steps of 5 ms; topographic distribution of amplitudes in -4 to 4 μV.

3.7 N100 amplitude

There were no significant differences between control subjects and MS-patients of amplitudes on stimulated left-hemisphere [A] in the chosen time-windows of 80 - 110 / 120 ms, 90 - 110 / 120 ms, and 95 - 110 / 120 ms on a *p*-level of .05.; all *p*-values were >.05. Neither there were any significant clusters on the non-stimulated right hemisphere [B] in the chosen time-windows of 80 - 120 ms and 90 - 130 / 140 / 150 ms. For right-hemispheric stimulation, *R-N100,* on the site of stimulation [C] we checked for significant clusters in the presumed TOI and the values exceeded by MS-subjects (-140 ms) without any significant results. Finally on the non-stimulated left hemisphere [D] we did not find a significant result in time-windows of 100 - 140 / 150 ms, 110 - 140 /150 ms and 120 - 140 ms (see table 3.7).

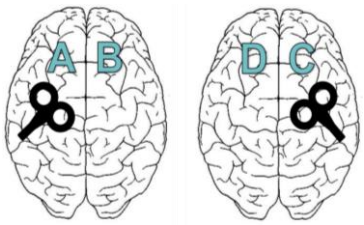


Figure 3.17 Scheme for evaluating ERPs in both trial conditions; L (left) and R (right) M1-stimulation

	TOI* A**	TOI B	TOI C	TOI D
L-N100	*~ 80 - 120*	*~ 80 - 150*		
R-N100			*~ 90 - 140*	*~ 100 - 150*

Table 3.7 Time windows of interest (*TOI in ms) were the same for control subjects and RRMS-patients. In terms of the amplitudes there were no significant differences between groups in the selected time windows (all *p*>.05) in left-hemispheric and right-hemispheric stimulation in the regions of interest. Results are presented following the scheme in figure 3.17.

4. DISCUSSION

"Il faut confronter les idées vagues avec des images claires." --- Jean-Luc Godard

4.1 Identification of TEPs

In the present study we delivered single-pulse TMS on M1 and simultaneously recorded the TMS-evoked EEG response in healthy controls and early-stage RRMS patients. TEPs in RRMS patients deviate from healthy controls, which may make it difficult to identify certain TEPs.

Since there is no general agreement on TEP identification in the literature, we adopted procedures for latency determination in visual, auditory, and somatosensory evoked potentials similar to those established in clinical neurophysiology (Luck, 2005; Misulis, 1994). We reviewed latencies in the context of the TMS-EEG literature: We assigned a clear peak at a certain given time-point to the most probable TEP component (figure 4.1). We labeled consecutive peaks according to known positive and negative deflections independent of peak latencies. In most cases we followed this simple way of peak-to-peak detection, as illustrated in a representative TEP in figure 4.3. For a split peak, with two peaks at similar amplitudes, we selected the latency to a point extrapolated from the rising and falling phases (figure 4.2d and figure 4.4); because it was the most appropriate way to deal with bifid peaks in a time-window known to have one single peak. There is no positive deflection described in literature between the P60 and the N100, so that we assigned a positive deflection at e.g. 80 ms to the P60. Same for the N100: The last negative deflection in the TEP is the N44, the next is the N280. This way we avoided confounding of latencies and we could label a negative peak at 130 ms as a delayed N100.

We have applied the same rules for detecting latencies in the MS patients as well, observing that the N100 differed in time between stimulated left-hemisphere and non-stimulated right hemisphere in comparison to healthy controls. This finding was what we refer to as *delayed interhemispheric propagation*.

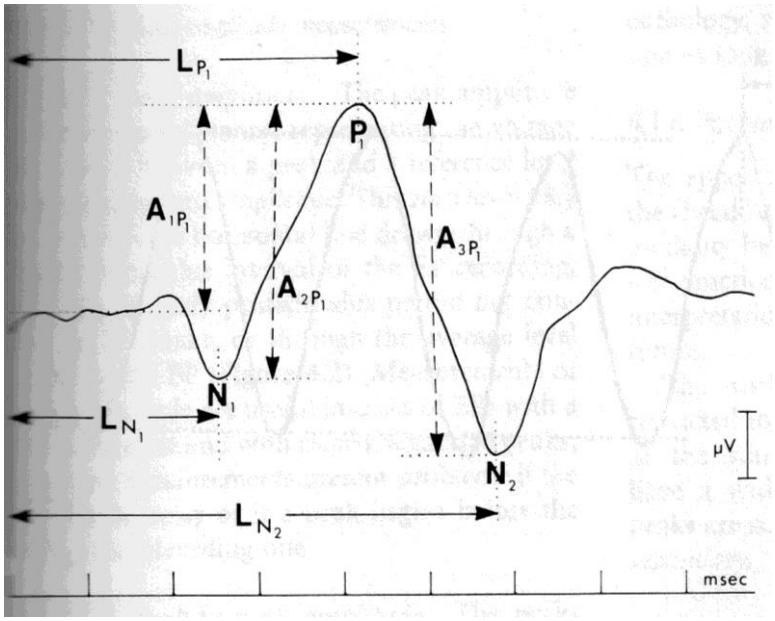


Figure 4.1 Measurement of EP parameters. Peak latency is measured from stimulus to first negative (LN1), first positive (LP1), and second negative (LN2) peak. [© 1994 by Butterworth-Heinemann Ltd., (Misulus, 1994)]

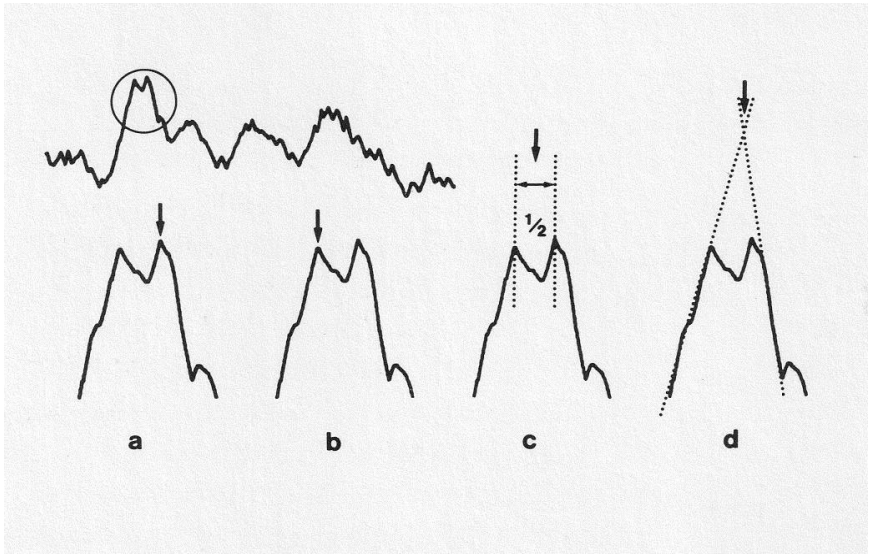


Figure 4.2 Methods of selecting the latency and amplitude of an EP peak that is split. (a) Highest point. (b) First point. (c) Latency to the midpoint between two tips. (d) Latency to a point extrapolated from the rising and falling phases.
[© 1994 by Butterworth-Heinemann Ltd., (Misulis, 1994)]

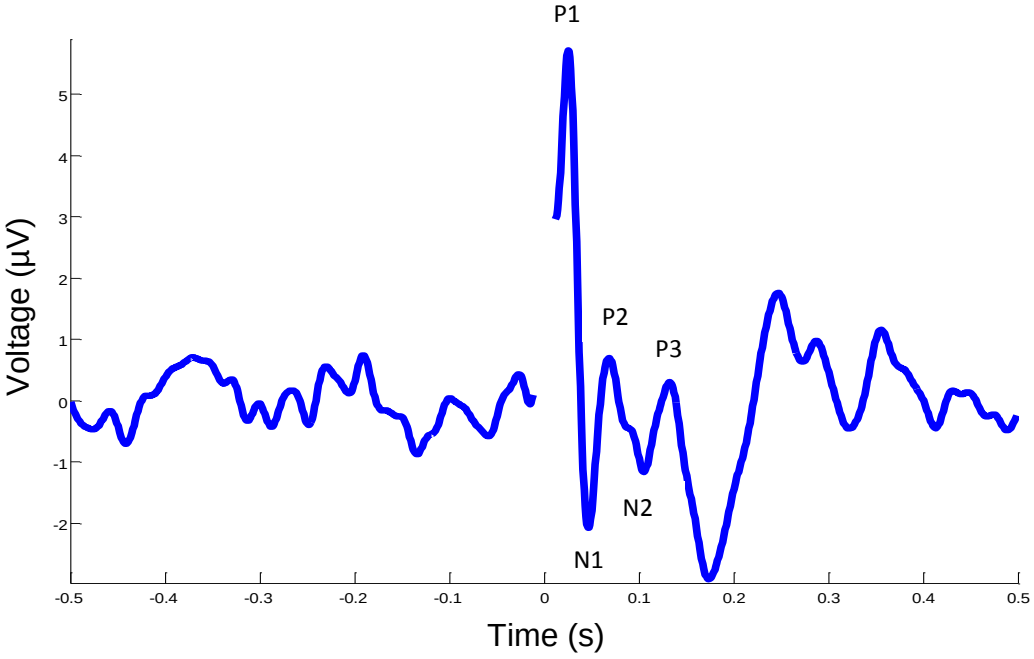


Figure 4.3 ERP of single representative control subject, grand average left-hemispheric stimulation / left ROI; latencies identified: P60 (P2), N104 (N2); y-axis: amplitude in µV, x-axis: time in seconds

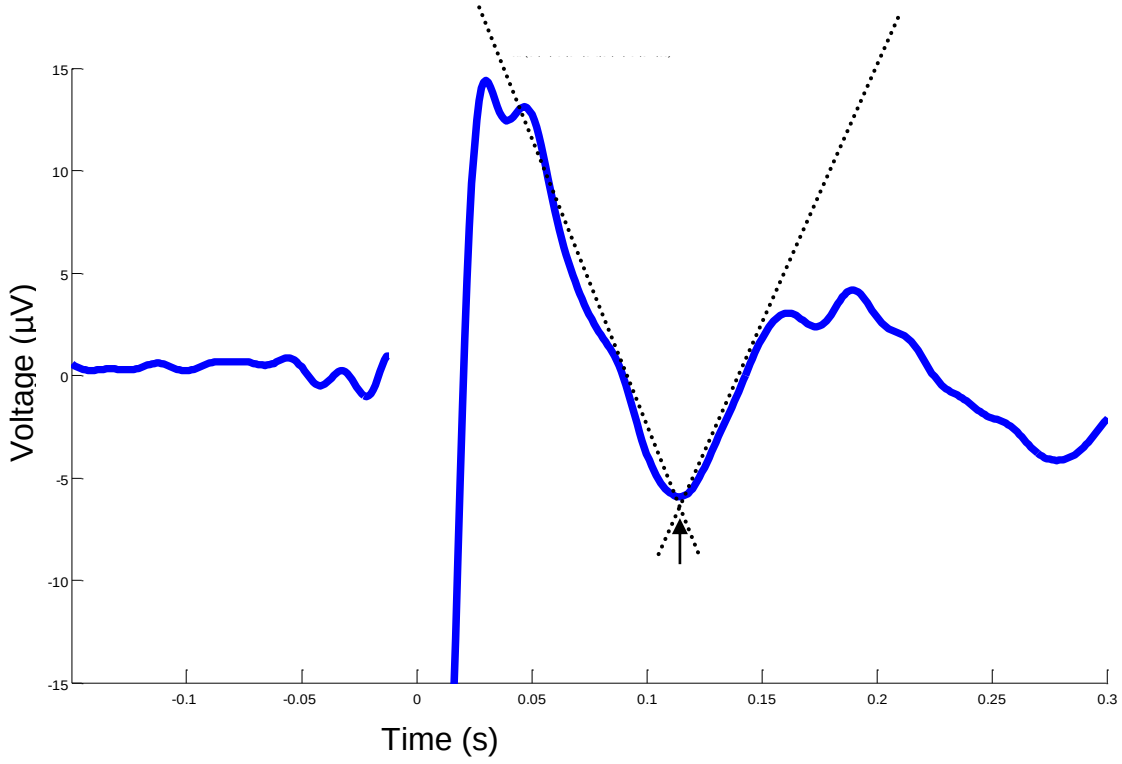


Figure 4.4 ERP of single control subject 016, grand average right stimulation / right ROI; latencies identified: P47, N115; y-axis: µV, x-axis: time in seconds

4.2 TEP latencies in healthy subjects in comparison to literature

We were able to obtain data from 16 healthy, right-handed, gender balanced volunteers across a relatively broad age-span.

Table 4.1 gives an overview of N100 peaks in absolute values recorded in earlier TMS-EEG in studies with healthy controls compared to our results. The values extracted from the literature, taking into account studies employing TMS-EEG in M1 in healthy adults with documented latencies, mostly match our absolute values for L-N100 and L-P60. For R-P60 and R-N100 only data from one study is available (Komssi, 2004); which matches with our results (R-P60: Komssi et al. 55 ± 6 vs. 61 ± 11 ms, R-N100: Komssi et al. 100 ± 18 vs. 102 ± 12 ms), whereas TEPs on the non-stimulated hemisphere were not reported. To our knowledge our study is the first to report statistical differences in L-N100 latencies between hemispheres. This has not been observed in a study conducted on 7 healthy male subjects with the primary purpose to test modulation of EEG responses to TMS by voluntary hand movement (Nikulin, 2003).

In the context of former studies it is necessary to discuss the selection of electrodes for EEG analysis in our study and as compared to the literature: Currently there is no general agreement on the selection of electrodes when investigating TMS evoked potentials, what results frequently in a choice of single, preferentially vertex electrodes (Cz) (Ferreri, 2011; Komssi, 2004; Bruckmann, 2012) or electrodes located around the left M1 (Daskalakis, 2008). We chose a ROI out of 10 electrodes, homologous on each hemisphere, including and surrounding motor cortex, because this electrode selection has been demonstrated to provide reproducible TEPs (Lioumis, 2009).

Study	N100 (A)	N100 (B)	P60 (A)	P60 (B)
Own data	104 ± 13 (86-134)	106 ± 12 (89-123)	62 ± 12 (42-90)	73 ± 11 (45-84)
Lioumis et al. 2009 [N=7]*	108 ± 9	108 ± 12	60 ± 11	50 ± 9
Kommsi et al. 2004 [N=7]**	105 ± 16	x	58 ± 8	x
Paus et al. 2001 [N=7]***	105 ± 12	x	x	x
Tiitinen et al. 1999 [N=4]‘	102	x	63 ± 10	x
Ferreri et al. 2011 [N=8]‘‘	103 ± 20	x	56 ± 3	x
Bonato et al. 2006 [N=6]‘‘‘	105 ± 15	x	x	x
Nikulin et al. 2003 [N=7]‘‘‘‘	~ 105	x	x	x

Table 4.1 N100 literature review: *(Lioumis, 2009) - 10 electrodes **(Komssi, 2004) - vertex electrode *** (Paus, 2001) - whole scalp ‘(Tiitinen, 1999) - Cz-electrode, 120% MT ‘’ (Ferreri, 2011) - whole scalp ‘’’(Bonato, 2006)- whole scalp ‘’’’ (Nikulin, 2003) - 10 electrodes, values extracted from fig. 3 of the publication; values rounded up to the next ms.

4.3 N100 TEP ‘propagation’:
A measure of interhemispheric transcallosal connectivity?

The activation of the contralateral (to the stimulated hemisphere) homologous cortical areas might be due to transcallosal signal propagation. This has been demonstrated by Voineskos et al. (2010) in a sham-controlled study in 30 healthy adult subjects by the finding of a significant inverse relationship between an TEP amplitude-based measure of interhemispheric signal propagation and microstructural (DTI-) integrity data of the motor corpus callosum (Voineskos, 2010). Furthermore Ilmoniemi et al. (1997) have proposed in their ground-breaking TMS-EEG study in 1997 for investigation of cortical connectivity (mainly on early potentials until 30 ms post-stimulus) that activation of contralateral homologous cortical areas might be transmitted via transcallosal connections (Ilmoniemi, 1997). Thus, TEP propagation can be used as a biomarker of cortico-cortical connectivity.

