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# *Effects of theta-burst transcranial magnetic stimulation over the human motor cortex: A neuroimaging study*

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| **A** | Adenine |
| **AC- PC** | Anterior comissure-posterior comissure |
| **ALS** | Amyotrophic lateral sclerosis |
| **AMPA** | α-Amino-3-Hydroxy-5-Methyl-4-isoxazolePropionic Acid |
| **AMT** | Active motor threshold |
| **ANOVA** | Analysis of variance |
| **APB** | Abductor pollicis brevis |
| **ASL** | Arterial spin labelling |
| **BA** | Brodmann area |
| **BDNF** | Brain-derived neurotrophic factor |
| **BOLD** | Blood oxygen level dependent |
| **BW** | Bandwidth |
| **C** | Carboxyl |
| **$Ca^{+2}$** | Calcium |
| **CaM** | Calmodulin |
| **CaMKII** | Calmodulin-dependent kinase II |
| **CASL** | Continuous arterial spin labelling |
| **cMAPs** | compound muscle action potentials |
| **cTBS** | continuous theta-burst stimulation |
| **D** | Direct |
| **dHb** | Deoxygenated hemoglobin |
| **e.g.** | Exempli gratia |
| **EMG** | Electromyography |
| **EEG** | Electroencephalography |
| **EPI** | Echo-planar imaging |
| **ε** | Epsilon (value) |
| ***F*** | Fisher (test) |
| **FDR** | False-discovery rate |
| **FWE** | Family-wise error |
| **fMRI** | Functional magnetic resonance imaging |
| **g** | Gram |
| **GABA** | γ-amino butyric acid |

| | |
|---|---|
| **GAD** | Glutamic acid decarboxylase |
| **GLM** | General linear model |
| **Glu** | Glutamate |
| **Glx** | Glutamate/glutamine |
| **G** | Guanine |
| **h** | Hour |
| **Hb** | Oxygenated hemoglobin |
| **Hz** | Hertz |
| **I** | Indirect |
| **i.e.** | id est |
| **IEGs** | Immediately early genes |
| **IH** | Intact hemisphere |
| **iTBS** | intermittent theta-burst stimulation |
| **LTD** | Long-term depression |
| **LTP** | Long-term potentiation |
| **M1** | Primary motor cortex |
| **MEP** | Motor evoked potential |
| **Met** | Metionine |
| **$Mg^{+2}$** | Magnesium |
| **min** | Minute |
| **ml** | Milliliter |
| **mm** | Millimetre |
| **MNI** | Montreal neurological institute |
| **MRI** | Magnetic resonance imaging |
| **MS** | Multiple sclerosis |
| **msec** | Millisecond |
| **mV** | Millivolt |
| **μs** | Microsecond |
| **μV** | Microvolt |
| ***n*** | Sample size (number of subjects) |
| **N** | Amino (terminal of a protein) |
| **NMDA** | N-methyl-D-aspartate |
| **PET** | Positron emission tomography |

| | |
|---|---|
| **PK** | Protein kinase |
| **PMA** | Pre-motor area |
| **PP** | Phosphatase pathway |
| **PV** | Parvalbumine |
| **rCBF** | Regional cerebral blood flow |
| **ROI** | Region of Interest |
| **RMT** | Resting motor threshold |
| **rTMS** | Repetitive transcranial magnetic stimulation |
| **RTs** | Reaction times |
| **s** | Second |
| **S1** | Primary somatosensory cortex |
| **S2** | Secondary somatosensory cortex |
| **SD** | Standard deviation |
| **SEM** | Standard error of the mean |
| **SH** | Stroke hemisphere |
| **SICI** | Short intracortical inhibition |
| **SICF** | Short intracortical facilitation |
| **SMA** | Supplementary motor area |
| **SNP** | Single nucleotide polymorphism |
| **SPECT** | Single-photon emission computerized tomography |
| **SPM** | Statistical parametric mapping |
| **TMS** | Transcranial magnetic stimulation |
| **TE** | Echo time |
| **TR** | Repetition time |
| **Val** | Valine |

# 1. INTRODUCTION

Transcranial magnetic stimulation (TMS) is a non-invasive technique able to modulate human cortical excitability not just at the site of stimulation, but also at remote areas. Several studies over the last years explored the therapeutic potential of repetitive pulses of TMS (rTMS) in the treatment of neurological and psychiatric disorders, but results are contradictory and its mechanisms are not completely understood (Fitzgerald et al. 2006). However, the effects of rTMS on cortical excitability seem to depend mainly on frequency and stimulation intensity (Chen et al. 1997; Maeda et al. 2000; Fitzgerald et al. 2002).

Recently, a new method of repetitive TMS called theta-burst stimulation (TBS) was developed (Huang et al. 2005). It requires lower stimulation intensity and a short stimulation time as compared to other rTMS protocols. The effects of TBS on motor cortex excitability have been mostly characterized by electrophysiological measurements of motor output showing differences in the individual response to stimulation (Huang et al. 2005; Huang et al. 2007; Gentner et al. 2008). Further, it has been suggested that the after-effects of rTMS stimulation are likely to be influenced by factors such as genetic variation. Thus, studying TBS effects should consider the different polymorphisms of genes involved in neuronal plasticity.

Little is known about cortical regions from a motor network that contribute to the modulation of excitability induced by rTMS. Given that functional magnetic resonance imaging (fMRI) does not require radiation, blood oxygenation level-dependent (BOLD) and arterial spin labeling (ASL) measurements are optimal methods to study the after-effects of rTMS. However, previous neuroimaging studies focusing on changes in rCBF or BOLD signal induced by conventional rTMS protocols over the primary motor cortex (M1) yielded ambiguous results.

Regarding TBS it remains unexplored, id est (i.e.) whether it affects the site of stimulation only or also remote regions, if the effects are present either during motor activity or at rest, and whether they rely on genetic factors. The elucidation of these factors would be helpful to monitor TBS treatment effects in further studies within a clinical framework.

## 1.1 Transcranial magnetic stimulation (TMS)

TMS is a technique of stimulating the brain through the intact scalp without generating strong pain. The use of TMS in the human brain was introduced by Antony Barker, who based his work on Faraday´s electromagnetic induction studies to stimulate the motor area corresponding to the hand muscles (Barker et al. 1985).

The equipment necessary for delivering TMS consists in a stimulator that generates brief pulses of electrical currents (peak 4000 Amperes after 110 μs) and a stimulation coil connected to the stimulator. Each pulse implies the pass of the current through the coil, which in turn induces a rapidly changing magnetic field. This magnetic field passes into the surrounding medium, where it again induces an electrical field and excites cortical neurons (Barker et al. 1985).The area of stimulation depends on the shape of the coil and the stimulation intensity.

There are basically two types of coils: round coils which are relatively non focal and figure-of-eight-shaped coils used to stimulate specific areas, producing maximal current at the intersection of the two round components. There are several variants of TMS, single pulse and rTMS are the most common modalities used in clinical studies.

### 1.1.1 Single pulse TMS

A single pulse TMS is useful to evaluate the cortical excitability and to understand changes in brain physiology such as cortical plasticity. After a single pulse over the hand motor area, a burst of activity is evoked lasting for 5-10 milliseconds (ms) and the targeting muscle of the opposite side responds with a slight twitch generating compound muscle action potentials (cMAPs) which reach an amplitude of several millivolts (mV) (Day et al. 1987). To elucidate this response, the coil must be positioned tangentially to the skull. After the pulse deliberation, the magnetic field passes through the skull and reaches the cortex and it is distributed parallel to the coil (Ridding and Rothwell 2007). Since the magnetic field decreases rapidly with distance, subcortical structures can not be stimulated directly (Barker et al. 1985). The mechanisms responsible for the after-effects of single pulse TMS are not completely known. Direct recordings of the corticospinal output showed that a series of volleys at a frequency of about 500 Hz is generated by one TMS pulse. The first volley, known as direct (D) - wave, is the result of direct activation of pyramidal tract neurons. It is only observed at higher stimulation intensities. Later waves are termed in the order of their appearance indirect (I) -waves I1, I2 and I3. They reflect synaptic activation of pyramidal neurons with interneurons, i.e. activity generated in the neural network of the motor cortex (Rothwell et al. 1991).The latency of indirect response is variable and at least approximately 1-2 ms longer than the direct one.

Motor cortical excitability is characterized in surface electromyographic (EMG) recordings considering cMAPs amplitude, also called motor evoked potential (MEP) amplitude (Figure 1). Basic values are the resting motor threshold (RMT) measured with relaxed muscles and the active motor threshold (AMT) established while the subject holds a slight activity of the targeted muscle (Rossini et al. 1994).