The L-N100 TEP is peaking significantly later in the non-stimulated right hemisphere than in stimulated left hemisphere in the MS-patients, compared to the healthy controls (F(1,26) = 8.298, p=.008). Given that the CC is affected at an early stage of MS (Wahl, 2011) we assume that the delayed appearance of the L-N100 in the right hemisphere provides further evidence that TEPs are conducted via transcallosal pathways. One limitation of our study is that we did not correlate this finding to structural data yet.

4.4 Possible mechanisms of P60 and N100 propagation

Although there was no overall group difference in L-N100 ($F(1, 26) = 0.208$, $p = .65$) we found that only the N100 on the non-stimulated right hemisphere did differ between groups ($F(1, 26) = 5.21$, $p = .002$). Strikingly these findings vary from our findings on the L-P60, where it turned out that there was also a strong effect of detection site ($F(1, 26) = 24.40$, $p < .001$) and no general group difference ($F(1, 26) = 0.17$, $p = .68$) or category by group interaction ($F(1,26) = 0.95$, $p = .34$). In both L-/ and R-P60 the peak on the non-stimulated hemisphere appears later, without any category by group difference ($F(1, 26) = 0.95$, $p = .34$ and $F(1, 26) = 0.003$, $p = .96$). As stated above we suppose that TEPs are conducted via specific interhemispheric pathways. Our results provide indirect evidence that the single TEP components P60 and N100 are transferred

via different networks. The finding of regular P60- but delayed N100-propagation in the MS patients could be explained by separate pathways, affected to a different degree by demyelination and axonal degeneration in MS.

We assume that the delayed N100 latency in MS at least partly reflects an impairment of cortical inhibitory mechanisms, even though amplitudes did not vary significantly between groups. The N100 is the best understood peak in TEP research. According to Bender et al., the N100 can be interpreted as a "wave" response to an externally generated spike. It might therefore provide an in vivo model to assess thalamocortical inhibitory processes in human subjects (Bender, 2005). Further studies supported this theory of the N100 to reflect inhibitory mechanisms, employing motor tasks and application of alcohol (Bikmullina, 2009; Nikulin, 2003; Kähkonen & Wilenius, 2007). Therefore we believe that the N100 plays a decisive role in cortical inhibitory mechanisms which might be impaired in MS.

Interestingly, we also found MS-subjects with regular P60 but prolonged N100 conduction but also with both components delayed (see figure 4.5, 4.6 and 4.7). It remains to be demonstrated in further trials whether different stages of MS lead to further abnormalities in TEP propagation. At this early stage we could demonstrate that the N100 appears to be the first component affected in RRMS-patients.

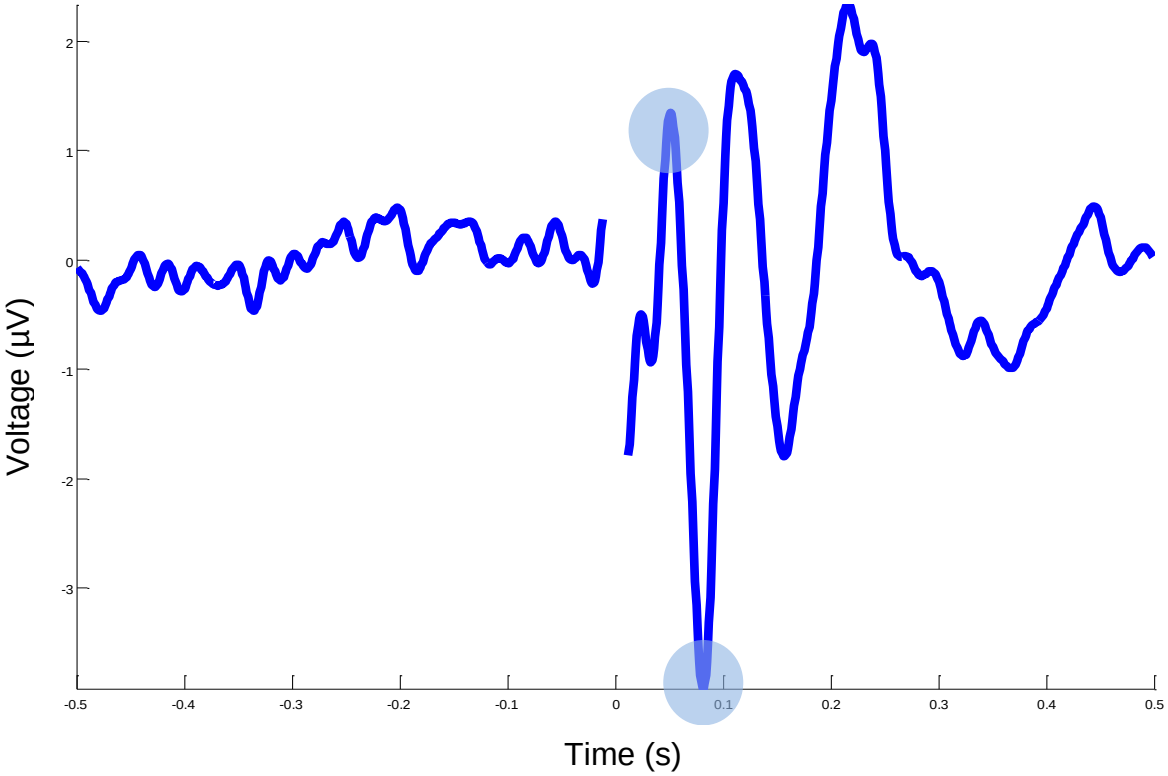


Figure 4.5 ERP of a single MS-subject 024; grand average right-hemispheric stimulation / right ROI [C]; latencies identified: P51 and N82; y-axis: amplitude in µV, x-axis: time in seconds

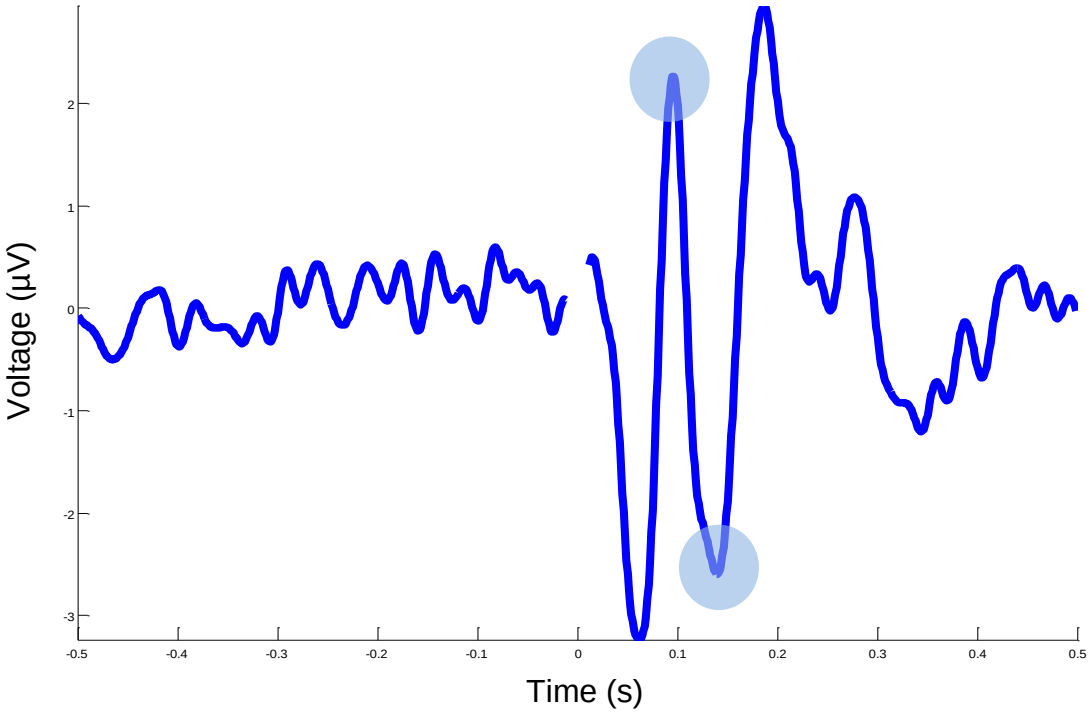


Figure 4.6 ERP of a single MS-subject 024; grand average right-hemispheric stimulation / left ROI [D]; latencies identified: P96 and N140; y-axis: amplitude in µV, x-axis: time in seconds

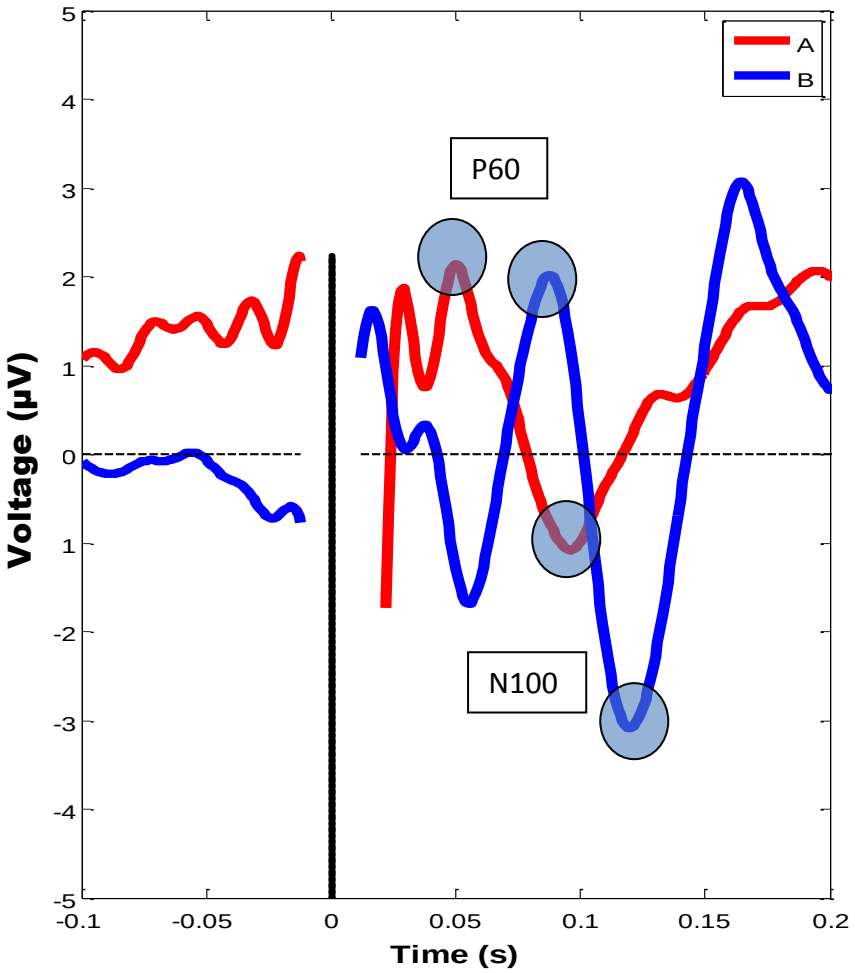


Figure 4.7 ERP of a single MS-subject S23 with both P60 and N100 delay in non-stimulated right hemisphere; 0 sec -> stimulus application; this figure displays P60 and N100 delay in a single MS-patient; Red Line (A) = stimulated left hemisphere, Blue Line (B) = non-stimulated right hemisphere; y-axis: amplitude in µV, x-axis: time in seconds

4.5 TEPs in right M1 stimulation: Cortical asymmetries?

For the R-N100 we did not confirm the finding of significantly delayed latencies between stimulated right hemisphere and non-stimulated left hemisphere and no general group difference. There was no significant delay of the N100 in the non-stimulated left hemisphere in the MS-group. We assume that this was due to the limited sample size as absolute values showed a strong trend towards a delay and the p-value showed at least a tendency towards a delayed interhemispheric N100 propagation in the MS patients (see

paragraphs 3.2 and 3.5). Our findings from R-P60 analysis strongly resemble L-P60 results, as the stimuli appeared later on the non-stimulated hemisphere in both settings (R-P60: $F(1, 26) = 9.43$, $p = .005$ vs. L-P60: $F(1, 26) = 24.40$, $p < .001$) without any differences between MS- and control group (L-P60: $F(1, 26) = 0.17$, $p = .68$ vs. R-P60: $F(1, 26) = 0.05$, $p = .83$).

The finding of the R-N100 appearing earlier in the non-stimulated left hemisphere than in stimulated right hemisphere in controls in *absolute values* (102 ± 12 ms on right hemisphere vs. 99 ± 16 ms on left hemisphere) was remarkable. This difference of interhemispheric N100 propagation between left- and right M1 stimulation is illustrated in the line diagrams in figures 4.8 a and b. Statistically there was no difference between N100 in stimulated right and non-stimulated left hemisphere ($F(1, 26) = 0.64$, $p = .43$), whereas in left M1 stimulation the N100 in non-stimulated right hemisphere appeared significantly later ($F(1, 26) = 8.298$, $p = .008$). This finding might provide evidence for asymmetries in interhemispheric conduction or differing velocity of potential generation in left and right M1. This assumption is supported by the large amount of studies describing callosal (Cherbuin, 2013) and motor-cortex (Amunts, 1996) asymmetries. Our findings provide evidence that TMS-EEG might be a useful tool for investigating cortical asymmetries.

Since the site of (left) M1 stimulation was remote from the non-stimulated right M1 we did not expect this finding to reflect 'false' stimulation of the contralateral M1. One previous study has reported a similar finding for the P60 propagation from the stimulated left to non-stimulated right hemisphere (50 ± 9 ms on right hemisphere vs. 60 ± 11 ms on left hemisphere) (Lioumis, 2009).

Future studies should explore underlying mechanisms of these 'precipitate' contralateral potentials. This could be done i.e. by comparing interhemispheric N100 TEP-propagation in right- and left-handers.

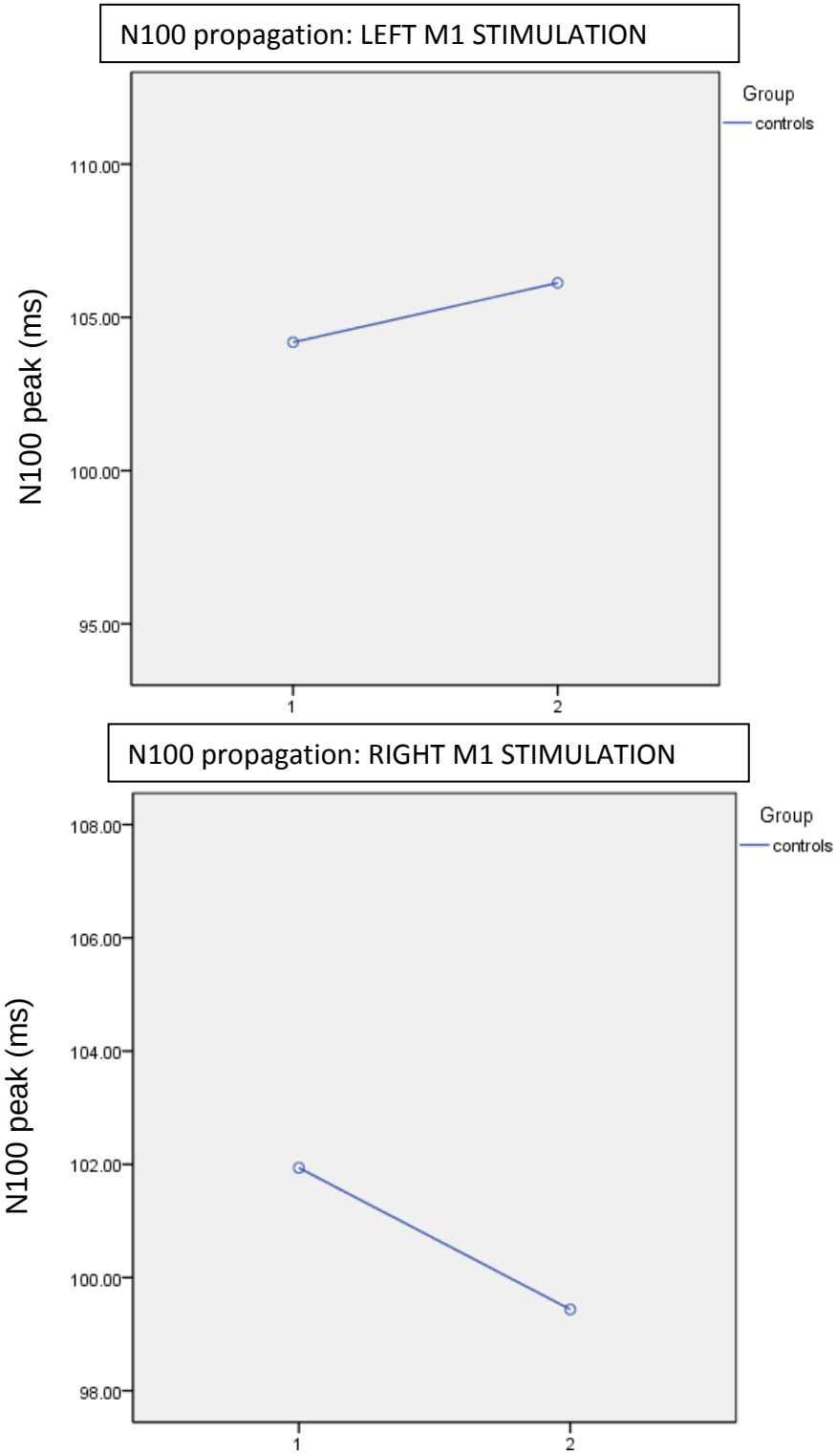


Figure 4.8 a, b Line diagrams displaying (average) interhemispheric propagation of L-N100 (a) and R-N100 (b) in healthy controls. Y-axes indicate N100 peak latency in the stimulated hemisphere (1) and non-stimulated hemisphere (2). Note the earlier appearance of the N100 in the non-stimulated left-hemisphere in the bottom diagram.

4.6 Amplitude analysis

We did not find any significant group differences in amplitude analysis on a low p-level (all $p>.05$). Application of basic knowledge to our results might indicate that the synchronization of neuronal firing was not affected at this early stage of disease. Thus, impaired interhemispheric connectivity does not affect the pattern of activation in the ROIs. This further supports the available evidence that the corpus callosum is affected at an early stage in MS, whereas there is no significant impairment of functional properties of M1. This should be further investigated by correlation to structural data and EEG source analysis to allow direct attribution to M1.

We decided to employ an automated approach due to our observation that in manual detection the baseline was stable but did not always start at 0 μV despite baseline-correction. Like that we have not been able to read out amplitudes but only approximate which was not sufficient for our purpose. The automated approach calculated the distance between groups in one time point, searching for clusters of difference. The involved time-points were chosen according to visual inspection of single subject peaks. We then widened our time-range, until 150 ms in L-N100, to cover affected MS-data. Respectively we narrowed our time-window in the P60, to 60 -85 ms in L-P60, in order to minimize time-points outside the peak.

4.7 Topographical plots

Plotting the spatial spreading of EEG patterns is a common way to visualize signal propagation. The main advantage of EEG is its high resolution in time, whereas its spatial resolution is rather poor. This is why we could not precisely assign any spreading to intercortical pathways. For the topographical plots, we refer to section 3.3 (page 25 and 26) for the P60 time window and to section 3.6 (page 32 and 33) for the N100 time window.

For the P60 we observed (time window 50-80 ms) the highest amplitude around 4 μV in the area of stimulation indicated by the cross. Then the signal was spreading mostly intracortical, to the same degree to frontal and occipital areas. The amplitude P60 deflection in the non-stimulated hemisphere was less strong than on the stimulated

hemisphere, around 0-1 μV from baseline. This finding might be caused by the development of the N100 TEP, which is already evolving (down-strike from P60 to the N100) and thereby may mask the contralateral peak. In accordance with our results in latency analysis there was no major difference between groups.

The N100 (time window 80-120 ms) was acting very differently, hence we could observe as well the highest amplitude around - 4 μV near the site of stimulation but then mostly direct trans-cortical spreading and, a strong signal in the non-stimulated hemisphere around - 4 μV. This might be explained by the longer duration of the N100 and later appearance of the following P280 component. Besides this, there was weak intracortical spreading on the stimulated hemisphere as well. Former studies have shown similar results in terms of topographical plots, which will be shown in the next section. Our observation from left and right stimulation did not differ to a great extent.

It appears that there is mainly interhemispheric spreading within the N100 range and intracortical spreading within the P60 range. This finding provides further evidence that callosal lesions could account for TEP abnormalities in MS. Still, we did not observe distinct differences between the topographical (P60- and N100) plots of the MS- and control group.

4.8 Topographical plots in literature

On a visual and descriptive level, our results likely match topographical plots of former studies. Former studies (see figures 4.9 - 4.11) including topographical plots showed similar results, i.e. high amplitudes at the stimulation site similar patterns of cortical spreading (see paragraph 4.7).

The highest amplitudes observed for the P60 were around 12 μV (fig. 4.9), 6 μV (fig. 4.10) and 5 μV (fig. 4.11) in the area of stimulation. Even though the signal spreading cannot be compared adequately due to larger time windows in previous studies the N100 showed as well the highest amplitude around - 8 μV (fig. 4.9), 0 μV (fig. 4.10) and -5 μV (at 120 ms fig. 4.11) near the site of stimulation.

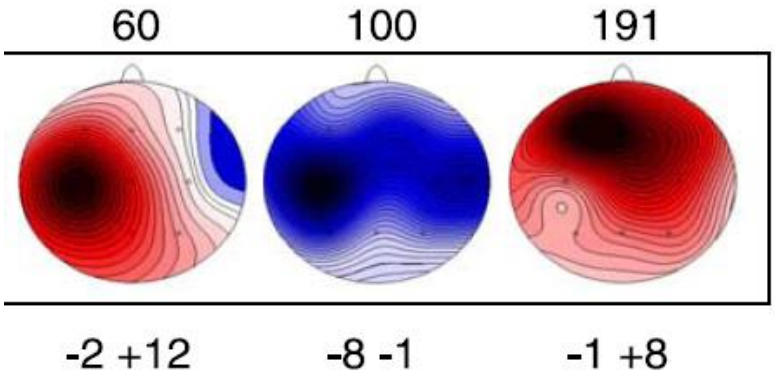


Figure 4.9 Topographical plots from previous studies, upper value: latency in ms; bottom value: amplitude in μV [RE-USE PERMISSION by Elsevier, © 2006, (Bonato, 2006)]

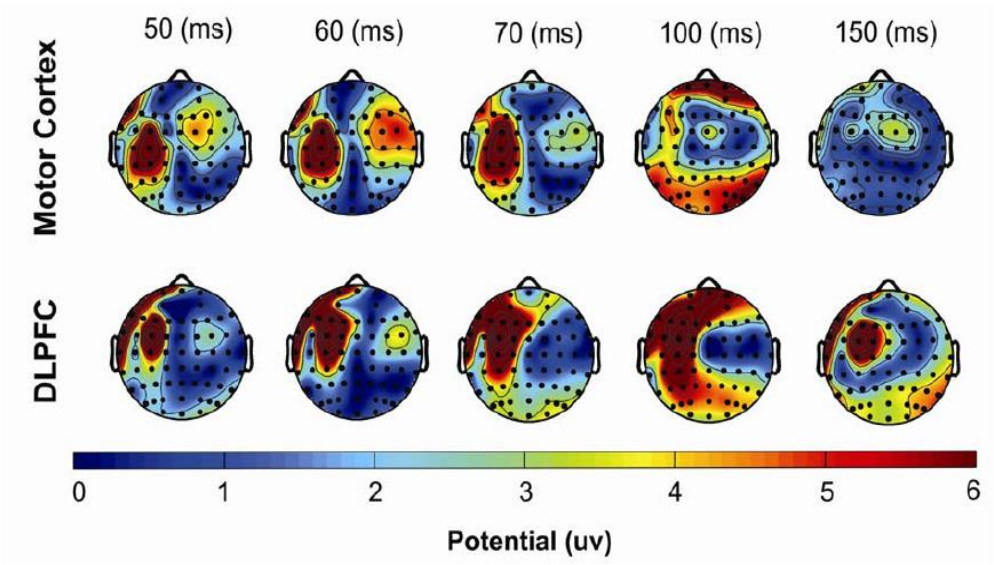


Figure 4.10 Topographical plots from previous studies [RE-USE PERMISSION by Elsevier, © 2010, (Voineskos, 2010)]

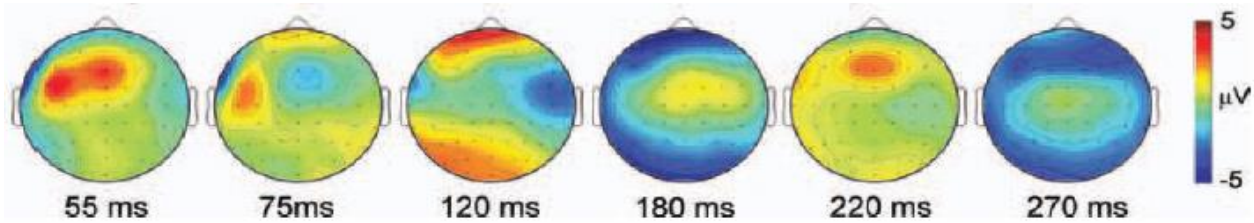


Figure 4.11 Topographical plots from previous studies [RE-USE PERMISSION by Wiley Periodicals Inc., © 2012, (Rogasch & Fitzgerald, 2012)]

5. CONCLUDING REMARKS

"Die Ideen sind nicht verantwortlich für das, was die Menschen aus ihnen machen"
Werner Heisenberg

5.1 Outlook

We have found a significant interhemispheric delay of the N100 propagation in MS-patients. Additionally, we showed a significant delay of the P60 in single MS-patients. Therefore TEPs should be further investigated in RRMS-patients with an EDSS >3, and in patients suffering from secondary- (SPMS) and primary progressive MS (PPMS) to evaluate whether later disease stages further affect TEP propagation. Future studies should include structural (imaging-) data to investigate to which degree TEP abnormalities correlate with structural changes in the CC.

There is furthermore evidence that CC lesions result in cognitive and motor disability (Sigal, 2012; Rimkus Cde, 2011; Van Hecke, 2010; Dineen, 2009). Thus, it is a promising approach to correlate TEP analysis with behavioral and cognitive data to explore the clinical significance of TEP abnormalities.

Prospective longitudinal studies should investigate whether TEPs are changing over the disease course and whether there is a possible influence of DMDs on TEPs. Such an approach is promising according to recent studies, which have demonstrated that measurement of evoked potentials (VEP and SEP) prior and one year after treatment with the humanized monoclonal antibody natalizumab might reflect treatment effects (Meuth, 2011). Future trials should obtain and correlate TMS-based measures of transcallosal inhibition to TEPs as it has been shown transcallosal inhibition has been suggested as an early marker of CC disconnection in MS (Schmierer, 2002).

Considering the rapidly growing knowledge on TEPs and our promising results we assume that TEP analysis in MS might be of interest for future investigations focusing on TEP physiology and on clinical applications as a biomarker.

5.2 Why are biomarkers for MS needed?

Within the last fifty years our view on multiple sclerosis changed radically, from an incurable burden to a manageable disease.

Basically the comparison of epidemiological studies in terms of MS-mortality across decades was limited because of methodological differences, new diagnostic definition and consequently earlier diagnosis of MS (Ragonese, 2008). Secondly the comparison between studies was limited because some of the studies were reporting mortality rates and other studies life expectancy (Ragonese, 2008). Despite these methodological challenges it appeared that the mortality rate in MS decreased world-wide between 1965 and 1984 (Lai, 1989).

Recent data from a retrospective cohort study including 1270 British RRMS-patients diagnosed between 2001 and 2008, compared to a matched-cohort still showed that MS-patients (unspecified type of MS) had a 3.5-fold increased mortality rate (Lalmohamed, 2012). A prospective study with 441 MS-patients in Wales (86% of the patients were diagnosed as probable or definite MS according to the criteria proposed by Poser et al., 1983) who were observed from 1985 to 2007 show a 3-fold increased mortality rate for all-cause mortality (Hirst, 2008). Both studies emphasized that increased mortality rates are mainly related to smoking and respiratory diseases.

Ragonese and colleagues suggested in a review article on epidemiological MS-studies that the main reason for higher mortality rates in MS would still be the evolving disability of MS-patient during the disease course (Ragonese, 2008). As already mentioned methodological differences between studies made a precise analysis of mortality in MS difficult. Nevertheless we might conclude that new therapeutic strategies are needed to improve the disease course of MS-patients.

The current revised McDonald diagnostic criteria (Polman, 2011) highly emphasize early diagnosis and treatment. Indeed, data from the BENEFIT study suggested that early treatment with interferon-beta 1b in 292 patients diagnosed as clinically isolated syndrome (CIS), compared to 176 patients with placebo, might decrease the rate of conversion to clinically definite MS (CDMS) (28% at two years in early intervention vs.

45% in placebo-group), and improve outcome in cognitive performance (PASAT-3 score over 5 years) without any significant loss of life-quality due to subcutaneous injection (Penner, 2012; Kappos, 2009).

Even though current disease-modifying therapies in MS are usually seen as safe and efficient in reducing relapse-rates, their long-term effect on disease progression is not clear (Katrych, 2009; Meuth, 2012). Still, there is no satisfying biomarker for MS as a neurodegenerative disease available (Ziemann, 2011; Rudick, 2012). As stated above TEPs, reflecting microstructural changes in the corpus callosum, might be a novel approach for monitoring disease activity, progression and treatment response in pharmacological trials and individual patient. Figure 5.1, extracted from a review by Ziemann et al. in 2011, summarizes the current biomarkers applied in MS for monitoring disease state.