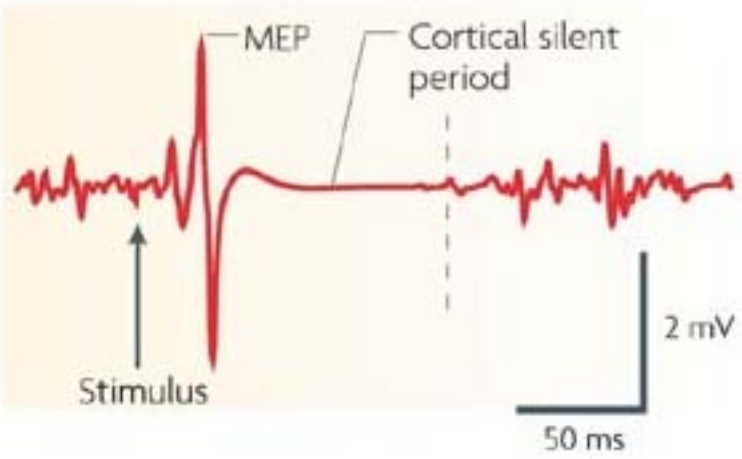



**Figure 1:** Electromyographic response of the hand muscle during a slight voluntary contraction to a single pulse of suprathreshold TMS. After the single pulse there is a motor-evoked potential (MEP) which reaches some millivolts (mV), followed by a transient silent period (SP) for some milliseconds (ms). The end of the SP is indicated by the dashed vertical line (taken from Ridding and Rothwell 2007).

### 1.1.2 Repetitive TMS (rTMS)

TMS pulse trains are able to trigger changes in cortical excitability for several minutes in analogy to those observed after electrical stimulation in hippocampal rat tissue (Mulkey and Malenka 1992; Malenka and Bear 2004). The modulatory effects of rTMS depend particularly on the intensity, frequency, train length, intertrain-interval, total number of magnetic pulses delivered in the stimulation session, as well as on the coil configuration, current direction, pulse waveform and position of the coil with respect to the cortex.

Pioneering studies on M1studying cMAPs amplitude evoked by single pulse TMS found that long lasting rTMS at 1 Herz (Hz) or lower causes a transient reduction in cortical excitability revealed by decreased cMAPs amplitude (Chen et al. 1997; Muellbacher et al. 2000; Gerschlager et al. 2001; Touge et al. 2001). In contrast, short rTMS trains at high frequencies ($\geq$5 Hz) increase cMAP amplitude (Pascual-Leone et al. 1994; Peinemann et al. 2000).

Although the modulatory after-effects of rTMS on the excitability of the motor circuitry have been mostly evaluated by using single pulse also paired-pulse (pp) TMS parameters have been used (Kujirai et al. 1993; Pascual-Leone et al. 1998; Ziemann 1999; Wu et al. 2000; Fitzgerald et al. 2002). Some pp-TMS parameters such as short intracortical inhibition (SICI) and short intracortical facilitation (SICF) explore inhibitory and excitatory interactions of different cortical regions within one hemisphere (for a review see (Reis et al. 2008). SICF is likely to be influenced by glutamatergic facilitacion modulated by γ–aminobutiric acid (GABA), whereas SICI is mediated by GABAergic activity (Ziemann et al. 1996; Ziemann 2004). However, neither changes in SICF nor SICI have been shown after conventional low and high-frequency rTMS protocols (Heide et al. 2006; Khedr et al. 2007).

Because of its potential for interfering with cortical function and for inducing plastic changes, rTMS has been widely evaluated as a therapeutic tool in several neurological and psychiatric disorders such as Parkinson's disease, Stroke, Epilepsy, Tinnitus, Depression and Schizophrenia (George et al. 1995; Tergau et al. 1999; Lee et al. 2005; Mansur et al. 2005; Kim et al. 2006).

However, the mechanisms by which rTMS modulates synaptic efficiency are to-date not completely understood. There is good evidence to support that observed changes in corticospinal excitability after rTMS occurs because of after-effects on cortical circuitry but not in the spinal cord or even at the neuromuscular junction (Berardelli et al. 1998; Di Lazzaro et al. 2008). Moreover, animal models demonstrated indeed that magnetic stimulation evokes changes in synaptic transmission directly (Ogiue-Ikeda et al. 2005; Ahmed and Wieraszko 2006).

A well-accepted assumption concerning the mechanism of synaptic modulation of rTMS is that the N-methyl-D-aspartate (NMDA)-receptors are involved in the rTMS after-effects (Ziemann 2004). This approach suggests that rTMS modulates cortical excitability by means of the activation of glutamate (Glu), NMDA and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, as is the case in the Long-Term Potentiation (LTP) and Long-Term Depression (LTD) processes of synaptic plasticity. Within this model, when Glu is released pre-

synaptically it binds both receptors and opens their channels. The NMDA receptor is permeable to calcium ($Ca^{+2)}$ influx once the postsynaptic depolarization is high enough to remove the magnesium ($Mg^{+2}$) that blocks it at rest. In the post-synaptic cell, there is a Calcium-binding protein called calmodulin (CaM). This protein mediates the $Ca^{+2}$ influx depending on the lobe of binding. A rapid increase in $Ca^{+2}$ binds to the carboxyl (C) lobe, and smaller concentrations bind to the amino (N) lobe. C-lobe binding triggers the activation of a protein kinase pathway, mainly protein kinase A (PKA), protein kinase C (PKC) and calcium-calmodulin-dependent kinase II (CaMKII) resulting in an increase of the conductance of the AMPA receptor and the addition of receptors into the membrane, facilitating the induction of LTP (Figure 2). Conversely, the N-lobe triggers a phosphatase pathway (especially phosphatase 1 and 2B) which induces a dephosphrylation of PKA or removes some phosphate groups from the AMPA receptor, causing a decrease in the number AMPA receptor and diminishing its permeability; as a consequence it triggers LTD of the synapse (Artola and Singer 1993; Malenka and Bear 2004).

The time course of LTP induction, stabilization and maintenance involves different pocesses. Immediately after stimulation or neuronal activity (phase 0), a specific group of transcription factors (i.e. the early growth response factors 1, 2 and 3) is rapidly elevated; $Ca^{+2}$, PKA, PKC, CaM activation and immediate early genes (IEGs) protein expression have also been reported in this short phase. Within one hour (phase 1), increases in NMDA-receptor subunits NR2A and NR2B, in AMPA-receptor phosphorylation and in synaptic Glu release capability have been observed. After some hours of LTP induction, further development of the transcriptional response occurs. This generates an increase in the receptor associated proteins at presynaptical and post-synaptical level such as brain-derived neurotrophic factor (BDNF). Changes found some days after induction include mainly maintenance of synaptic structure, contact area plus increased Glu receptor (Ouyang et al. 1999; Abraham and Williams 2003).

In-vitro studies have shown that rTMS at 15 Hz and 100 Hz induce a persistent increase in field excitatory post-synaptic potentials, which results in a stable LTP in

the rat hippocampus slices, while stimulation at a frequency of 1 Hz had no influence on LTP for at least 60 min. Additionally, it was found that the LTP was blocked by the preapplication of NMDA receptor antagonist D-AP5, suggesting the involvement of NMDA-receptor in this process (Ahmed and Wieraszko 2006; Tokay et al. 2009). In-vivo studies exploring the protein expression of IEGs have demonstrated that rTMS at 1, 10 and 25 Hz enhance c-fos protein in diverse regions of the rat including motor cortex, paraventricular nucleus of the thalamus and hippocampus, whereas 10 Hz rTMS increased Zif268 expression only in the M1 and sensory cortices and 1 Hz rTMS had no effect (Ji et al. 1998; Aydin-Abidin et al. 2008).

Several investigations have proposed that both low- and high-frequency rTMS might influence the BDNF production (Müller et al. 2000; Angelucci et al. 2004). Further, BDNF is of relevant relevance since it is the most abundant protein of the neurotrophins family (for review see Allen and Dawbarn 2006). It is involved not only in synaptic plasticity mechanisms such as LTP, but also in neurogenesis, survival for motoneurons, neuronal cell migration, response to motor skill training and to refinement of synaptic architecture (Gottschalk et al. 1998; Karege et al. 2002; Baquet et al. 2004; Klintsova et al. 2004; Laske and Eschweiler 2006). Taken all this together, if high frequency rTMS effects induce LTP, it is likely that the modulation induced imply several events in more than one structure that varies over time. Moreover, synaptic plasticity always involves interconnected areas, and it does not take place at a single site. Therefore, there are many interconnected areas that can produce forms of plasticity which might change with repetition (Izquierdo et al. 2006).