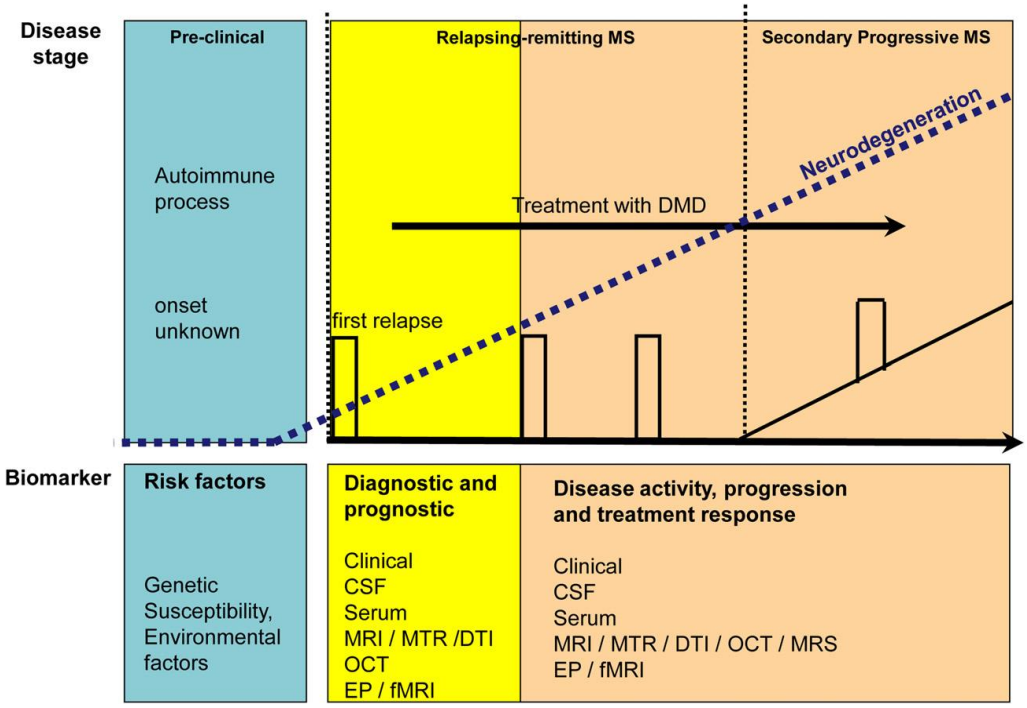


Figure 5.1 Biomarkers for MS related to the disease stage
[RE-USE PERMISSION by Elsevier, © 2011, (Ziemann, 2011)]

By developing new biomarkers we would like to address one further important issue in ongoing MS-research: *The high prevalence of anxiety and depression in MS-patients*. This higher prevalence compared to the general population is proposed to result from the actual disease burden (lesions, brain atrophy), coping, conceptions of the self and illness, and stress (Jones, 2012; Arnett, 2008; Feinstein, 2011). In terms of disease burden particularly cognitive impairment, i.e. attention deficits, problems in information processing and memory is highly affecting the social functioning and quality of life in MS-patients (Rao, 1991; Amato, 2006). Even the rate of suicide (as a result of severe depression) was reported to be seven times (Stenager, 1992) or twofold higher (Sadovnick, 1991) compared to that in the general population in the 1990s. More recent data from 2012 did no longer support these findings and did not find higher rates of suicide related deaths of MS-patients (Lalmohamed, 2012).

During our trials and in clinical practice we had the chance to interview our patients concerning their mental status, observing that most of them are sharing the problem of feeling insecure when evaluating their state of health. Frequently they reported difficulties in discriminating physiological mood changes and actual disease-related changes. Uncertainty is described as one independent predictor of depression in MS by Lynch et al., along with emotion based coping, loss of hope and the degree of disability (Lynch, 2001) (see textbox 5.1). This strongly supports our assumption that a robust biomarker for disease progression might contribute to a better psychological outcome by reassuring patients of drug efficacy, more efficient therapy regimes and hereby providing more certainty about the actual progress of the disease.

(1) Uncertainty
(2) Emotion based coping
(3) Loss of hope
(4) Degree of disability

Textbox 5.1 Independent predictors of depression in MS (Lynch, 2001)

Up-to-date, the reduction of relapse-rates was most frequently used to determine effects of disease modifying drugs. To conclude, there still is need for novel biomarkers to investigate long-term effects of these pharmaceutics on a microstructural level and thereby assure MS-patients of the drug efficacy.

6. Abstract

Title: Spatiotemporal dynamics of brain activation in multiple sclerosis patients and healthy control subjects: A TMS-EEG study

Aim: The aim of this study was to measure cortico-cortical connectivity in multiple sclerosis (MS) patients by TMS-evoked potential (TEP) latencies in EEG evoked by transcranial magnetic stimulation (TMS) of the hand area of the primary motor cortex of one hemisphere. TEPs were recorded on the stimulated- and at the homologue site in the non-stimulated contralateral hemisphere. Both interhemispheric directions were tested. Interhemispheric latencies of the two main reproducible TEPs, the positive component at 60 ms and the negative component at 100 ms (P60 and N100, respectively), were expected to be significantly prolonged in MS-patients compared to healthy volunteers.

Material and methods: The study compared interhemispheric propagation of P60 and N100 in groups of 12 patients with early-stage relapsing-remitting MS (RRMS) and 16 age- and gender-matched healthy controls. The study was approved by the Ethics Committee of the Medical Faculty of the Goethe-University of Frankfurt/Main and conformed to the latest revision of the Declaration of Helsinki of 2008. TEPs were recorded by means of EEG and their latencies were statistically evaluated in 10 channels around the stimulation site and in 10 corresponding electrodes in the non-stimulated contralateral hemisphere. Interhemispheric conduction time was calculated by the difference of TEP latency in non-stimulated vs. stimulated hemisphere.

Results: An ANOVA on interhemispheric conduction time showed a significant prolongation for the N100 from left to right hemisphere in MS compared to controls, while no group differences were found for the P60 and the N100 from right to left hemisphere.

Conclusion: The results provide first evidence that the N100 may constitute an interesting marker to measure interhemispheric conduction delays in early-stage RRMS. The specificity of the present finding and its relation to fiber tract pathology should be examined in further correlative analyses with diffusion tensor imaging and other structural MRI data.

7. Zusammenfassung

Titel: Raumzeitliche Dynamik kortikaler Aktivität bei Patienten mit Multiple Sklerose und gesunden Probanden: Eine TMS-EEG Studie

Fragestellung: Das Ziel dieser Studie war die Messung kortiko-kortikaler Konnektivität bei Patienten mit Multiple Sklerose (MS) mittels TMS-evozierter Potenziale (TEP) im EEG, welche durch transkranielle Magnetstimulation (TMS) im Handareal des primären Motorkortex ausgelöst werden. TEPs wurden auf der stimulierten und der nicht-stimulierten Hemisphäre aufgezeichent. Die Erregungsausbreitung bei rechter sowie linker Stimulation wurde untersucht. Wir erwarteten eine signifikante interhemisphärische Verzögerung der beiden reproduzierbaren Haupt-Latenzen, einer positiven Komponente bei 60 ms und einer negativen Komponente bei 100 ms (abgekürzt P60 und N100), beim Patientenkollektiv im Vergleich zu gesunden Probanden.

Material und Methoden: Die Studie vergleicht die interhemisphärische Leitung vom P60 und N100 in einer Gruppe von 12 Patienten mit früher schubförmiger MS (RRMS) und einer Vergleichsgruppe von 16 gesunden Probanden vergleichbaren Alters und Geschlecht. Die Studie wurde von der Ethikkommission des Fachbereichs Medizin der Goethe-Universität genehmigt und entspricht den Vorgaben der Deklaration von Helsinki von 2008. TEPs wurden mittels EEG aufgenommen und in 10 EEG-Kanälen über der stimulierten Region und in 10 entsprechenden EEG-Kanälen der gegenüberliegenden Seite statistisch ausgewertet. Die interhemisphärische Leitungszeit der TEPs entsprach der Differenz zwischen der TEP-Latenz der nicht-stimulierten und der stimulierten Hemisphäre.

Ergebnisse: Eine Varianzanalyse der interhemisphärischen Leitungszeit zeigte eine signifikante Verlängerung der N100-Latenz von der linken zur rechten Hemisphäre bei MS-Patienten verglichen mit der Vergleichsgruppe, wohingegen sich keine signifikanten Unterschiede für das P60 und N100 von der rechten zur linken Hemisphäre zeigten.

Schlussfolgerung: Unsere Ergebnisse deuten darauf hin, dass die N100-Latenz ein potentieller Marker zur Messung interhemisphärischer Leitungsverzögerung bei MS-Patienten in einem frühen Erkrankungsstadium darstellt. Die Spezifität dieser Beobachtung sollte in weiteren Korrelationsanalysen, z.B. mit diffusionsgewichteter- (DTI) und weiterer struktureller (MRT-) Bildgebung untersucht werden.

II

8. Road to the setup

"The road is always better than the inn" --- *Cervantes*

The following paragraph intended to describe stepwise the development of our experimental setup - in the descriptive style of a lab-diary. The TMS-EEG setup has been established for the first time at the lab in the course of this study. In this section our early attempts and landmarks for the recording are summarized.

Prof. Dr. Ulf Ziemann and his motor cortex group at the University Hospital of Frankfurt can list a considerable amount of TMS research, mostly on neural circuits and plasticity and their modulation by pharmacological intervention (Muller-Dahlhaus, 2008; Murakami, 2011; Cash, 2011). Main expertise and interest of Ulf Ziemann is investigating cortical networks by combined usage of TMS and brain active substances (Ziemann, 2004; Ziemann, 2011; Ziemann, 1996; Ziemann, 1996).

In the first pilot trials (see figure 8.1) conducted, we obtained data with a permanent, monomorph 50 Hz signal in all channels, and a physical (coil-) induced artifact resting about 25 ms and an amplitude of more than 3000μV. At this stage we did neither apply EEG filters, nor did we orient the electrodes radially away from the TMS- coil (Sekiguchi, 2011). Furthermore the position of the reference electrode was near the TMS- coil. Thus, in this stage, the long-lasting artifact did not allow further analysis. By comparison, in our final setup we were able to record TMS-EEG with an artifact resting 5-10 ms and an amplitude maximum of 20 μV.

With increasing experience we achieved better signal quality. We rearranged the wires according to Sekiguchi et al. (Sekiguchi, 2011) and we changed the reference electrodes to both mastoids (EEG-channels TP9 / TP10). Regarding the hardware, we have completed our experimental setup at this stage. We then focused on the software.

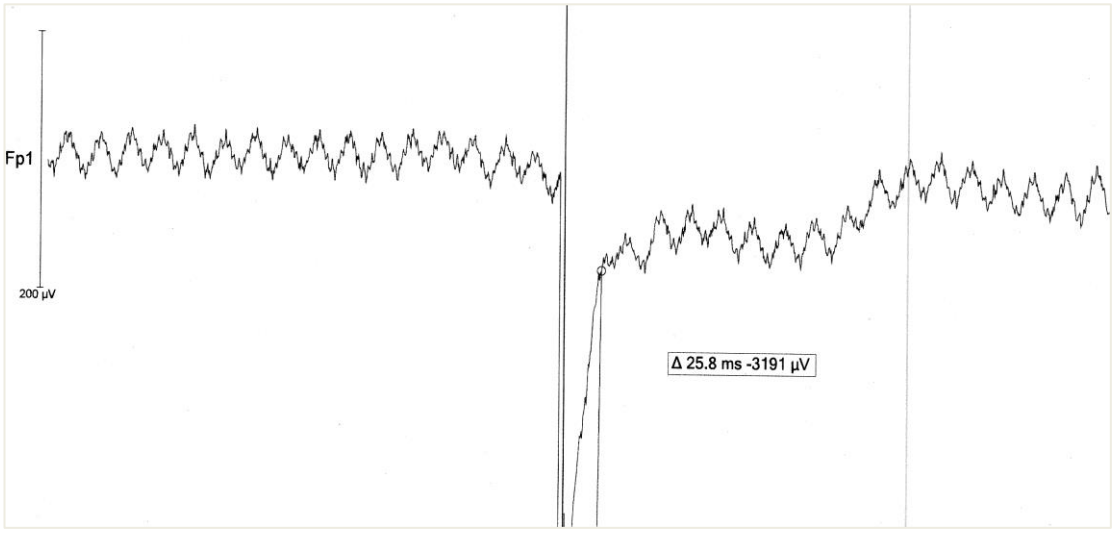


Figure 8.1 Early TMS-EEG recording, channel Fp1

The adoption of 'linear interpolation', an artifact-correction tool provided by the EEG-analysis software BrainVision Analyzer, was essential for the further analysis. Linear interpolation simulates a linear connection between a start- and endpoint (we selected a time range between -10 to 10 ms) to smoothen the initial artifact without affecting the raw signal. Importantly, we have confirmed that this way of correcting the artifact will not bias the final results by demonstrating that neither the power spectrum (range below 100 Hz) nor the wave morphology post-intervention were changed from pre- to post-correction (see figure 8.2, 8.3).

With the completion of the technical hard- and software setup we had to verify inner-subject reproducibility and inter-subject variability.

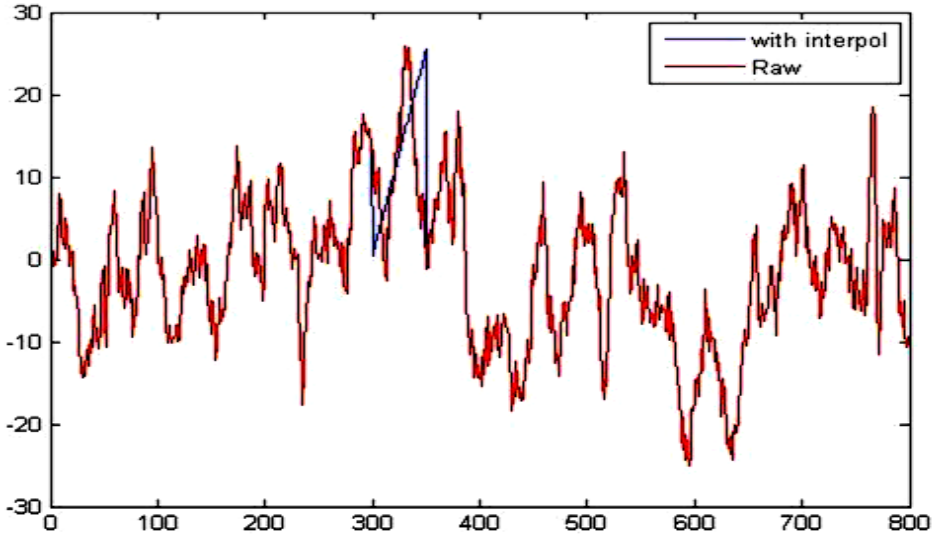


Figure 8.2 EEG raw signal morphology compared to interpolated data

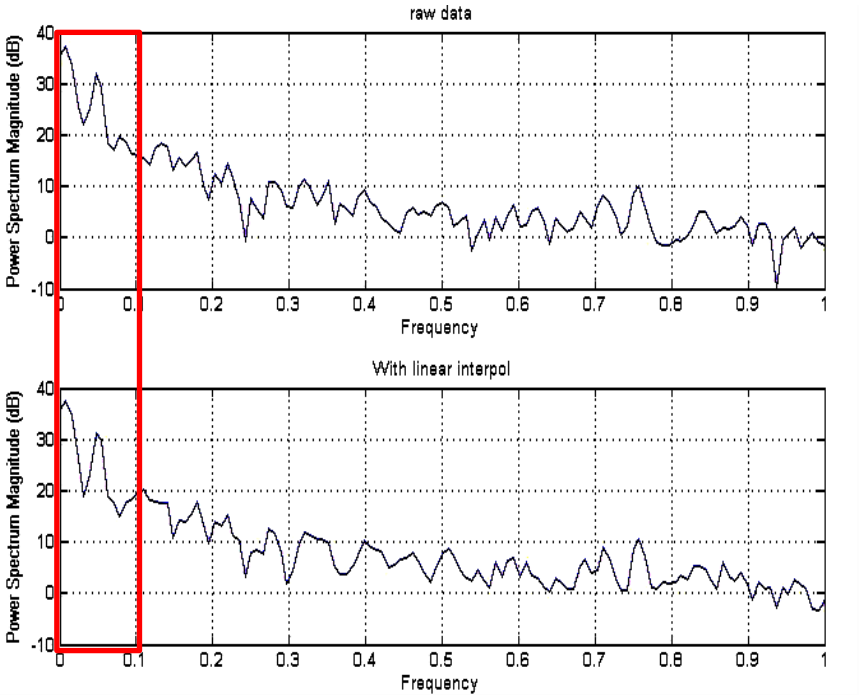


Figure 8.3 Analysis of the power spectrum in raw data vs. linear interpolation

We performed the pilot-experiment with four healthy, age- and gender balanced volunteers who underwent the planned TMS-EEG protocol three times under the same trial conditions. We then evaluated quantitatively the butterfly plots of each subject and across subjects. The following plots show the results of subject 0, in three consecutive trials (Figures 8.4 a-c), the last plot, subject 2, trial 1 (Figure 8.5), demonstrates the low inter-subject variability. We were able to identify the peaks in the time of interest (P60 and N100) which were described previously in literature (Lioumis, 2009; Ferreri, 2011). We then considered our setup to be suitable and reliable for our final projects.

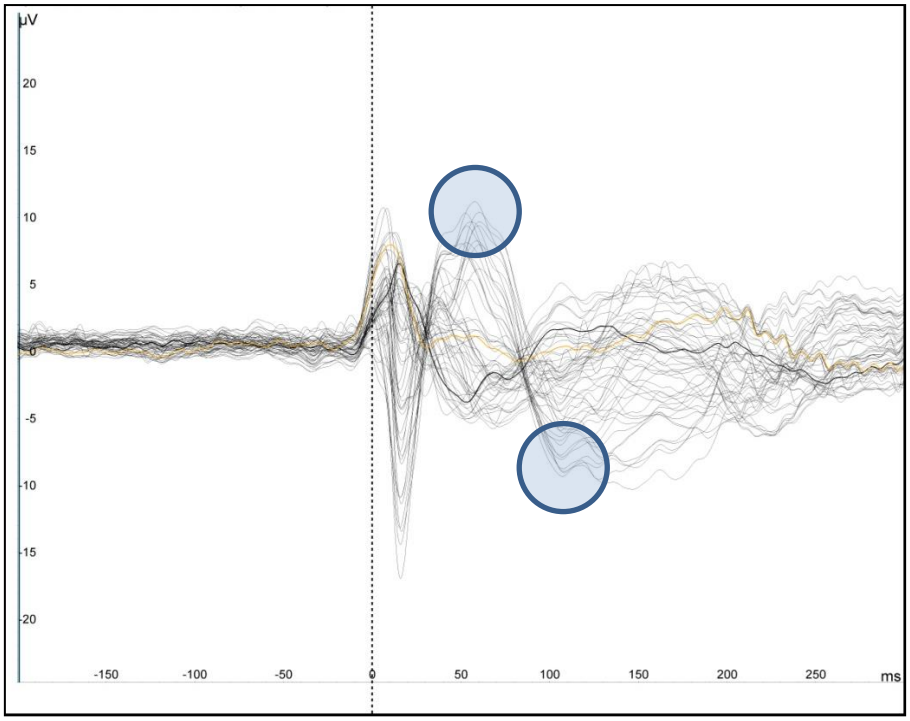


Figure 8.4a Grand average of Subject 0 (trial 1) for left-hemispheric stimulation; dotted line at 0 ms = stimulus application; x-axis: time in milliseconds, y-axis: amplitude in µV; light blue ellipsoids label the P60 and N100.

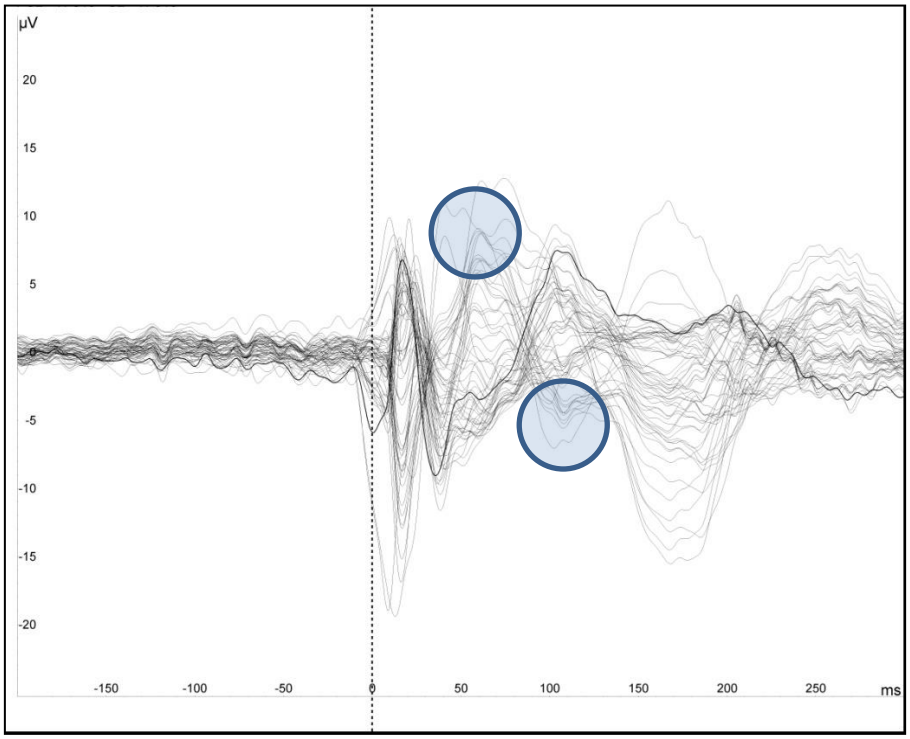


Figure 8.4b Grand average of Subject 0 (trial 2) for left-hemispheric stimulation; dotted line at 0 ms = stimulus application; x-axis: time in milliseconds, y-axis: amplitude in µV; light blue ellipsoids label the P60 and N100.

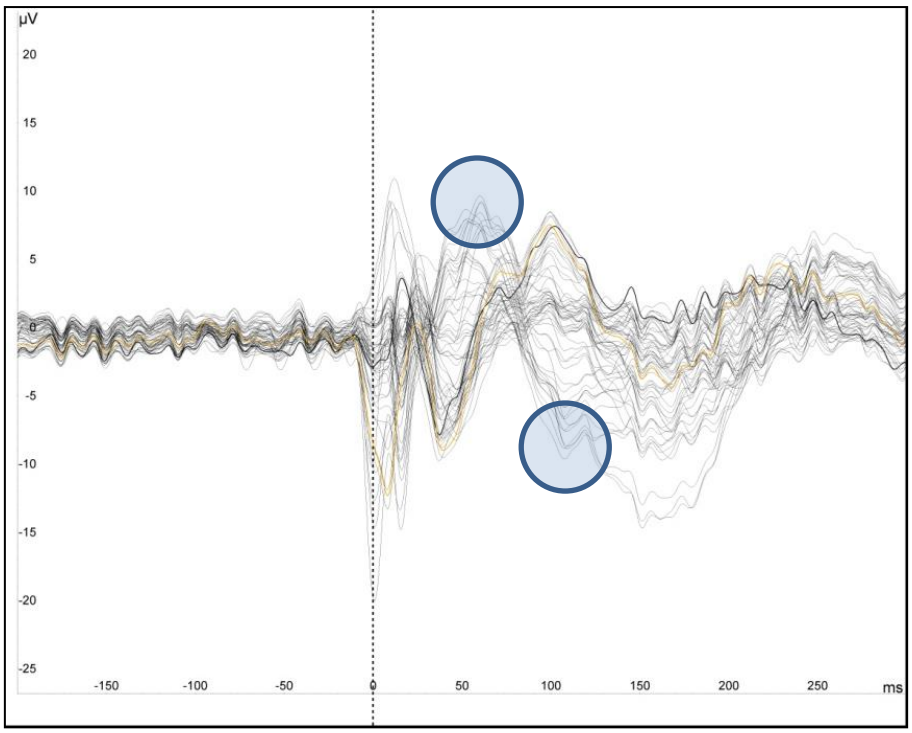


Figure 8.4c Grand average of Subject 0 (trial 3) for left-hemispheric stimulation; dotted line at 0 ms = stimulus application; x-axis: time in milliseconds, y-axis: amplitude in µV; light blue ellipsoids label the P60 and N100.

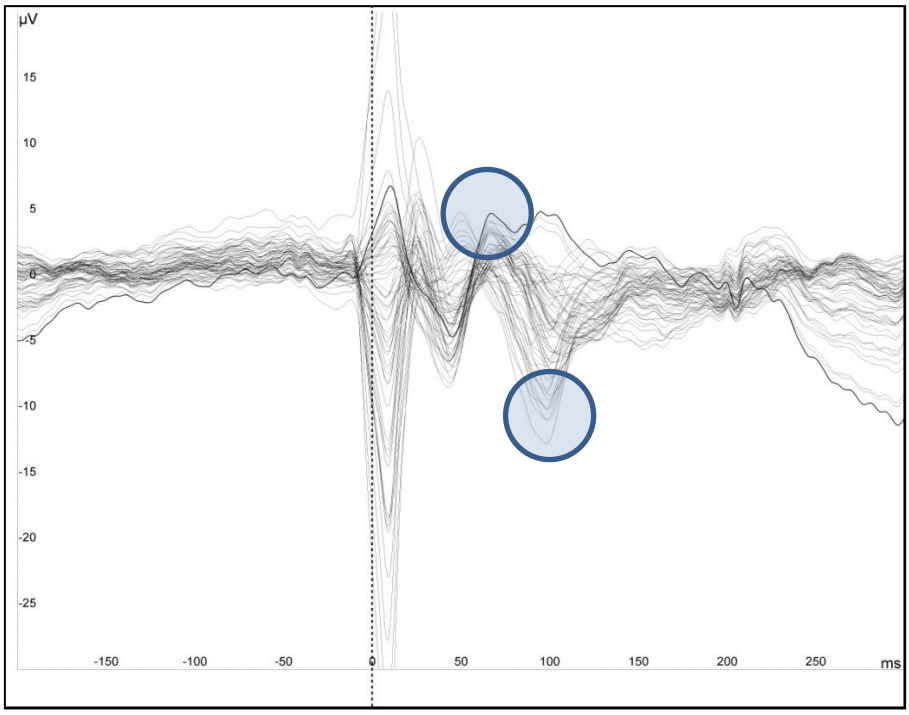
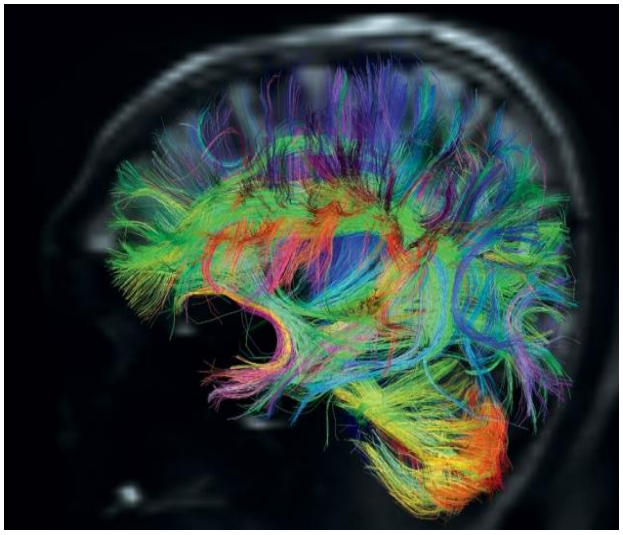


Figure 8.5 Grand average of Subject 2 (trial 1) for left-hemispheric stimulation; dotted line at 0 ms = stimulus application; x-axis: time in milliseconds, y-axis: amplitude in µV; light blue ellipsoids label the P60 and N100.

9. DTI



DTI, a non-invasive MR-imaging technique probing brain white matter microstructure, is a novel tool to investigate the structural integrity of the CC (Jones, 1999; Le Bihan, 2001). The detection of fiber tracts is based on the directionality of Brownian molecular water motion directionality (anisotropy) in brain tissue.

Figure 9.1 Fiber tracking with DTI [RE-USE PERMISSION by Nature Publishing Group, © 2012, (Bardin, 2012)]

DTI is a procedure to measure the anisotropic diffusion of H_2O in body tissue, with no need for ionizing radiation or radiocontrast agents. Basic physical law for this method is diffusion, spreading of particles in space. The integrity of space-limiting elements, such as the myelin in the central-nervous system, is influencing molecule movements. The basic parameters in MRI are Spin and Echo, whereas the spin is changed depending on the wall integrity. Thus, in demyelinating diseases such as MS the neuronal-integrity can be evaluated by DTI. Relative to non-organized fluid-filled brain components such as the ventricles, anisotropy is distinct in organized white matter fibers (Le Bihan, 2001), which is why the degree and direction of anisotropy serve as indicators for tissue organization. Evidently, disability in MS results at an early stage from axonal damage (De Stefano, 2001). Importantly DTI made acquisition of highly detailed structural images of cortical networks possible (figure 9.1) (Jones, 2002; Bardin, 2012).

Due to its color- and playful look, DTI images recently found their way into pop-culture (see figure 9.2). But they are as well subject of print media and media studies discussing their esthetic value (Reents, 2008).

Figure 9.2 6th studio album "The 2nd law" by British rock band Muse, 2012
[© 2012 by Warner Bros. Records / Human Connectome Project]

9.1 Image acquisition

DTI data acquisition was accomplished by means of the 3 T Magnetom Allegra MRI scanner (Siemens, Erlangen, Germany). Ms. Julia Dieter performed the scan, the author served as qualified (MR-) assistant. A diffusion-weighted SE-EPI sequence (TE=100 ms, TR=140 ms, 60 slices with a slice thickness of 2 mm, FOV=195 mm, 10 b_0-reference scans, b=1000 s/mm^2, voxel size =2x2x2 mm, 3 averages) was applied. A T1-weighted modified driven equilibrium Fourier transform (MDEFT) sequence served as anatomical reference scan.

9.2 Data analysis

In order to enable an FMRIB Software Library (FSL)-based DTI data analysis, DICOM-formatted scanner output files were converted to the NIfTI file format via MRIcron-to-NIfTI converter (general command: “mricron-dcm2nii <options> <name of first input image>”). Options were set in a way to enable anonymization and gzipping, whilst reorientation and cropping of data were deactivated. The image orientation of converted files was checked for correctness retrospectively by loading the FA map (created within the processing step of diffusion tensor calculation via the dtifit-command, see below) into FSLview and overlaying the λ_1-eigenvectors (also obtained via dtifit) as voxel-wise lines to this map. Secondly we obtained the λ_{2-} and λ_3 eigenvectors by dtifit.

Proper image orientation was given when the direction of the λ_1-eigenvectors within the corpus callosum matched the inherent horizontal arrangement and diffusion direction of corpus callosal white matter fibers. Subsequently, the three converted image files were merged in time (option "-t") into a single data file. Merging achieved stacking of all three dti-sequences' images, beginning with those having been acquired first (general command: "fslmerge <options> <name output file> <name input for merging1> <name input for merging2>…"). To serve the purpose of correcting for coil-induced eddy currents distorting the acquired images and head movements, an eddy current correction was accomplished. The operation was initiated by entering an adapted version of the general FSL command. Here, all images were corrected relative to the reference volume 0 of the merged image file. Afterwards, the merged image file was split again into the original partition in order to enable averaging across the three dti-sequences. Averaging was achieved by making use of fslmaths. The removal of non-brain image elements was accomplished by means of the brain extraction tool of the FSL toolbox (Smith, 2002).

The command's general scheme is "bet <name input image> <name output image> <options>". Here, the options of –m (request for brain mask generation), -f (fractional intensity; values ranging from 0 to 1; threshold determining the strength with which brain tissue is distinguished from non-brain structures; high f-factors set a stricter threshold leading to increased brain tissue removal) and –g [vertical gradient in fractional intensity threshold; values ranging from -1 to 1; enables the overall fractional threshold (controlled via f) to be varied linearly with slice number; high g values lead to cutting of upper slices] were defined. For the factors of f=0.2 and g=-0.1, the best compromise was found between having removed as much non-brain elements as possible and having cut away as less brain tissue as necessary. In order to achieve brain extraction for all volumes within the 4D-input multivolume file, the option of –F (a bet2 default variation) was applied additionally. In order to obtain information about diffusion processes within the white matter brain tissue, the diffusion tensor was calculated by fitting a diffusion tensor model at each voxel (Jung, 2010) ["dtifit -k data -m nodif_brain_mask -r bvecs -b bvals -o outputname", the input files were renamed to "data", "nodif_brain_mask", "bvecs" and "bvals" to be recognized by the program].
The processing steps outlined above build the basis for specific DTI data analyses.