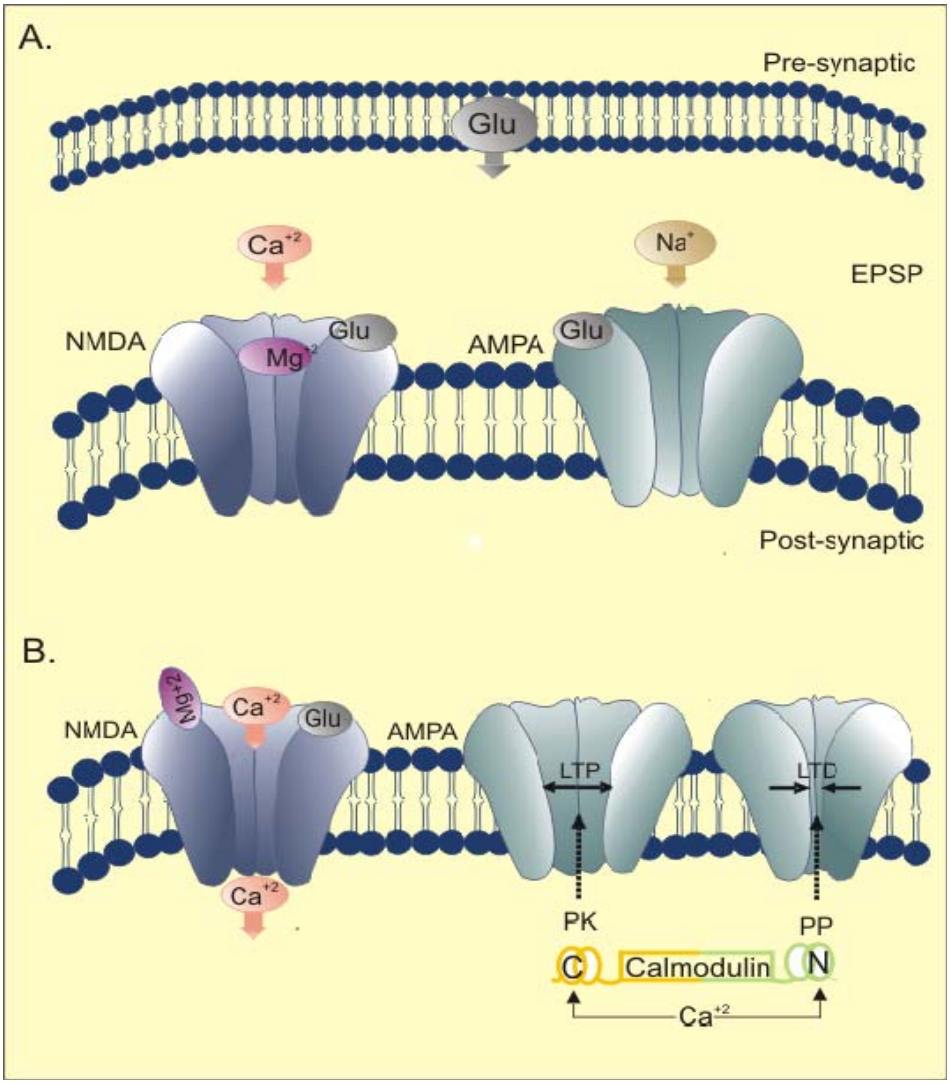



**Figure 2:** Long-term potentiation (LTP) and long-term depression (LTD) processes. Once glutamate (Glu) is released pre-synaptically, it binds N-methyl-D-aspartate (NMDA)- and to α-Amino-3-hydroxy-5-Methyl-4-isoxazole propionic acid (AMPA)-receptors and triggers Calcium ($Ca^{+2}$) and sodium ($Na^{+2}$) influx. After an excitatory post-tsynaptic potential (EPSP), calmodulin mediates the $Ca^{+2}$ influx depending on the lobe of binding. A rapid increase in $Ca^{+2}$ binds to the carboxyl (C) lobe of CaM, and smaller concentrations bind to the amino (N) lobe. C-lobe binding triggers the activation of a protein kinase (PK) pathway that induces an increment in the number AMPA receptors and hence it results in LTP. Conversely, the N-lobe triggers a phosphatase pathway (PP), removing phosphate groups from the AMPA receptor, causing a decrease in the number AMPA receptor and diminishing its permeability; as a consequence, it triggers LTD.

On the other hand, it is well known that motor cortical excitability is controlled by diverse inhibitory GABAergic interneurons (Markram et al. 2004). Within this group, a vast population of fast-spiking basket cells expressing the calcium-binding protein parvalbumine (PV) are recruited predominantly for motor execution, with pyramidal cells producing a command-like activity (Isomura et al. 2009).

Given the activation of excitatory and inhibitory neurons in the organization of voluntary movement and the capability of rTMS to enhance or reduce cortical excitability (depending on the protocol), it results challenging to analyze the effects of rTMS on inhibitory GABAergic cells. The expected potentiation of synapses between excitatory neurons that follows rTMS cannot be the only mechanisms involved; thus, it has been alternatively suggested that rTMS modulates also the GABA receptor activity (Thickbroom 2007; Stagg et al. 2009). Actually, evidence in animal data has demonstrated that low-frequency rTMS affects the expression of two forms of the glutamic acid decarboxylase (GAD) expressed in cortical inhibitory interneurons of the rat, which catalyze the decarboxylation of Glu to GABA (Trippe et al. 2009). 1 Hz rTMS reduced somatic expression of GAD-67 in frontal, motor, somatosensory and visual cortex, but increased pre-synaptic GAD-65 level after 2 hours (h) of stimulation. The decrease in GAD-67 was reversed after one day and led to increase up to one week. However, the increase in GAD-65 continued even after seven days. Further, a transient decrease in the number of fast-spiking neurons expressing Calcium-binding protein calbindin (CB) after 1 Hz rTMS was recently reported (Benali et al. 2009).This observation demonstrated that low-frequency rTMS targets specifically inhibitory neurons controlling the dendritic integration of inputs to pyramidal cells, since no changes in other Calcium-binding proteins expressed in different inhnibitory interneurons were found.

### 1.1.3 Theta-burst stimulation (TBS) pattern

TBS is a novel pattern of rTMS developed to produce after-effects of rTMS in the cerebral cortex (Huang et al. 2005). The main difference between TBS paradigm and conventional rTMS protocols is that a brief period of subthreshold stimulation (between 20 and 190 s) causes changes in cortical excitability that outlast the time of stimulation for at least 20-30 min. Huang proposed a TBS protocol that consists of bursts of 3 pulses given at 50 Hz repeated every 200 ms; thus, mimicking the coupling of theta and gamma rhythms (Figure 3). The stimulation intensity used in this protocol is 80% of the AMT. Two main modalities have been tested showing opposite effects on motor cortex excitability. The intermittent TBS (iTBS) consists of 1840 ms of stimulation repeated every 10 s for a total of 191.84 s (600 pulses). It induces a facilitation of cortical excitability reflected as an increase in cMAPs amplitude and SICI for 20 min. The second modality, called continuous TBS (cTBS) contains 3 pulses at 50 Hz repeated every 200 ms for 20 s or 40 s. It decreases cMAPs amplitude, SICI, and SICF for 20 or 60 min after application depending on the number of pulses (300 or 600 pulses) respectively. Thus, only the difference in grouping pattern of TBS determines the direction of modulatory after-effects. The after-effects of TBS on cMAPs amplitude and SICI turn into the opposite direction when testing the non-stimulated hemisphere, which might be caused as an effect of transcallosal cortico-cortical connections (Suppa et al. 2008). Both modalities of TBS induce after-effects which last longer than any other known short rTMS protocol (Table 1).

This TBS protocol was based on animal studies which have shown that bursts of 3-5 pulses at 50-100 Hz, repeated at 5 Hz (theta rhythm), induced LTP when applied to the motor cortex or hippocampus (Larson and Lynch 1986; Davies et al. 1991, Hess et al. 1996). The use of TBS relies on the observation that during locomotion and other voluntary motor behaviors both in animals and humans, theta oscillations are present in cortical areas (Kahana 2006). It has been suggested that theta-frequency oscillations modulate high-frequency gamma oscillations in human neocortex (Lisman and Idiart 1995). The coupling between theta and gamma rhythms coordinates activity in cortical areas and provides a

mechanism for an effective communication during cognitive processing in humans (Canolty et al. 2006).

**Table 1:** Comparison of different conventional patterns of repetitive magnetic stimulation

| | Conventional rTMS | | TBS | |
|---|---|---|---|---|
| **Type of effect** | **Excitatory [1] (5 - 20 Hz)** | **Inhibitory[2,3] (0.9 -1 Hz)** | **Excitatory[4] (intermittent)** | **Inhibitory[5] (continuous)** |
| **Number of pulses** | 20 - 50 | 180 -810 | 600 | 300 or 600 |
| **Intensity** | 110% - 200% RMT | 115% RMT | 80% AMT | 80% AMT |
| **Duration of effects** | 3 - 4 min | 15 min | 15 min | 20 - 60 min |

***Note:*** AMT= active motor threshold; Hz= Herz; min= minutes; RMT=resting motor threshold; rTMS= repetitive transcranial magnetic stimulation; TBS= theta-burst stimulation; [1] (Pascual-Leone et al. 1994); [2] (Chen et al. 1997); [3] (Wassermann et al. 1996); [4] and [5] (Huang et al. 2005).

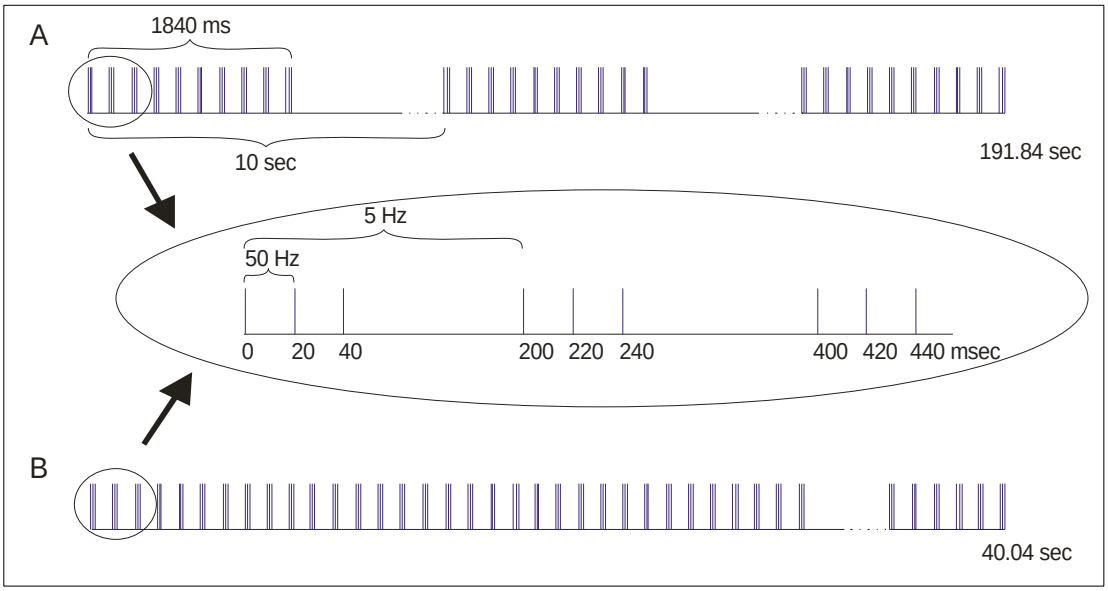



**Figure 3**: Different modalities of theta-burst stimulation (TBS): (A) intermittent TBS (iTBS) and (B) continous TBS (cTBS). TBS pattern applied in humans consists in 3 pulses delivered at 50 Herz (Hz) every 200 milliseconds (ms). The figure was taken from (Cárdenas-Morales et al. 2010).

The mechanisms underlying TBS are not completely understood but they might be at least partially similar to those involved in other rTMS protocols. There is evidence for the cortical origin of the after-effects of cTBS and iTBS in humans (Di Lazzaro et al. 2008b). In the referred study, using spinal epidural recordings the authors measured the corticospinal volleys evoked by single-pulse TMS and showed that cTBS decreased the amplitude of the earliest indirect (I)-wave, whereas iTBS increased later I-waves. They suggested that iTBS develops its after-effects by increasing the intrinsic circuitry of the motor cortex.

Studies in humans with drugs have demonstrated that the after-effects of iTBS and cTBS depend at least partially on NMDA receptor activity. In one study, the NMDA-receptor antagonist memantine was administrated and compared with placebo in healthy subjects. It blocked the effect of both cTBS and iTBS whereas placebo did not modify the after-effects of TBS (Huang et al. 2007). These results are in agreement with other studies in which the use of NMDA receptor antagonists suppressed induction of LTP and LTD in animal preparations and the training-induced motor cortical plasticity (Hrabetova and Sacktor 1997; Schwenkreis, 2005). In a second study, the NMDA-receptor partial agonist D-Cycloserine was administrated before iTBS. It was found that the facilitatory effect of iTBS was switched to inhibitory in presence of the drug. On the one hand, these findings reflect the participation of the NMDA receptor activity in the after-effects of TBS. On the other hand, these effects could be due to simultaneous effects of TBS (excitatory and inhibitory) with differing time courses and strengths (Teo et al. 2007). Additionally, studying rats it was shown that iTBS increased the expression of the IEGs protein Zif268 in all cortical areas, whereas it had no effect on c-fos expression, supporting the idea that iTBS after-effects are involved at least in the early phase of LTP (Aydin-Abidin et al. 2008).

Moreover, it is likely that TBS targets both excitatory and inhibitory networks within the motor cortex (Thickbroom 2007).The assumption is based on the finding that a priming stimulation 200 ms before a high-frequency burst decreases the effectiveness of inhibitory synapses at the time that the burst is delivered. This effect is mediated by GABAergic activity (Davies et al. 1991). Since TBS involves

stimulation at intervals of 200 ms (as a 5 Hz theta-frequency), it is likely that in the motor cortex this theta-frequency may be a means of reducing the efficacy of inhibitory inputs and facilitating activity-dependent up-regulation of excitatory networks. Intracortical inhibition tested by pp- pulse TMS was increased following iTBS and reduced after cTBS , whereas other rTMS protocols did not have any effect on it (Pascual-Leone et al. 1998; Wu et al. 2000; Fitzgerald et al. 2002; Daskalakis et al. 2006). Stagg et al. (2009), by using magnetic resonance spectroscopy demonstrated that cTBS to the M1 increased GABA concentration, but not glutamate/glutamine (Glx) levels. They suggested therefore, that GABAergic activity might be a mechanism by which long-lasting after-effects of cTBS on cortico-spinal excitability are generated (Stagg et al. 2009).

Animal studies have shown that in interneurons both TBS protocols reduced somatic expression of GAD-67 and increased pre-synaptic GAD-65 level after 2 h of stimulation. The decrease in GAD-67 and the increase in GAD65 were reversed after one day recovering baseline levels (Trippe et al. 2009). Regarding changes in Calcium-binding proteins, ITBS reduced the number of PV-positive cells without changes in CB expression (Benali et al. 2009). These findings lead to the assumption that iTBS affects the perisomatic inhibitory control of pyramidal cells, since PV-containing basket interneurons operate as a precision clockwork for gamma and theta oscillations, indispensable for cortical processing such as motor execution (Freund 2003). Further, cTBS significantly reduced the number of cells expressing CB, recovering to control level within one day, indicating that it affects transiently inhibitory neurons controlling the dendritic integration of inputs to pyramidal cells. These findings show how different TBS protocols lead to changes in the activity of different classes of inhibitory cortical neurons and how these effects vary over time (see Table 2).

**Table 2:** Acute and chronic effects of rTMS protocols on inhibitory interneurons

| | iTBS | | cTBS | | 1 Hz | |
|---|---|---|---|---|---|---|
| | *Acute* | *Chronic* | *Acute* | *Chronic* | *Acute* | *Chronic* |
| **Parvalbumin**[1] | ↓ | ↓ | ↔ | ↔ | ↔ | ↔ |
| **Calbindin**[1] | ↔ | ↔ | ↓ | R | ↓ | R |
| **Calretinin**[1] | ↔ | ↔ | ↓ | ↔ | ↔ | ↔ |
| **GAD67**[2] | ↓ | ↑ | ↓ | ↑ | ↓ | ↑ |
| **GAD65**[2] | ↑ | R | ↑ | R | ↑ | ↑ |

***Note:*** cTBS= continuous theta-burst stimulation; Hz= Herz; iTBS= intermittent theta-burst stimulation; GAD= Glutamic acid decarboxylase; R= recover to baseline level; ↓=decrease; ↑=increase; ↔=no change; [1]( Trippe et al. 2009) [2] (Benali et al. 2010)

### 1.1.4 Factors influencing the individual response to TMS

During the last years, genetic diversity in human population has been a crucial topic in clinical research. It has been hypothesized that common genetic variants may contribute to genetic risk for some diseases and that they might influence the subject's response to TMS (Kleim et al. 2006; Cheeran et al. 2008). One could speculate that a profound knowledge on genetic variants might help to predict whether participants will respond or not to magnetic stimulation and in which direction the modulation will take place.

The BDNF gene has been associated to the individual response to TMS. This gene has 13 exons and it encodes a precursor peptide (pro-BDNF) which in turn is cleaved to form the mature protein. A single nucleotide polymorphism (SNP) located at nucleotide 196 (guanine (G)/adenosine (A)) has been identified (Table 3). The result is an amino acid substitution Valine (Val)-to-Methionine (Met) at

codon 66, and it has been hypothesized that this SNP though located in the pro-BDNF alters intracellular processing and secretion of BDNF (Egan et al. 2003). In healthy subjects it has been associated with mild memory impairments, reduction in hippocampal and frontal cortical areas and some personality traits (Egan et al. 2003; Hariri et al. 2003; Pezawas et al. 2004). This Val66Met polymorphism could be also associated to psychiatric disorders such as depression and risk of schizophrenia, as well as to the pathogenesis of some neurodegenerative diseases, i.e. Alzheimer´s disease, Parkinson´s disease and amyotrophic lateral sclerosis (Egan et al. 2003).

**Table 3:** BDNF polymorfisms distribution within healthy people

| Author | Val/Val | Non-Val/Val |
|---|---|---|
| Egan et al. (2003) *n*= 133 | 79% | 26% |
| Pezawas et al. (2004) *n*=111 | 62% | 38% |
| Kleim (2006) *n*=78 | 63% | 37% |

***Note:*** BDNF= brain-derived neurotrophic factor; *n*= number of subjects; Val= Valine

The strong evidence, on the one hand, of a functional role for this BDNF common polymorphism, and on the other hand, the implication of this gene in LTP process yielded to analyze whether a BDNF genotype influences the response to TMS delivered over M1. Little is known regarding this topic. The first investigation by Kleim et al. (2006) demonstrated that the facilitation following the performance of fine-motor tasks, reflected as an increase in the amplitude of cMAPs, was more pronounced in Val/Val polymorphism carriers as compared to Val/Met or Met/Met carriers. A second study explored the inhibitory effect of the cTBS protocol in healthy carriers of different polymorphisms of the BDNF gene. The findings suggested that Val/Met or Met/Met (Non-Val/Val) carriers have a reduced

response to cTBS as compared to those subjects with Val66Val polymorphism (Cheeran et al. 2008).

Beside genetic variations a second factor influences the individual response to TMS: the physiological state of neurons at the time of stimulation. Synaptic plasticity can be modulated by prior synaptic activity. The direction and the degree of modulation seem to depend on the previous state of the network. This kind of plasticity is called metaplasticity (Abraham and Bear 1996; Turrigiano et al. 1998). For example, external stimulation that activates the resting network could decrease the same network if it was not at rest at the moment of stimulation. In animal models, it has been related to the NMDA- receptor activation, $Ca^{+2}$ influx, CaM, CaMKII and to modifications of inhibition of GABA release (Davies et al. 1991).