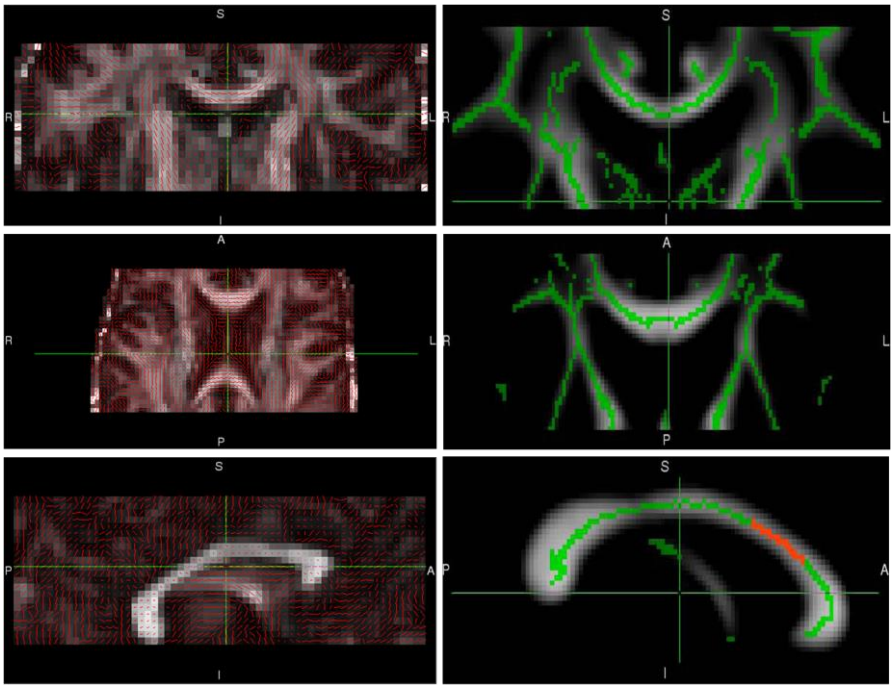


Figure 9.3 FA-map in frontal, transversal and sagittal view and TBSS quantification

9.3 DTI quantification

To reach the study aim, FA-values were extracted from the whole corpus callosum (CC) as well as from CC-segments via tract-based spatial statistics (TBSS) and FSL. Within the frame of TBSS, the output of the diffusion tensor model fitting (i.e. one FA-map per participant) was preprocessed (removal of outliers), nonlinearly registered to the FMRIB58_FA template and transformed to 1x1x1 mm standard space. All FA-maps were merged into a single 4D-file, averaged and skeletonized. Once created, the mean FA skeleton (representing fiber bundle centers common to all study participants; {Smith, 2006} was thresholded to 0.25 (Smith, 2006; Qiu, 2008; Damoiseaux, 2009). The threshold was decided based on subjective optical control in FSL view ensuring coverage of the mean FA map's main fiber tracts by the mean FA skeleton but preventing the latter to reach beyond the common tracts. For the extraction of whole-CC FA-values, a mask (ROI) was drawn upon the CC of the midsagittal slice within the mean FA skeleton map by means of FSLview. FA-values per participant were then obtained by applying the drawn mask upon the file containing all subjects' skeletonized FA-data. Next, based upon the mean FA-map, the CC was segmented according to the revised Witelson proposed by Hofer and Frahm (Witelson, 1989; Hofer & Frahm, 2006). Segment-masks were drawn upon the mean FA-skeleton's part lying within the

respective CC-segment. Both the mean FA map as well as the mean FA skeleton had been thresholded from 0.25 to 1 (Rimkus Cde, 2011). Segment-specific FA-values of each subject were extracted by applying the drawn mask upon the file containing all subjects' skeletonized FA-data.

Tract-based spatial statistics- (TBSS-) toolbox was favored for extraction of parameters compared to structural T2-weighted images because of its higher objectivity, lower variability and high comparability, which outweighs the loss of values in other regions than mean skeleton, demonstrated in figures 9.4 and 9.5. TBSS is a toolbox for aligning FA images from multiple subjects to a skeleton. The CC of different subjects varies in size and, particularly in MS-subjects, in integrity. Which is why selection of the actual CC is challenging and somehow arbitrary. Employing TBSS we aimed to objectify the chosen area, calculating the mean FA-skeleton in all subjects (see figure 9.3). Hereby we aimed to improve sensitivity and interpretability, as well as transferability of our test results.

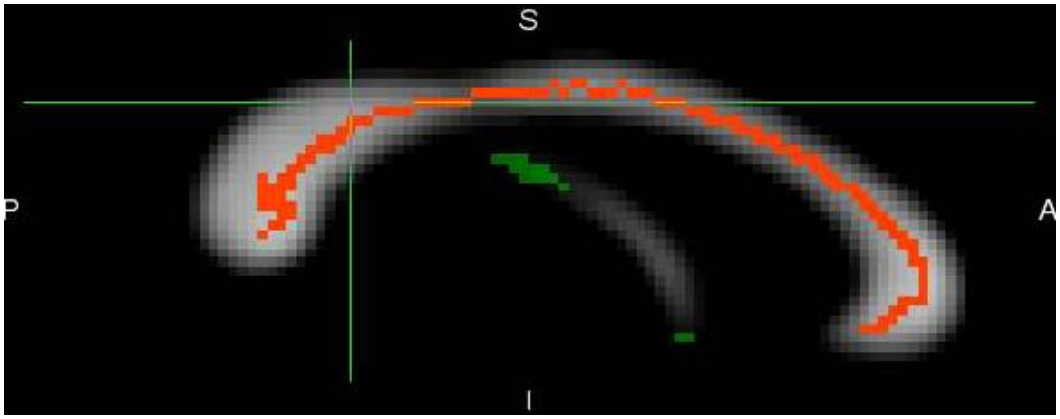


Figure 9.4 Extraction of CC-FA values via mean skeleton in TBSS

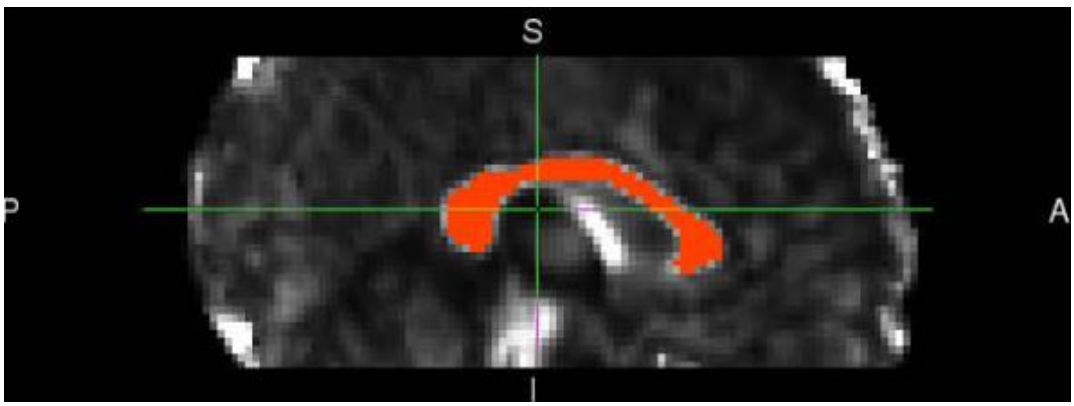


Figure 9.5 Extraction of CC-FA values via T2 weighted images

9.4 DTI troubleshooting

Due to an artifact, occurring rather homogeneously in all MS-subjects (see figure 9.6), we were not able to proceed with skeletonized FA-data, because it would then contaminate the mean values. It appeared in all MS-subjects, went from areas above the CC, crossed segments 1 and 2 (see figures 9.6 and 9.7), ending in subcallosal areas. We were then exploring reasons for the artifact. Henceforth we consulted assigned experts and neuroradiologists for identifying causes, without any conclusive explanation. Evidently it appeared during the recording and not within the quantification. Former studies from the department of Neurology of Frankfurt, employing the same imaging setup were not facing this problem (Hubers, 2012). Within this work we could not present the DTI-data due to the contamination by this artifact, which, very unfortunately, rendered further analysis using TBSS useless.

We therefore applied a manual voxel-wise approach of extracting FA-values of CC-regions 3 and 4. Hereby we avoid contamination by the artifact, which is limited to CC-region 1 and 2. Since region 3 is presumed to contain fibers projecting to the primary motor cortex it represents the most valuable region for investigation of interhemispheric motor connectivity (Hofer & Frahm, 2006). As region 4 is presumed to refer to primary sensory fibers we adopted this neighboring segment as a control region. This part of our analysis still is in progress at this point in time.

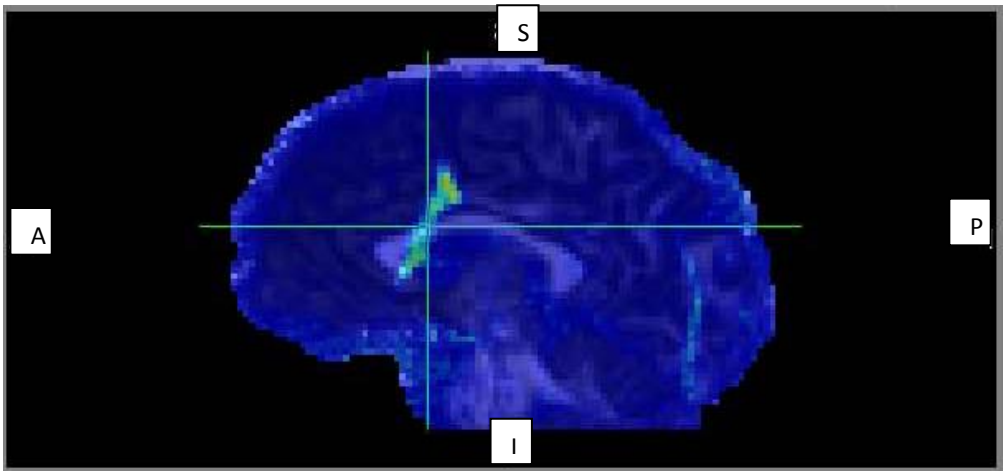


Figure 9.6 Artifact in DTI-recording

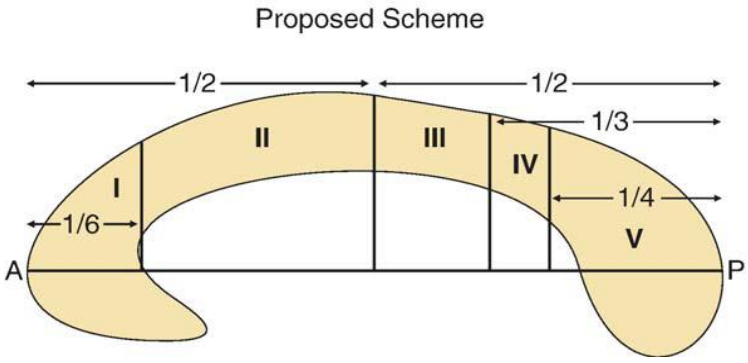


Figure 9.7 Corpus Callosum segmentation - according to the scheme by Hofer and Frahm, [RE-USE PERMISSION by Elsevier, © 2006, (Hofer & Frahm, 2006)]

9.5 Correlating DTI and electrophysiological data

We aimed to correlate the electrophysiological measures of the P60 and N100 latencies with following DTI parameters:

(i) FA (Obtained via FA-map)

(ii) MD (mean diffusivity = $(\lambda_{1+}\lambda_{2+}\lambda_3) / 3$)

(iii) RD (radial diffusivity = λ_1)

(iv) AD (axial diffusivity = $(\lambda_{2+}\lambda_3) / 2$)

For this purpose we employed a Pearson's correlation. This correlation is a statistical procedure for measuring the strength of association between two variables, e.g. TEP latencies and DTI-parameters. The Pearson's correlation coefficient (r) ranges from -1 to +1, means from very strong negative relationship (directing -1) to no relationship (towards 0) until very strong positive relationship (directing +1) (see fig. 9.8).

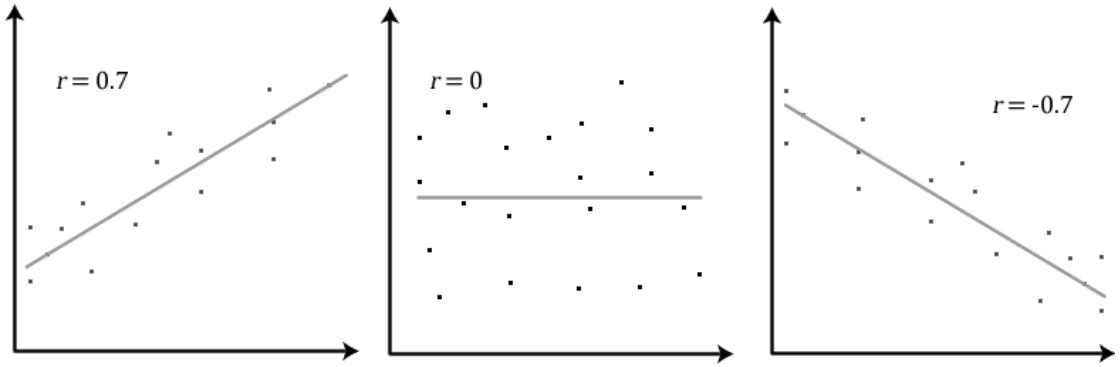


Figure 9.8 Pearson's correlation [© 2013 Laerd Statistics by Lund Research]

10. Abbreviations

ADHD	**attention deficit-hyperactivity disorder**
AD	**axial diffusivity**
AEP	**auditory evoked potential**
b	**diffusion-weighting factor**
CC	**corpus callosum**
CMCT	**central motor conduction time**
CNS	**central nervous system**
DMD	**disease-modifying drug**
DTI	**diffusion-tensor imaging**
EDSS	**expanded disability status scale**
EEG	**electroencephalography**
EMG	**electromyography**
EP	**evoked potential**
ERP	**event-related potential**
FA	**fractional anisotropy**
FOV	**field of view**
FSL	**FMRIB Software Library**
Hz	**hertz**
ISI	**inter-stimulus interval**
ITI	**inter-trial interval**
LICI	**long-interval intracortical inhibition**
M1	**motor cortex**
MD	**mean diffusivity**
MDEFT	**modified driven equilibrium fourier transform**
MEG	**magnetoencephalography**
MEP	**motor-evoked potential**
(f)MRI	**(functional) magnetic resonance imaging**
ms	**milliseconds**
MS	**multiple sclerosis**
MSO	**maximum stimulator output**
µV	**microvolt**
N+number	**negative deflection at time x**
NIfTI	**neuroimaging informatics technology initiative**

P+number	**positive deflection at time x**
PPMS	**primary progressive multiple sclerosis**
RD	**radial diffusivity**
RMT	**resting motor threshold**
ROI	**region of interest**
RRMS	**relapsing-remitting multiple sclerosis**
SE-EPI	**spin-echo echo planar imaging**
SEM	**standard error of the mean**
SEP	**somatosensory evoked potential**
SPMS	**secondary progressive multiple sclerosis**
SPSS	**statistical package for the social sciences**
TBSS	**tract-based spatial statistics**
TE	**echo time**
TE_2	**transfer entropy**
TEP	**TMS-evoked potential**
TMS	**transcranial magnetic stimulation**
TOI	**time of interest**
TR	**repetition time**

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12. Erklärung

Schriftliche Erklärung

Ich erkläre ehrenwörtlich, dass ich die dem Fachbereich Medizin der Johann Wolfgang Goethe-Universität Frankfurt am Main zur Promotionsprüfung eingereichte Dissertation mit dem Titel

Spatiotemporal dynamics of brain activation
in multiple sclerosis patients and control subjects:
A TMS-EEG study

im Zentrum der Neurologie der Universitätsklinik der Goethe-Universität, Arbeitsgruppe Motorkortex, unter Betreuung und Anleitung von Prof. Dr. med. Ulf Ziemann mit Unterstützung von Dr. med. Florian Müller-Dahlhaus ohne sonstige Hilfe selbst durchgeführt und bei der Abfassung der Arbeit keine anderen als die in der Dissertation angeführten Hilfsmittel benutzt habe. Darüber hinaus versichere ich, nicht die Hilfe einer kommerziellen Promotionsvermittlung in Anspruch genommen zu haben.