The phenomenon of metaplasticity has been demonstrated applying rTMS at cortical regions that have previously been modulated by means of cathodal or anodal transcranial direct current stimulation (Siebner et al. 2004). Huang et al. (2008) studied metaplasticity in healthy subjects. One-minute of muscular contraction of the abductor pollicis brevis (APB) during TBS over M1 suppressed the effect of the cTBS and iTBS effect on the cMAPS amplitude. When the contraction was hold immediately after TBS, it enhanced the facilitatory effect of iTBS and reversed the usual inhibitory effect of cTBS into facilitation. In a second study, the application of 300 pulses of cTBS facilitated cMAPs amplitude, whereas the same train of stimulation preceded by voluntary contraction of 5 min or 600 pulses of cTBS with the muscle at rest decreased it. The results suggest that 300 pulses of cTBS may have a similar mechanism than iTBS and may prime neuronal elements to undergo inhibition by the late cTBS with 600 pulses. Similarly, the change in the TBS effects before or after a muscular contraction provides evidence for metaplasticity of corticospinal excitability in the human M1. These findings must be considered when applying TBS in clinical trials (Gentner et al. 2008). A summary of the studies using TBS over the motor cortex in healthy volunteers is shown in Table 4.

**Table 4:** Effects of theta-burst stimulation on the motor cortex excitability evaluated by single pulse transcranial magnetic stimulation

| Author | Type TBS | Procedure | Effects on cMAP amplitude | Conclusion |
|---|---|---|---|---|
| Huang et al. (2005) *n* = 8 | i<br>c300<br>c600 | MEP size, short intracortical inhibition and facilitation | i = ↑ for 15 min<br>c600 = ↓ for 60 min<br>c300 = ↓ for 20 min | iTBS favoured facilitation<br>cTBS initially produced facilitation, but then inhibitory effects dominated |
| Huang et al. (2008) *n* =9 | i<br>c600 | slight muscle contraction during stimulation or 1 min contraction after TBS. | During contraction:<br>i= No effect<br>c= No effect<br><br>Contraction after:<br>i= ↑<br>c600 = ↑ | Contraction of the target muscle during or after TBS influences the size and direction of the after-effects of TBS |
| Huang et al. (2007) *n* = 6 | i<br>c300 | 4 sessions of TBS with add-on:<br>- NMDA-antagnist memantine<br>-placebo | Memantine blocked the after-effects of both TBS protocols | The findings lead to the conclusion, that after-effects of TBS on the motor cortex rely on NMDA receptor activity |
| Teo et al. (2007) *n* = 6 | i | Intake of:<br>-D-Cycloserine (agonist of the NMDA receptor)<br>- Placebo administrated one day before | D-Cycloserine inverted the classic facilitatory effect of i into an inhibitory effect | The after-effects of TBS depend at least partially on NMDA receptors |
| Talelli et al., (2007) *n* = 18 | i<br>c300 | cTBS and iTBS at 80 and 100% AMT with P-A or A-P current direction. | i= No changes in effect<br>c = ↓ lasted longer using A- P current direction | c300 with A-P current at a higher intensity is more effective to suppress cMAP. |
| Gentner et al. (2008) *n* = 14 | c300<br>c600 | The effects of c300 (at rest) & c300 preceded by a 1.5 or 5 min contraction were compared with c600 | c300 = ↑<br>c600 = ↓<br>5 min contraction prior to<br>c300 = ↓ | c300 did not induce depression on neuronal excitation unless conditioned by prior activity when applied on M1 (Hand) |

***Note*:** AMT= active motor threshold; A-P= antero-posterior; c300= continuous theta-burst stimulation with 300 pulses; c600= continuous theta-burst stimulation with 600 pulses; cMAP= compound motor action potential; i =intermittent theta-burst stimulation; M1= primary motor cortex; *n*= number of subjects included in the study; P-A= posterior-anterior; RMT= resting motor threshold; TBS= theta-burst stimulation.

### 1.1.5 Safety aspects of TMS and clinical use of TBS

Single-pulse TMS has shown to be safe. However, after the application of rTMS the induction of epileptic seizures without recurrence as well as other undesirable effects such as pain or headache has been described (Pascual-Leone et al. 1992; Classen et al. 1995; Nowak et al. 2006). In order to reduce the risk of induced seizures when delivering rTMS, safety guidelines have been developed in several studies (Pascual-Leone et al. 1993; Wassermann et al. 1996; Wassermann 1998). These guidelines list upper limits of stimulation parameters (i.e. frequency, intensity, duration and site of stimulation) as well as contra-indications to rTMS such as metal parts in the brain or implanted brain stimulators. Furthermore, they consider some ethical issues including medical and psychosocial management of induced seizures. Despite the potential risks of rTMS, in October 2008 the United States Food and Drug Administration approved rTMS for the treatment of patients with medically intractable depression. This approval based on a study with 301 patients suffering from major depression which had not benefited from medical treatment. All study patients tolerated four weeks of rTMS as antidepressive monotherapy (O'Reardon et al. 2007) without any severe side effects.

Concerning TBS, no induced seizure as major adverse event has been reported so far, suggesting that the risk to induce a seizure with TBS might be lower compared to conventional rTMS. An explanation for the reduced risk may be the lower stimulation intensity required and the shorter stimulation time (Huang et al. 2005; Cheeran et al. 2008; Di Lazzaro et al. 2008b; Hubl et al. 2008). When applied over prefrontal regions, the most prominent side effect has been the occurrence of vagal reactions during stimulation, without impact on mood (Grossheinrich et al. 2009). Nevertheless, a consensus with respect to optimal stimulation parameters is lacking at present, not least due to the fact that several study-groups have modified the original TBS protocols, changing the number of pulses, stimulation intensity and current directions (Franca et al. 2006; Nyffeler et al. 2006; Ishikawa et al. 2007; Talelli et al. 2007; Nowak et al. 2008; Stefan et al.

2008; Zafar et al. 2008). Further studies need to address the occurrence of vagal reactions and to explore the safety limits regarding seizures in both healthy subjects and patients by testing different intensities and number of pulses delivered over different brain regions. Some preliminary investigations suggest that TBS could be a tool for understanding the pathogenesis of some neurological disorders (i.e. multiple sclerosis, amyotrophic lateral sclerosis and dystonia), and its potential therapeutic use in movement disorders has been initiated to be evaluated with positive results (Table 5). In all these studies no adverse effects were observed and TBS was well tolerated by patients and healthy controls even when stimulation was repeatedly applied among several days, showing that TBS is a safe rTMS protocol (Di Lazzaro et al. 2006; Di Lazzaro et al. 2008a).

**Table 5:** Clinical studies applying theta-burst stimulation over the motor cortex

| Author | Type of TBS | Procedure | Conclusions |
|---|---|---|---|
| Di Lazzaro et al. (2006) *n*=15 | c<br>sham | In two groups of patients with ALS:<br>- Stimulation was delivered bilaterally 5 consecutive days, every month for six months | Patients who received cTBS deteriorated clinically slower as compared to the sham group. |
| Edwards et al. (2006) *n* =30 | c | A single session of cTBS was delivered in patients:<br>- DYT1-gene carriers with dystonia<br>- DYT1-gene carriers with torticollis<br>- control subjects | DYT1 gene carriers with dystonia and subjects with torticollis had a more prolonged response to cTBS. |
| Talelli et al. (2007) *n* =6 | i<br>c<br>sham | In patients after stroke:<br>- A single session of i or c<br>- cMAP and RMT were measured | RMT decreased and cMAP increased on stroke side after iTBS over SH improved motor behavior in paretic hands. cTBS did not have effects. |
| Di Lazzaro et al. (2008a) *n* =12 | i<br>c | In patients with stroke a single session of TBS in each hemisphere was delivered:<br>- i on SH<br>- c on IH | Both protocols increased the cMAP amplitude of the IH. |
| Codecà et al. (2008) *n* =10 | i | In MS patients with lower limb spasticity:<br>- A single session, or<br>- A treatment of two weeks | More significant improvement with two weeks of I during two weeks compared to a single session. |

***Note:*** ALS=Amyotrophic lateral sclerosis; c=continuous; cMAP= compund motor action potential; i= intermittent; TBS= theta-burst stimulation; IH= intact hemisphere; RMT= resting motor threshold; SH= stroke hemisphere; MS= Multiple Sclerosis.

## 1.2 Combining neuroimaging techniques and rTMS over the M1

The idea of creating brain maps for specific activities is not new. Early studies of the cerebral cortex used the distinctive cell size and architecture to define borders between cortical regions. The most classic analysis on the human brain was carried out by Brodmann in 1909, who divided the cerebral cortex into 47 cytoarchitectonic areas (Figure 4) which coincide partially with recent information on the functions of the cortex (Brodmman 1909). Penfield and Rasmussen proposed in 1950 an association of the motor and sensory cerebral cortex with body members including the hand not according to their actual size, but rather to the degree of subtlety in their movements. They created maps of the motor cortices of the brain known as homunculus and suggested that specific motor cortex regions exert influence on the activity of certain groups of muscles.

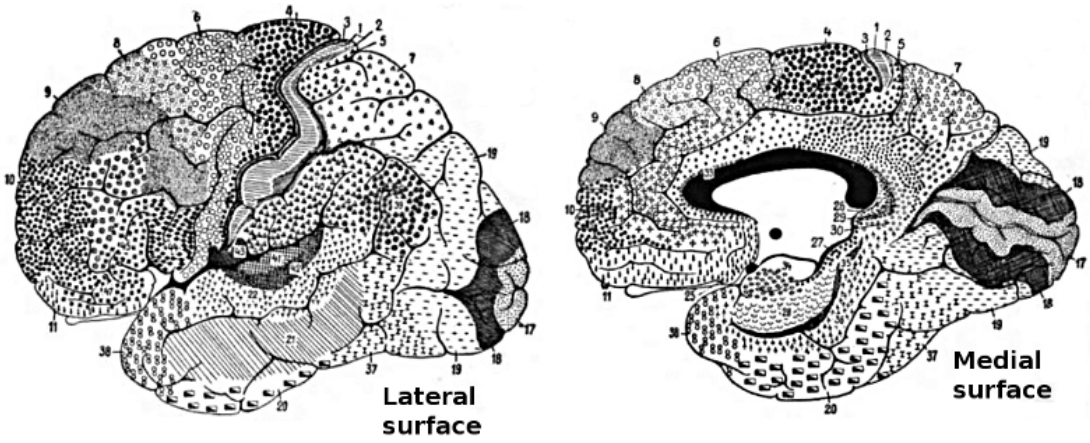



**Figure 4:** Brodmann's areas in the brain divided by cytoarchitectonic differences. Figure was taken from Penfield and Rasmussen (1950).

Later on it was feasible to map the activity of the living brain in space and time by means of a group of techniques known as functional neuroimaging. They include single-photon emission computerized tomography (SPECT), positron emission tomography (PET), and functional magnetic resonance imaging (fMRI). In advantage to the other methods, fMRI provides various approaches for visualizing brain activity without the exposure to radioactivity.

Two mapping techniques are mainly exploited for fMRI. The first is based on blood oxygenation level dependent (BOLD) signal (Ogawa et al. 1990). It results of an interaction between changes in blood flow, volume and oxygenation consumption accompanying neural activity (Buxton et al. 1998). Functional contrast is obtained because deoxygenated hemoglobin (dHb) is paramagnetic, thus, has a greater magnetic susceptibility than oxygenated hemoglobin (Hb). Paramagnetic dHb is confined in the intracellular space of the red blood cells that in turn are restricted to the blood vessels. In the resting state of a brain region, the majority of red blood cells in veins are deoxygenated. In contrast, in the active state, blood supply greatly exceeds oxygen demand and more of the red cells are oxygenated. The change in hemoglobin oxygenation produces a field distortion (0.5-5%) manifested as a signal intensity increase (Detre and Wang 2002). This increase in signal intensity has a good temporal resolution (in the order of seconds) and has been the basis for the majority of fMRI studies.

Although the mechanisms underlying the hemodynamic response remain under debate, it correlates with local-field potentials and reflects the post-synaptic input signals in a given area and may involve not only neurons but also astrocytes and vascular cells (Buxton et al. 1998; Magistretti and Pellerin 1999; Logothetis et al. 2001). It has to be considered that changes in excitation-inhibition activity strongly affect the regional metabolic energy demands and the concomitant regulation of CBF which in turn alters the fMRI signal. The limitations of BOLD response are related to the circuitry and functional organization of the brain, as well as to inappropriate experimental protocols that ignore this organization. Therefore, the accurate interpretation of BOLD signal partially depends on how effectively one characterizes the nature of the underlying neural activity that gives rise to the hemodynamic response (Nair 2005; Logothetis 2008).

Regarding motor activity a pioneer study using fMRI was capable to generate somatotopic maps of M1 in response to voluntary movements of the hand in humans (Rao et al. 1995). Activation of the contralateral M1, premotor area, supplementary motor area (SMA) primary somatosensory area (S1), insular region, ipsilateral cerebellum and bilateral cingulate cortex (Figure 5) has been

also reported during self-paced finger movement and choice reaction time tasks (Kim et al. 1993; Rao et al. 1995; Mima et al. 1999; Toni et al. 1999; Solodkin et al. 2001). A linear decreasing activity in frontal, prefrontal, parietal premotor and motor cortex has been observed over sessions during simple motor task and short-term motor learning without changes in task performance (Floyer-Lea and Matthews 2005; Goodyear and Douglas 2009). Moreover, GABA concentrations in the primary M1 and S1 cortex contralateral to the hand used showed to be reduced after 30 min of motor learning, suggesting that specific modulation of GABAergic input might facilitate motor learning (Floyer-Lea et al. 2006). On the other hand, increase in activity within M1, S1 and putamen has been associated with long-term motor learning, suggesting that different networks are recruited during different phases of motor learning (Floyer-Lea and Matthews 2005).

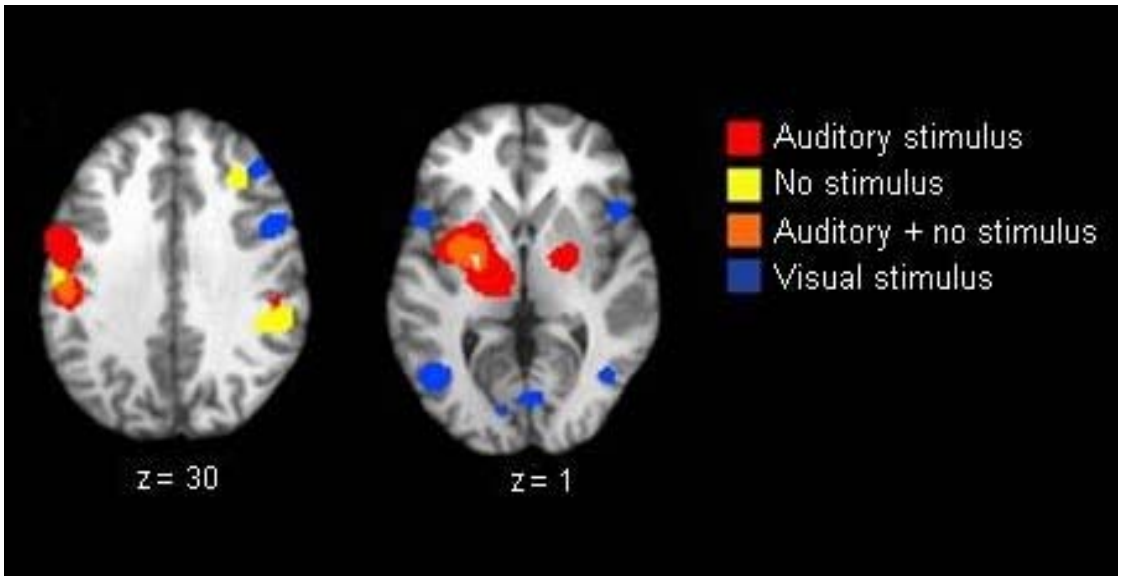



**Figure 5:** Representative axial slices for simple motor tasks activations when paced by auditory, visual or no stimuli in blood oxygen level dependent signal in functional magnetic resonance imaging studies. Figure was taken from Witt et al. (2008).

The second fMRI mapping technique is based on arterial spin labelling (ASL) perfusion (Detre et al. 1992). It has been applied to a broad range of quantitative measures of hemodynamic properties, including cerebral blood flow (CBF) and cerebral blood volume (Raichle 1998; Bartsch et al. 2006). In ASL arterial blood water protons within the left and right carotid artery are used as endogenous contrast agent. These water protons are labelled with radiofrequency pulses using a transmitter coil before they reach cortical tissue. They flow through the vascular tree and are distributed according to the local perfusion. The perfusion image is obtained by the subtraction of the labelled blood protons signal from the unlabeled blood image. Within ASL, a modality called continuous ASL (CASL) contrast provides the highest labelling efficiency and therefore, has been widely used in the last years (Detre and Wang 2002). BOLD-signal fMRI has been the contrast of choice for mapping the neural response to brief tasks or events. In contrast, ASL is more useful for measuring neural states of the brain (such as the resting state) over time with considerable test-retest reliability (Hermes et al. 2007; Detre et al. 2009).

The combination of TMS with functional magnetic imaging is technically feasible and contributes to study the localization, physiology and connectivity of the human motor system (Bohning et al. 1997; Bohning et al.1999; Bestmann et al. 2003; Moisa et al. 2008; Moisa et al. 2009). It allows measurement of any activity changes throughout the brain that result from this direct application of TMS to the targeted region and could be useful to promote the development of combined therapeutic approaches for neuropsychiatric diseases (Bestmann et al. 2008). TMS/fMRI studies demonstrated that subthreshold high frequency rTMS increased BOLD signal during motor activity only in remotes areas but not at the site of stimulation (Bestmann, 2003; Yoo et al. 2008). In contrast, suprathreshold high frequency rTMS induces activation both at the stimulated area and at a range of cortical and subcortical motor pathways (Bestmann et al. 2005). The difference in the threshold for electrophysiological and BOLD effects has been attributed to an intrinsic difference in the sensitivity of the two measures (Bestmann et al. 2008).

Combined TMS/PET studies during a motor activity reported changes in rCBF after the deliberation of rTMS over the left M1which lasted for up to 1 h. An increase in the rCBF at bilateral M1 and remote areas (i.e. left SMA, premotor area and bilateral cingulate motor areas) as well as decreases in cerebellum and superior temporal gyri was reported after the application of both excitatory and inhibitory rTM protocols over M1 (Siebner et al. 2000; Siebner et al. 2001; Chouinard et al. 2003; Lee et al. 2003; Rounis et al. 2005). These data are incongruent to the known electrophysiological effects (Lee et al. 2003; Rounis et al. 2005). Regarding resting state of the brain data are heterogenous, whereas some studies showed an increase in rCBF at the site of stimulation and other remotes areas (Lee et al. 2003; Eisenegger et al. 2008) other did not find changes (Conchou et al. 2008).