Ich habe bisher an keiner in- oder ausländischen Universität ein Gesuch um Zulassung zur Promotion eingereicht*. Die vorliegende Arbeit wurde bisher nicht als Dissertation eingereicht.

Vorliegende Ergebnisse der Arbeit werden veröffentlicht von:

Zipser, CM; Premoli, I; Castellanos, N; Rivolta, D; Dieter, J; Heidegger, T; Mueller-Dahlhaus F; Ziemann U

_____ _____

(Ort, Datum) (Unterschrift)

13. Curriculum vitae

Carl Moritz Zipser
Geb. am 05.09.1986 in Frankfurt

AUSBILDUNG

Seit 08/2006	**J.W. Goethe Universität, Frankfurt** Doppelstudium der Humanmedizin und Philosophie
03/2009	1. Abschnitt der ärztlichen Prüfung
Seit 12/2010	**Arbeitsgruppe Motorcortex, Frankfurt** Dissertation zum Dr. med. bei Herrn Prof. Dr. U. Ziemann
01-06/2011	**Université Claude Bernard, Lyon 1** Auslandssemester Humanmedizin
1994-2005	**Elisabethengymnasium, Frankfurt** Abitur

STUDIENFÖRDERUNG

03/2013	**Reisestipendium der deutschen Gesellschaft für klinische Neurophysiologie (DGKN)**
01-10/2012	**Promotionsstipendium der FAZIT-Stiftung** Gemeinnützige Verlagsgesellschaft GmbH
01-06/2011	**Erasmus-Stipendium Université Claude Bernard, Lyon**

ARBEITSERFAHRUNG

12/12 – 07/2013	**Hospital zum Heiligen Geist Frankfurt** Praktisches Jahr in der inneren Medizin und Chirurgie
09-12/2012	**Universitätsklinikum Frankfurt** Praktisches Jahr in der allg. Neurologie und Schlaganfall-Station
04-05/2011	**Hôpital Neurologique Pierre Wertheimer, Lyon** Famulatur in der Neuro-Onkologie und Schlaganfall-Station

02-04/2011 **Centre Hospitalier Le Vinatier, Lyon**
Famulatur in der Psychiatrie

08-10/2010 **Universitätsklinikum Frankfurt**
Famulatur in der allgemeine Neurologie
Famulatur in der diagnostischen und interventionellen Radiologie

05-06/2010 **Columbia University Medical Center/ NY Presbyterian Hospital, New York City**
Famulatur in der Kardiologie/Intensivmedizin

08-09/2007 **Hôpital Européen Georges-Pompidou, Paris**
Krankenpflegepraktikum in der Kardiologie

WISSENSCHAFTLICHE ERFAHRUNG

03/2013 **Jahrestagung der DGKN**
Poster-Präsentation auf dem Kongress der deutschen Gesellschaft für klinische Neurophysiologie (DGKN)

06/2012 **Rhine-Main Neuroscience network (rmn²)**
Poster-Präsentation der Dissertation auf dem biennal meeting

05/2011 **TMS Summer School/ University of Oxford**
Teilnahme an der zweitägigen TMS Summerschool 2011

11/09-02/10 **Institut für medizinische Mikrobiologie, Frankfurt**
Studentische Hilfskraft im Praktikum für medizinische Mikrobiologie und Krankenhaushygiene

10-12/2008 **Dr. Senckenbergische Anatomie, Frankfurt**
Wissenschaftliche Hilfskraft Anatomie - Unter Anleitung von Prof. Dr. T. Deller, Betreuung einer Gruppe von 15 Studierenden

SPRACHKENNTNISSE

Englisch, fließend. IELTS 7.5/ 8
Französisch, fließend. C1
Spanisch und **Japanisch**. Grundkenntnisse

SOZIALES ENGAGEMENT

09-10/2008 **Drogenhilfe VANDU, Vancouver, B.C., Kanada**
Ehrenamtliche Tätigkeit in der Drogenhilfe

2005-2006, **Albert-Griesinger Schule für praktisch Bildbare, Frankfurt**
Zivildienst – Unterstützung und Begleitung der Lehrkräfte

KULTURELLE INTERESSEN

Musik, Literatur, Kunst, Sport

Frankfurt am Main, 04.11.2013

14. Study documents

14.1 Study information and informed consent for MS-patients (separate documents for control group participants were provided)

PATIENTENAUFKLÄRUNG

Titel der Studie:

Untersuchung kortiko-kortikaler Konnektivität mittels TMS-EEG und MRT bei Patienten mit Multipler Sklerose im Vergleich mit gesunden Probanden

Protokoll-Nr.: 01-2012

Prüfarzt (Ihr Ansprechpartner):
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Studienleiter:
Prof. Dr. Ulf Ziemann
Leitender Oberarzt der Klinik für Neurologie der
Goethe-Universität Frankfurt am Main
Schleusenweg 2-16
60528 Frankfurt am Main

Sehr geehrte Studienpatientin,
sehr geehrter Studienpatient,

Ihr Studienarzt bietet Ihnen an, an einer klinischen Prüfung teilzunehmen. Sie erhalten mit dieser Patientenaufklärung Informationen zur Studie. Nehmen Sie sich ausreichend Zeit, um diese sorgfältig durchzulesen und Ihrem Studienarzt bzw. seinen Mitarbeitern eventuelle Fragen zu stellen. Entscheiden Sie erst anschließend, ob Sie an der Studie teilnehmen möchten oder nicht.

Warum wird diese Studie durchgeführt?

Sie leiden an Multipler Sklerose und sind nach Meinung Ihres behandelnden Arztes geeignet, an einer klinischen Prüfung teilzunehmen. Bei dieser Erkrankung ist auch die Verbindung zwischen den beiden Hirnhälften (Balken, Corpus Callosum) betroffen. Die transkranielle Magnetstimulation in Kombination mit Elektroenzephalographie (TMS-EEG) stellt ein nicht-invasives und schmerzfreies Verfahren dar, mit dem die Verbindung zwischen den Hirnhälften gemessen werden kann. Ziel dieser Studie ist es, die Grundlagen der Multiplen Sklerose genauer zu untersuchen. Hierbei wird insbesondere der Frage nachgegangen, inwieweit die Ergebnisse des TMS-EEG mit Ergebnissen der Magnetresonanztomographie (MRT) zusammenhängen. Es handelt sich nicht um eine therapeutische Studie, aber die Untersuchungen können der Vorbereitung klinischer Studien dienen, die neue Kontrollmöglichkeiten für die Wirksamkeit der MS-Therapie ermöglichen.

Informationen zu dieser Studie

Es sind Gründe denkbar, die gegen Ihre Teilnahme an dieser Studie sprechen. Der Studienarzt wird diese mit Ihnen besprechen. Die Studie besteht aus zwei Versuchstagen, die jeweils ca. 2-3 Stunden dauern werden. Zwischen den Versuchstagen liegt jeweils ein Abstand von mindestens einer Woche. Gegebenenfalls kommt in einigen Fällen ein zusätzlicher Besuch vor Beginn der Studie hinzu, an dem Ihre Eignung für die Studie untersucht wird.

An den einzelnen Versuchstagen werden mehrere Messungen und Stimulationsprotokolle in unterschiedlicher Reihenfolge durchgeführt werden. Diese sind im nächsten Abschnitt erläutert. Sie werden diese Erklärungen benötigen, um den Studienablauf zu verstehen.

Transkranielle Magnetstimulation (TMS) mit Elektroencephalographie (EEG)

Bei der transkraniellen Magnetstimulation handelt es sich um eine nicht-invasive (d.h. Sie werden dabei nicht gestochen oder sonst irgendwie verletzt) Technologie, bei der mit Hilfe eines Magnetfelds Bereiche des Gehirns mit schwachen Stromstößen stimuliert werden. Hierfür werden Oberflächenelektroden über einem kleinen Handmuskel aufgeklebt, um zu messen, welche Reaktion auf solche schwachen Stromstöße von den Muskeln generiert wird. Auf diese Weise können verschiedene Reaktionen gemessen werden, zum Beispiel die "motorische Schwelle" (= der kleinstmögliche Reiz, der noch an die für Bewegung zuständigen Teile des Gehirns weitergeleitet wird). Außerdem kann mit der Methode der TMS die Erregbarkeit der motorischen Hirnrinde bestimmt werden.
Zu Beginn jeder Studienvisite werden mittels TMS Ihre motorische Schwelle und Ihre aktive Erregbarkeitsschwelle ermittelt. Anschließend werden während der Visite zweimalig über etwa 15 Minuten je 150 TMS-Pulse appliziert werden. Gleichzeitig werden Hirnströme mittels EEG aufgezeichnet. Einen Großteil der Zeit wird die Vorbereitung der EEG-Kappe in Anspruch nehmen. Hierbei wird Ihnen eine Stoffkappe mit Öffnungen auf den Kopf gesetzt und ein Gel in die Löcher eingebracht. In dieser Zeit sitzen Sie auf einem komfortablen Stuhl in Halbliegeposition.

Magnetresonanztomographie (MRT)

Zusätzlich zur TMS-EEG wird eine etwa 45 min. umfassende MRT-Untersuchung stattfinden. Da bei der Untersuchung starke Magnetfelder wirken, findet die Messung in einem separaten Raum statt, der während der einzelnen Messungen geschlossen sein muss. Zudem müssen alle Metallgegenstände am Körper entfernt werden (Schmuck, Schlüssel, Gürtel, Reißverschlüsse, BHs mit Metallverschlüssen usw.). Als Bekleidung stehen bei Bedarf OP-Kittel und -Hosen zur Verfügung. Während der Untersuchung können Sie eventuell ein Engegefühl empfinden. Sie befinden sich in ständigem Sprachkontakt zum Untersucher und können die Untersuchung jederzeit abbrechen. Es sind jedoch grundsätzlich keine gesundheitlichen Risiken bekannt, die aus dem Experiment entstehen könnten.

Zu Beginn der Untersuchung werden Sie auf einer Liege Platz nehmen. Um brauchbare Daten zu gewährleisten, ist es unbedingt notwendig, dass Sie sich während der Messung sehr ruhig verhalten.

Wie ist der Ablauf der Studie, und was müssen Sie bei Teilnahme beachten?

Zu Beginn der ersten Visite wird Sie der Studienarzt zu Ihrer Krankengeschichte befragen, um zu prüfen, ob Sie für die Teilnahme an der Studie geeignet sind. Zu den Ausschlusskriterien gehören unter anderem neurologische oder psychiatrische Vorerkrankungen. Um mögliche Risiken zu vermeiden, dürfen sich in Ihrem Kopf keine Metallteile (z.B. Gefäßclips, Granatsplitter) befinden.

Wenn Sie an der Studie teilnehmen, wird im Detail Folgendes von Ihnen erwartet:

- Die Menge an koffeinhaltigen Getränken und Alkohol, die Sie während der Studie trinken dürfen, ist begrenzt. Ihr Studienarzt oder seine Mitarbeiter werden Sie genauer informieren.
- Sie sollten an den einzelnen Terminen ausgeruht erscheinen, da Müdigkeit Auswirkungen auf die Erregbarkeit der Hirnrinde hat.

Was geschieht während Ihrer Besuche beim Studienarzt?

Der Studienarzt wird in den beiden Visiten die folgenden Maßnahmen durchführen:

- Befragung zur Krankengeschichte und den Studieneinschlusskriterien
- Befragung zu Nebenwirkungen nach der letzten Visite
- TMS-EEG Messungen und -Stimulationen
- MRT-Messung

Welchen persönlichen Nutzen haben Sie von der Teilnahme an der Studie?

Sie werden durch die Teilnahme an dieser Studie außer einer ärztlichen Untersuchung voraussichtlich keinen persönlichen Gesundheitsnutzen haben. Es ist jedoch denkbar, dass die Ergebnisse der Studie zur Entwicklung neuer Biomarker zur Therapiekontrolle der Multiplen Sklerose beitragen.

Im Rahmen von bildgebenden Studien wie dieser werden erfahrungsgemäß bei ca. jedem fünften Teilnehmer von der Norm abweichende Befunde der Hirnstruktur festgestellt. Je nach Untersuchung wird angenommen, dass jedoch bei lediglich jedem 12. bis 100.

Teilnehmer einer Studie eine hirnstrukturelle Auffälligkeit gefunden wird, welche eine klinische Relevanz hat, d.h. eine Auswirkung auf Ihren gegenwärtigen oder zukünftigen Gesundheitszustand.

Es ist nicht Ziel dieser Studie, nach hirnstrukturellen Auffälligkeiten zu suchen. Die im Rahmen der Studie erhobenen strukturellen Hirnbilder kommen nicht der Wertigkeit von strukturellen Hirnbildern bei klinisch-diagnostischen Fragestellungen gleich, zudem erfolgt die Auswertung der strukturellen Bilder nicht notwendigerweise durch einen Fachmediziner. D.h. eine Nichterkennung auffälliger Befunde bedeutet keinen sicheren Ausschluss klinisch relevanter hirnpathologischer Befunde. Voraussetzung zur Teilnahme an dieser Studie ist Ihre Einwilligung, dass Ihnen klinisch relevante Befunde mitgeteilt werden sollen. Auf besonderen Wunsch des Teilnehmers können auch nicht klinisch relevante Zufallsbefunde zur Kenntnis gebracht werden. Im Falle der Entdeckung klinisch relevanter Befunde, erfolgt eine unverzügliche persönliche Mitteilung und es wird seitens der Untersucher für eine Möglichkeit zur klinischen Diagnostik Sorge getragen. Eine weitere Bedingung zur Teilnahme an dieser Studie stellt ein ausreichender Krankenversicherungsschutz dar, durch den die Kosten gedeckt sind, die im Falle einer bei Zufallsbefunden notwendigen weiteren Diagnostik bzw. Therapie entstehen.

Welche anderen Möglichkeiten haben Sie, wenn Sie nicht an der Studie teilnehmen?

Da die Studie lediglich zu Forschungszwecken durchgeführt wird, ist die einzige Alternative ein Verzicht auf die Teilnahme an der Studie.

Welche Risiken sind mit der Teilnahme an der Studie verbunden?

Die Magnetstimulation ist schmerzfrei und die elektromagnetischen Impulse, die für dieses Verfahren verwendet werden, sind harmlos. Durch die Entladung der Reizspule tritt ein Klick auf, vor dem Sie sich durch von uns gestellte Ohrstöpsel schützen können. Während oder nach der Messung können leichte Kopfschmerzen auftreten, die in der Regel ohne weitere Behandlung zurückgehen. Es gibt Personengruppen, die nicht an TMS-Untersuchungen teilnehmen dürfen. Um herauszufinden, ob Sie hierzu gehören, wird der Studienarzt vor Beginn der Studie einen separaten Fragebogen mit Ihnen durchgehen.

Die Untersuchung mittels Elektroencephalographie (EEG) hat keine bekannten Nebenwirkungen, ebenso die Untersuchung mittels Magnetresonanztomographie (MRT). Es gibt Personengruppen, die nicht an MRT-Untersuchungen teilnehmen dürfen. Um herauszufinden, ob Sie hierzu gehören, wird der Studienarzt vor Beginn der Studie einen separaten Fragebogen mit Ihnen durchgehen.

Die Experimente haben keine Auswirkungen auf Ihre Lebensführung.

Bitte teilen Sie den Mitarbeitern der Prüfstelle alle Beschwerden, Erkrankungen oder Verletzungen mit, die im Verlauf der Studie auftreten. Falls diese schwerwiegend sind, teilen Sie den Mitarbeitern der Prüfstelle diese bitte umgehend mit, ggf. telefonisch.

Gibt es eine Nachbeobachtung bzw. Nachuntersuchung?

Nach Abschluss der Messungen werden Sie am Ende jedes Studientages zu Nebenwirkungen befragt. Sollten dabei Auffälligkeiten festgestellt werden oder sollten Sie sich in irgendeiner Weise unwohl oder beeinträchtigt fühlen, wird Ihr Studienarzt Sie bitten, solange im Untersuchungsraum unter Beobachtung zu bleiben, bis Sie sich wieder gut fühlen bzw. bis die festgestellten Auffälligkeiten abgeklungen sind. Es werden lediglich gesunde Probanden in die Studie eingeschlossen.

Wer darf an dieser Studie nicht teilnehmen?

Sie können an dieser Studie nur teilnehmen, wenn Sie an Multipler Sklerose erkrankt sind, die Ein- und Ausschlusskriterien erfüllen und sich nicht gleichzeitig für andere Studien oder andere klinische Forschungsprojekte zur Verfügung stellen oder bis vor kurzem an solchen teilgenommen haben.

Entstehen für Sie Kosten durch die Teilnahme an der Studie? Erhalten Sie eine Aufwandsentschädigung?

Durch Ihre Teilnahme an dieser Studie entstehen für Sie keine Kosten. Für Ihre Teilnahme an dieser Studie werden Sie wie folgt bezahlt: Sie erhalten 10 Euro pro abgeleisteter Stunde. Falls Ihr Prüfarzt Sie aus der laufenden Studie ausschließt, z.B. aus medizinischen Gründen, werden nur die bereits stattgefundenen Visiten bezahlt. Für die für die Studie erforderlichen Untersuchungen und Leistungen dürfen die Krankenkassen nicht belastet werden.

Werden Ihnen neue Erkenntnisse während der Studie mitgeteilt?

Sie werden über neue Erkenntnisse, die in Bezug auf diese Studie bekannt werden und die für Ihre Bereitschaft zur weiteren Teilnahme wesentlich sein können, informiert. Auf dieser Basis können Sie dann Ihre Entscheidung zur weiteren Teilnahme an dieser Studie überdenken.

Kann Ihre Teilnahme an der Studie vorzeitig beendet werden?

Ihre Studienteilnahme erfolgt freiwillig. Sie haben die Möglichkeit, die Beteiligung an der Studie ohne Angabe von Gründen abzulehnen bzw. Ihre Teilnahme zu jedem Zeitpunkt zu beenden. Daraus entstehen Ihnen keine Nachteile. Darüber hinaus kann Ihre Beteiligung auch durch den Studienarzt unabhängig von Ihrer Einwilligung beendet werden (z.B. falls bei Ihnen eine andere Behandlung erforderlich wird, Sie den Studienplan nicht einhalten, eine studienbedingte Gesundheitsschädigung auftritt oder ein anderer wichtiger Grund vorliegt).

Sollte Ihre Teilnahme an der Studie vorzeitig beendet werden, so werden Ihre bis dahin verwendeten Daten in der Art und Weise, wie unter „Wie werden meine persönlichen Daten geschützt?“ beschrieben, weiterverwendet, sofern dies zur Auswertung der Studie oder zur Wahrung Ihrer schutzwürdigen Interessen erforderlich ist.

Wie werden Ihre persönlichen Daten geschützt?

Vergleichen Sie hierzu bitte die Angaben zum Datenschutz auf Seite 7 (Einverständniserklärung).

An wen wenden Sie sich bei weiteren Fragen?

Sie haben stets die Gelegenheit zu weiteren Beratungsgesprächen mit dem auf Seite 1 genannten oder einem anderen Prüfarzt.

Einverständniserklärung und Datenschutz

Titel der Studie

Untersuchung kortiko-kortikaler Konnektivität mittels TMS-EEG und MRT bei Patienten mit Multipler Sklerose im Vergleich mit gesunden Probanden

Name des Studienteilnehmers in Druckbuchstaben:.....

Teilnehmer-Nr.:.....

Ich erkläre mich bereit, an der Studie teilzunehmen.

- Ich bin von Herrn / Frau (Dr. med.)_____ ausführlich und verständlich über mögliche Belastungen und Risiken sowie über Wesen, Bedeutung und Tragweite der Studie, sowie die sich für mich daraus ergebenden Anforderungen aufgeklärt worden. Ich habe darüber hinaus den Text der Probandenaufklärung und dieser Einverständniserklärung gelesen und verstanden. Aufgetretene Fragen wurden mir vom Prüfarzt verständlich und ausreichend beantwortet.
- Ich hatte ausreichend Zeit, Fragen zu stellen und mich zu entscheiden.
- Ich werde den ärztlichen Anforderungen, die für die Durchführung der Studie erforderlich sind, Folge leisten. Ich behalte mir jedoch das Recht vor, meine freiwillige Mitwirkung jederzeit zu beenden, ohne dass mir daraus Nachteile entstehen.

Datenschutz

Ich bin mit der Aufzeichnung der im Rahmen der Studie an mir erhobenen Daten und ihrer anonymisierten Verwendung, z. B. für Veröffentlichungen, einverstanden.

Eine Kopie der Probandenaufklärung und der Einverständniserklärung habe ich erhalten. Das Original verbleibt beim Prüfarzt.

_____ _____

(Datum, Unterschrift d. Teilnehmers) (Datum, Unterschrift d. Arztes/Ärztin)

Fragen zur Mitteilung bei Zufallsbefunden in der Magnetresonanztomographie

Titel der Studie

Untersuchung kortiko-kortikaler Konnektivität mittels TMS-EEG und MRT bei Patienten mit Multipler Sklerose im Vergleich mit gesunden Probanden

Name des Studienteilnehmers in Druckbuchstaben:.....

Teilnehmer-Nr.:.....

Ich bin damit einverstanden, dass mir im Rahmen der Studie erhobene klinisch relevante Zufallsbefunde mitgeteilt werden.

Anmerkungen:

(Falls z.B. klinisch nicht relevante Zufallsbefunde mitgeteilt werden sollen, ist dies unter Anmerkungen festzuhalten)

Ich erkläre, dass für meine Person ein hinreichender Krankenversicherungsschutz besteht, durch den die Kosten gedeckt sind, die im Falle einer bei klinisch relevanten Zufallsbefunden notwendigen weiteren Diagnostik bzw. Therapie entstehen.

_____ _____

(Datum, Unterschrift d. Teilnehmers) (Datum, Unterschrift d. Arztes/Ärztin)

14.2 Declaration of anonymity for study participants

Hiermit bestätigen wir, dass betroffene Personen über die Weitergabe ihrer pseudonymisierten Daten im Rahmen der Dokumentations- und Mitteilungspflichten nach § 12 und § 13 an die dort genannten Empfänger aufgeklärt werden.

Betroffene Personen, die der Weitergabe nicht zu stimmen, werden nicht in die klinische Studie eingeschlossen.

14.3 Case report form

Studie: Untersuchung kortiko-kortikaler Konnektivität mittels TMS-EEG und MRT bei Patienten mit Multipler Sklerose im Vergleich mit gesunden Probanden

Teilnehmer-ID _____
Datum _____
Visite _____

Neurologische Untersuchung

Kann der Teilnehmer die Studienvisite durchführen?

Sind nach der letzten Studienvisite Nebenwirkungen aufgetreten?

TMS_EEG

RMT

Besonderheiten während der Messungen?

MRT

Besonderheiten während der Messungen?

Neurologische Untersuchung: Änderungen im Vgl. zur Baseline?

Kann der Teilnehmer aus medizinischer Sicht entlassen werden?

Begleitung des Probanden notwendig?

Kommentar

14.4 Detection of handedness

Edinburgh Handedness Inventory

Bitte kreuzen Sie an, welche Hand Sie bei folgenden Aktivitäten benutzen. Nur wenn Sie beide Hände etwa gleich häufig benutzen, wählen Sie bitte "**Beide**". Wenn Sie für eine bestimmte Tätigkeit niemals die andere Hand benutzen, wählen Sie bitte "**Nein**".

Wenn Sie...	**Welche Hand benutzen Sie?**		
	Links	**Rechts**	**Beide**
...schreiben:			
...malen:			
...etwas werfen:			
...eine Schere benutzen:			
...sich die Zähne putzen:			
...ein Messer benutzen (ohne Gabel):			
...einen Löffel benutzen:			
...einen Besen benutzen (obere Hand am Stiel):			
...ein Streichholz anzünden:			
...eine Schachtel öffnen (Hand am Deckel):			

_____

Datum, Unterschrift Prüfarzt

14.5 MRI Screening form

Klinikum der Goethe-Universität

Brain Imaging Center (BIC)

Schleusenweg 2-16

60528 Frankfurt am Main

Sehr geehrte Probandin, sehr geehrter Proband,

wegen des sehr starken Magnetfeldes, das in der Kernspintomographie eingesetzt wird, möchten wir Sie bitten, die folgenden Fragen sorgfältig zu beantworten.

Name:_____

Geburtsdatum:_____

Gewicht:_____kg.

Zutreffendes bitte ankreuzen:

Haben Sie Fieber?	Ja	Nein
Tragen Sie einen Herzschrittmacher?	Ja	Nein
Sind Sie schon einmal operiert worden?	Ja	Nein
Sind in ihrem Körper evtl. Metallplatten, Schrauben, Drähte? (z.B. nach einem Knochenbruch)	Ja	Nein
Hatten Sie schon einmal eine Metallsplitterverletzung? (z.B. am Auge)	Ja	Nein
Haben Sie Implantate? (z.B. Shunt, Cochlea-Implantat)	Ja	Nein

Haben Sie Tätowierungen, Piercings oder Permanent-Make-Up? (dies könnte evtl. zu Verbrennungen führen)	Ja	Nein
Haben Sie einen Neurostimulator oder eine Insulin- oder Morphinpumpe?	Ja	Nein
Tragen Sie ein Hörgerät? (dieses sollte zur Untersuchung herausgenommen werden)	Ja	Nein
Tragen Sie Zahnersatz zum Herausnehmen? (dieser sollte zur Untersuchung herausgenommen werden)	Ja	Nein
Haben Sie einen Retainer (Metalldraht z.B. hinter den Schneidezähnen)	Ja	Nein
Sind Sie geschminkt? (Wimperntusche könnte sich erhitzen, bitte schminken Sie sich ab)	Ja	Nein
Tragen Sie einen Bügel-BH? (Metallbügel können sich erhitzen, bitte legen Sie ihn ab)	Ja	Nein
Könnte eine Schwangerschaft bestehen?	Ja	Nein
Tragen Sie eine Spirale? (Kupferspiralen sollten nach der Untersuchung vom Frauenarzt kontrolliert werden)	Ja	Nein
Leiden Sie unter “Platzangst“? (z.B. beim Aufzug fahren)	Ja	Nein
Tragen sie Kontaktlinsen? (bitte weiche Kontaktlinsen herausnehmen, diese könnten evtl. an der Hornhaut kleben bleiben)	Ja	Nein

Bitte beachten Sie beim Betreten des Untersuchungsbereiches:

. Legen Sie alle Metallgegenstände ab (z.B. Taschenmesser, Feuerzeug, Kleingeld, Schlüssel, Haarnadel usw.). Diese könnten in den Magneten hineingezogen werden und zu Verletzungen führen bzw. eine Beeinträchtigung der entstehenden Bilder verursachen.

. Legen Sie Ihren Schmuck und Uhren. Uhren können im Magnetfeld stehen bleiben oder dauerhaft beschädigt werden.

. Nehmen Sie keine Scheckkarten oder Kreditkarten mit in den Untersuchungsraum, die Magnetstreifen werden unwiederbringlich gelöscht.

Ich bestätige hiermit, dass ich die obigen Fragen gewissenhaft beantwortet und die Patienteninformation zur Kenntnis genommen habe.

Datum:_____

Unterschrift:_____

14.6 TMS Screening form

TMS - Eignungsfragebogen für Erwachsene

Haben Sie jemals

1.) ...eine TMS-Anwendung bekommen?

2.) ...Nebenwirkungen durch eine TMS-Anwendung verspürt?

3.) ...einen Krampfanfall erlitten?

4.) ...einen Schlaganfall erlitten?

5.) ...eine Kopfverletzung oder eine Kopf-/Gehirnoperation gehabt?

6.) Tragen Sie Metall, z.B. in Form von Clips oder Splittern, irgendwo im Kopfbereich?

7.) Tragen Sie implantierte Geräte wie Herzschrittmacher, Insulin- oder Schmerzpumpen?

8.) Leiden Sie unter häufigen und/oder schweren Kopfschmerzen?

9.) Hatten Sie jemals eine Erkrankung des Hirns oder der Hirnhäute?

10.) Hatten Sie jemals eine andere Erkrankung, die zu einer begleitenden Erkrankung oder Verletzung des Gehirns führte?

11.) Nehmen Sie Medikamente ein?

12.) Für Frauen im gebärfähigen Alter: verwenden Sie eine sichere Verhütungsmethode?

13.) Gibt es in Ihrer Familie Fälle von Epilepsie/Krampfleiden?

14.) Haben Sie noch Fragen zur Transkraniellen Magnetstimulation?

Sollten Sie eine oder mehrere der Fragen mit „ja“ beantwortet haben, so bedeutet dies nicht automatisch, dass Sie an der Studie nicht teilnehmen können. Der Studienarzt wird diese Fragen nochmals ausführlich mit Ihnen besprechen und danach entscheiden, ob Sie in die Studie eingeschlossen werden können.

_____

Unterschrift Studienarzt Ort, Datum

14.7 Expense allowance for study participants

Ethik-Kommission des Fachbereichs Medizin
der Goethe-Universität Frankfurt
Haus 1, Theodor-Stern-Kai 7

60590 Frankfurt

Kompensation der Studienteilnehmer

Vollständiger Studientitel: Untersuchung kortiko-kortikaler Konnektivität mittels TMS-EEG und MRT bei Patienten mit Multipler Sklerose im Vergleich mit gesunden Probanden

Studienleiter: Prof. Dr. Ulf Ziemann

Hiermit wird erklärt, dass die Studienteilnehmer an o.g. Studie wie folgt aus Mitteln der Klinik für Neurologie (Kostenstelle 6580028) für ihre Studienteilnahme entschädigt werden. Es wird eine Aufwandsentschädigung von € 10.- pro begonnener Stunde erstattet. Diese Erstattung erfolgt unbar nach Abschluss aller Studienvisiten.

Datum, Unterschrift

_____

14.8 Author disclosure

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_____

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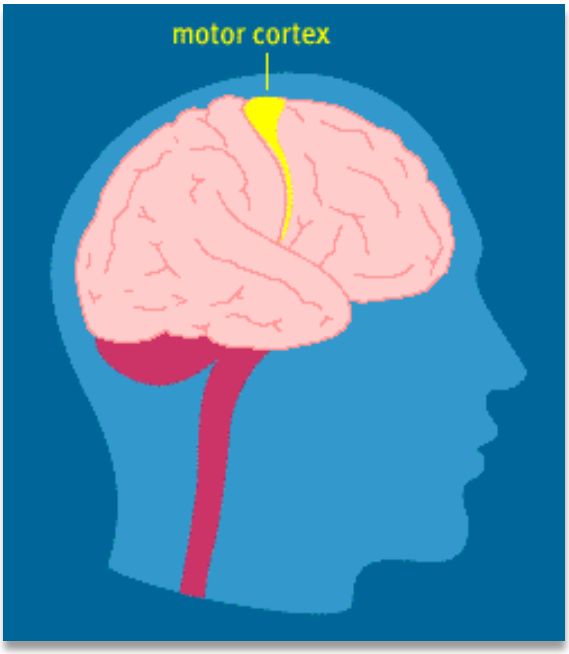

[PHOTO COURTESY of pbs.com, © 1998]

I would like to deeply thank my family: My mother, my father, my brother Daniel and my sister Marie, my brother-in-law Aleksis, my sister-in-law Linlin and my nephew Paul for supporting me all through my personal and professional life.

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"Fare thee well, and if for ever. Still for ever fare thee well." --- *Lord Byron*