Additionally, combined TMS/electroencephalography (EEG) studies have been conducted to characterize the TMS effects. After one session of conventional rTMS induced changes in the EEG comparable in magnitude to those reported after learning or with fatigue up to one hour. In line with EMG recordings few EEG studies observed that TBS on non-motor regions induced similar effect-size but longer effect-durations when compared to conventional rTMS protocols (Sağlam et al. 2008; Grossheinrich et al. 2009). In addition, some of these studies demonstrated that the major impact of TBS occurs directly in the stimulated area itself and a lower effect on remote areas can also be observed (Hubl et al. 2008; Sağlam et al. 2008; Schindler et al. 2008).

In summary, regarding iTBS it remains unexplored whether it exerts an effect at the site of stimulation only or also in remote regions, if its after-effects are present either during motor activity or at rest, and for how long do they last under these two different conditions.

## 1.3 Aims of the study

1. To analyze the effects of iTBS on the motor cortical excitability of healthy subjects evaluated by surface EMG responses evoked by single-pulse TMS.

2. To compare the motor cortical excitability after delivering iTBS between healthy carriers of different polymorphisms of BDNF gene.

3. To explore BOLD signal fMRI changes at local and remote regions during a choice reaction time task after iTBS.

4. To explore rCBF changes evaluated by CASL fMRI in motor and non-motor regions in a resting state after iTBS.

# 2. MATERIAL AND METHODS

## 2.1 Subjects

20 healthy male volunteers aged between 24 and 33 years (mean age: 27.3 ± 2.6 years) were recruited by local advertisement. They were right handed according to the Edinburgh inventory (Oldfield 1971, ratio > 0.5). Exclusion criteria were a history of brain injury, the presence of major medical illness, neurological and/or psychiatric clinical history, intake of medication during the study, implanted devices or foreign metal particles, and history of drug intake. All participants gave their written informed consent for the experiments and were paid for participation. The project followed the Declaration of Helsinki and was approved by the Ethics Committee of the University of Ulm.

Two subjects presented hyperventilation symptoms after a few single pulses of TMS and thus, discontinued the study. In one subject an anatomical abnormality was discovered during the first MRI session (sham). He was referred to the Department of Neuroradiology for further diagnosis. The remaining 17 subjects completed the study. Since the minimum sample size (*n*) required for comparisons between groups according to genetic polymorphism (*n*=10 in each group) in fMRI studies was not reached, further fMRI analysis was carried out considering only one group (*n*=17).

## 2.2 Equipment

### 2.2.1 Transcranial magnetic stimulation

Single-pulse TMS and intermittent theta-burst stimulation (iTBS) were delivered over the left motor cortex using a Magpro X100 stimulator (Medtronic, Skovlunde, Denmark) with the figure-of-eight coil MC-B70. Stimulation parameters were biphasic pulse waveform and antero-posterior (A-P) current direction (first induced current direction in the brain) in both, single-pulse TMS and iTBS. According to Huang et al. (2005) iTBS consisted of 3 pulses delivered at 50 Hz every 200 ms during 2 s (10 bursts) repeated every 10 s for a total duration of 191.84 s (600 pulses). Intensity of iTBS was set to 90% of active motor threshold (AMT, see below). The pattern was triggered using an audio file (wav) generated by a custom program written in Python (2.4, Python Software Foundation, Hampton, NH, USA) and played by Windows Media player.

CMAP responses were measured from the right APB muscle with a belly-tendon montage using a universal amplifier (Toennies-Jaeger, Höchberg, Germany, bandpass 20 – 2000 Hz). Signals were digitalized with an A/D data acquisition board (DAP 4200a, Microstar Laboratories, Bellevue, WA, U.S.A) at a sample rate of 5000 Hz, and stored on a PC. A custom program in DasyLab (V 9.0, National Instruments, measX GmbH & Co KG, Mönchengladbach, Germany) calculated and stored peak-to-peak amplitudes in an appropriate time window.

Coil position was monitored and stored with the frameless stereotactic positioning system BrainView (V2, Fraunhofer IPA, Stuttgart, Germany, see Kammer et al. 2007). The motor hot spot over left M1 was defined as the point of maximum cMAP response in the right APB muscle at rest (Figure 6). Resting motor threshold (RMT) was defined as the lowest stimulus intensity that elicited at least six responses ≥ 50 µV within 10 consecutive single pulses (Rossini et al. 1994). AMT was defined as the lowest stimulus intensity that elicited an averaged response ≥ 200 µV during voluntary contraction (10% of maximum force, online measured and visualized as average of quadratic mean amplitude).

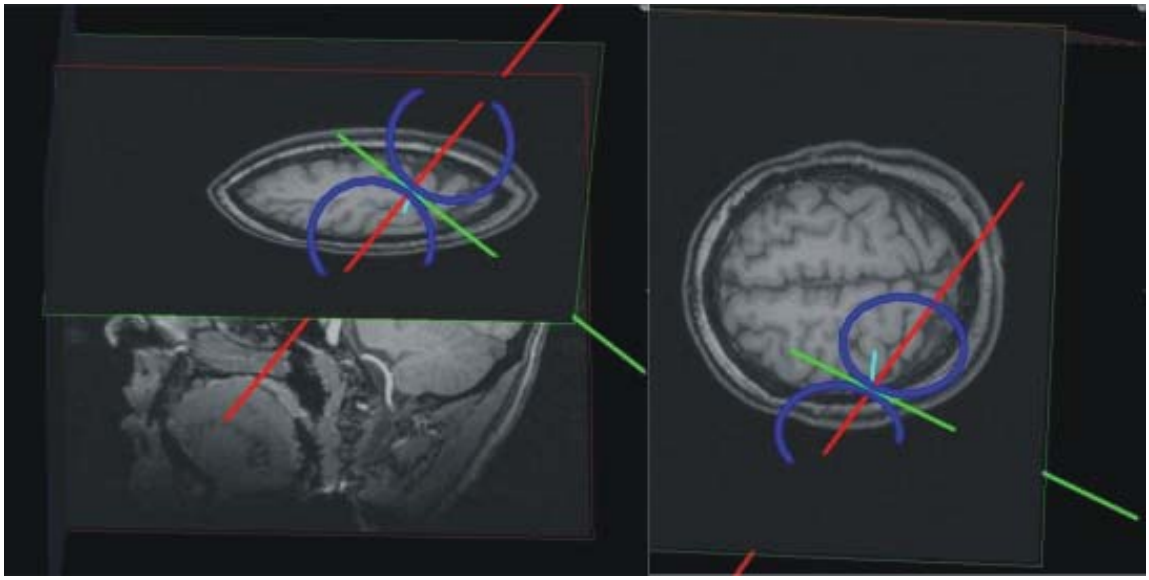


**Figure 6:** Site of stimulation visualized by the neuronavigation system BrainView. 3 dimentional and axial views display the focus of the coil over the left hand “knob” in one prototypical subject.

### 2.2.2 Magnetic resonance imaging

A 3-Tesla MRI system with a whole-brain coil (Siemens Allegra, Siemens Medical system, Erlangen, Germany) was used for the experiment. Visual stimuli were projected by means of MR-compatible LCD goggles (VisuaStim Digital Interface Box, Resonance Technology Inc. CA, USA) that covered a horizontal visual angle of 30° and allowed an individual correction for the refraction abnormalities.

The visual cued reaction time task was written in Presentation (V 11.0 Neurobehavioral Systems; Inc., San Francisco, CA, USA). Motor responses of the task were recorded with a precision of 1 ms using a custom made keyboard.

#### 2.2.2.1 Structural imaging

High resolution T1-weighted anatomical images were obtained using a 3D MPRAGE sequence with repetition time (TR) 2.08 s, inversion time 1 s, echo time (TE) 3.93 ms, bandwidth (BW) 130 Hz/ Pixel, flip angle 12°, matrix 256 x 256 pixels (1 x 1 $mm^2$). The volume consisted of 256 contiguous slices of 1 mm thickness in sagittal direction, depending on the left-to-right extension of the subject's brain. Total scan time was about 7.5 min.

#### 2.2.2.2 BOLD imaging

T2*- weighted functional MR images were obtained using gradient echo echo-planar imaging in axial orientation along the anterior comissure (AC) – posterior comissure (PC) line with TR 2 s, TE 36 ms, BW 3906 Hz/Pixel, flip angle 90°, matrix 64 x 64 pixels (3.6 x 3.6 $mm^2$). The volumes consisted of 30 slices of 3 mm with a gap of 0.6 mm. A total of 180 volumes were acquired for each scan lasting 6 min. The first four volumes of each session were discarded to allow for T1 equilibration.

#### 2.2.3.3 Perfusion imaging

In CASL (Wang et al. 2005), positioning of the labeling plane was 8 cm below the center of the imaging sections. Labeling was accomplished by means of 20 radio-frequency pulses of 100 ms duration in combination with a gap of 7.5 ms between labeling pulses. Mean duration of the control or labeling sequence was 2142.5 ms. A delay of 1 s between the end of the labeling pulses and the image acquisition was inserted to reduce transit-related effects. Control for off-resonance artifacts was accomplished by applying a sinusoidal amplitude-modulated version of the labeling pulse. T2*-weighted interleaved (label, control) images with and without labeling were acquired using a gradient echo echo-planar imaging (EPI) sequence with TR 4 s, TE 16 ms BW 3004 Hz/Pixel, matrix 64×64 Pixels (3.44 × 3.44 $mm^2$).

Along the AC-PC line 18 transversal slices with a thickness of 5mm and a gap of 1.5 mm were acquired in ascending order. One perfusion block comprised 60 acquisitions of labelled and control images each. Scan time for one perfusion scan was 4 min.

### 2.2.4 BDNF genotyping

A 7 ml single-blood sample was taken from each subject at the beginning of the experiment into EDTA-tubes. The amplification of the genetic material was processed by means of Polymerase Reaction Chain (PCR) at the Institute of clinical Pharmacology, University of Ulm. A common coding variant in the BDNF gene was identified (G→A polymorphism responsible for a Val66Met change).

## 2.3 Experimental design

The study design comprised three different measurements executed on separate days. All sessions, however, started always at the same time of the day. The first measurement was the electrophysiological monitoring after the application of iTBS, the second and third sessions corresponded to fMRI measurement with real iTBS and fMRI measurement without iTBS (control). Electrophysiological monitoring was always the first measurement, while the order of real and control fMRI sessions was randomized and counterbalanced across subjects.

### 2.3.1 Electrophysiological monitoring

CMAPs amplitude evoked by single-pulse TMS was evaluated before and after the delivery of iTBS. Baseline corticospinal excitability was assessed during 10 min by measuring the amplitude of cMAPs in the right APB muscle at rest to single pulse TMS applied with an intensity of 110% of the RMT. Every minute eight single-pulses were applied at a frequency of 0.25 Hz. After the baseline period, iTBS was delivered over left M1 and immediately followed by a one minute lasting contraction of the right APB muscle at 10% of maximal individual force, as

controlled online using DASYLab. The muscle contraction was performed in order to increase the facilitatory effect of iTBS as demonstrated previously (Huang et al. 2008). The amplitude of the cMAPs was recorded immediately after the application of iTBS every minute for 60 minutes with the same procedure used for the baseline measurement.

### 2.3.2 MRI sessions

A slow event-related design was applied to measure the BOLD fMRI signal during a choice reaction-time task. On average, every 25 s (± 3 s jitter) an arrow appeared on the screen directing either to the left or to the right. Upon appearance of the stimulus, subjects had to press a button with the corresponding index finger as fast as possible. In each BOLD session a total of 18 arrows (nine pointing to the left and nine pointing to the right) were presented in pseudo-randomized order. After each BOLD scan subjects were asked to close their eyes for of 4 min while running the CASL protocol (Figure 7). All MRI sessions started with an anatomical T1 scan in order to equilibrate the cardio-vascular system to the supine position. Then a baseline sequence comprising a BOLD scan with a choice reaction time task, a CASL perfusion session at rest and a second BOLD scan with RT task were performed prior to the TMS intervention. The two BOLD baseline measurements served only to compute a functional motor mask and were not considered for further analysis (see results). Similarly, the baseline CASL session was not included in the analysis. TMS was performed outside the scanner in a separate room. For transportation, subjects were carefully laid on a MR compatible examination couch and rolled into the separate room. Neuronavigated iTBS over the left M1 hot spot as determined in the electrophysiological session was applied in the real session, followed by the one minute contraction of the right APB muscle. In the control session no TMS was applied, and subjects waited for the time interval the real stimulation had lasted, and then performed the muscle contraction. In both MRI sessions subjects laid on the MR compatible couch during stimulation or control session in a separate room. Immediately after the end of the muscle contraction, subjects were replaced in the MR scanner. Across all subjects

the first BOLD scan comprising the choice reaction time task consistently started after 14 minutes with respect to the beginning of iTBS. A total of ten scans (five BOLD, five CASL) were performed in an interleaved order.

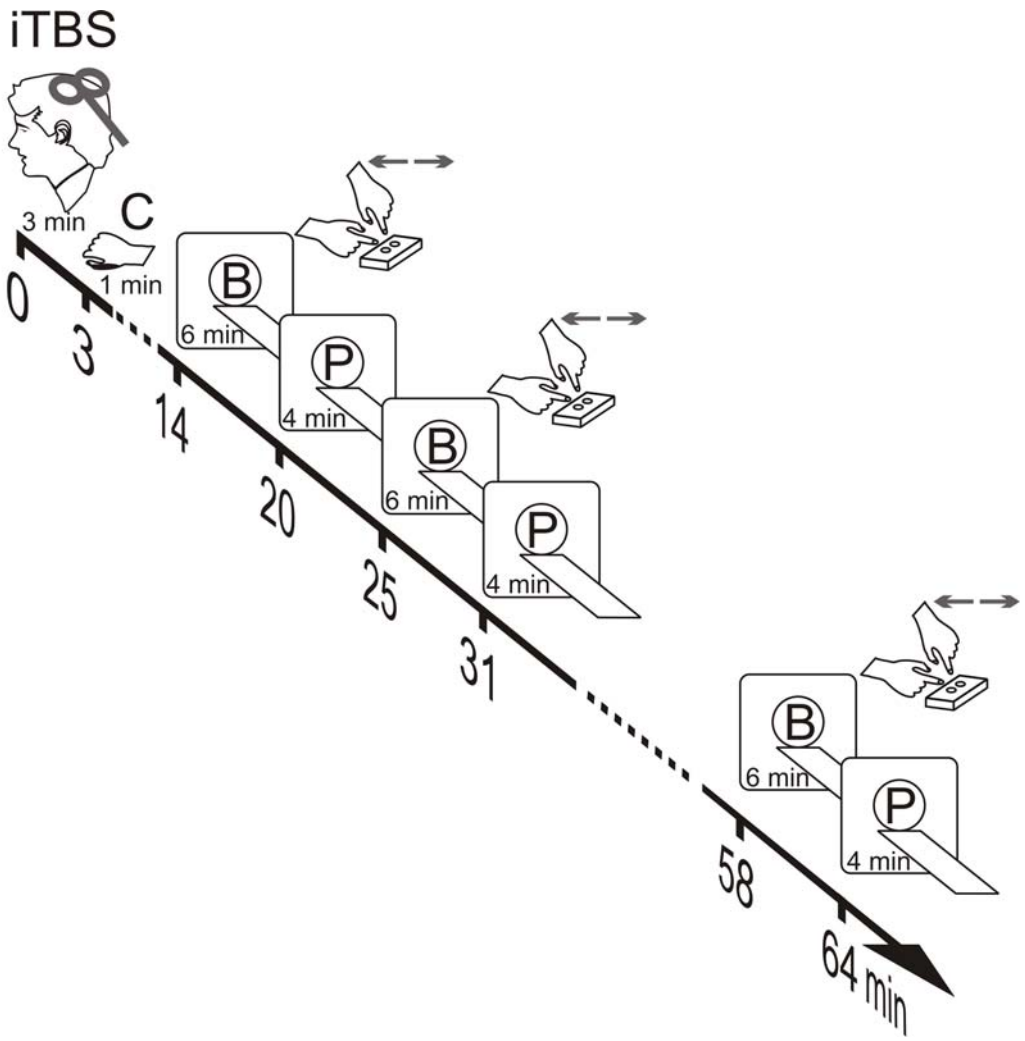



**Figure 7: Experimental design**. Participants received intermittent theta-burst stimulation (iTBS) with a total of 600 stimuli over the left motor cortex followed by 1 minute (min) of contraction of the abductor pollicis brevis muscle. After iTBS, blood oxygen level dependent scans (B) were acquired during a choice reaction-time task with duration of six min per session. B acquisitions were alternated with four minutes lasting perfusion scans (P) at rest (eyes closed). A total of ten fMRI sessions were measured within 69 min per test day. For the control session, scans were acquired without applying TBS but in presence of the 1 minute lasting muscular contraction as on the days with real iTBS applied.

## 2.4 Data analysis

### 2.4.1 Behavioral responses and cMAPs amplitude

Statistical analyses were computed using Statistica (V 8.0, StatSoft Inc., Tulsa, OK, USA). Data from the electrophysiological monitoring (cMAPs amplitude) were averaged to a baseline value and across five separate blocks after stimulation, according to the timing of BOLD scans. They were analyzed using a repeated-measures analysis of variance (ANOVA) with the factor *time* (six levels). In addition, a second ANOVA with within-subject factor *time* and between-subjects factor *group* (two levels: Val/Val and Non-Val/Val) was carried out. One-way ANOVAs were carried out to compare motor thresholds (RMT and AMT) and baseline cMAPs amplitudes between both groups of subjects (Val/Val and Non-Val/Val).

The same analysis was used to test on significant *treatment* effects on choice reaction time data obtained during fMRI. For each scan and hand median values of individual response times were considered for analysis. Within-subject factors were *hand* (two levels: right and left), *time* (five levels: corresponding to the five BOLD scans) and *treatment* (two levels: control and real iTBS). Mean error rates during the motor task in both sessions (control and iTBS) were compared by means of a Wilcoxon signed-rank test. Repeated measures were accounted for by using Greenhouse-Geisser corrections (epsilon values are reported together with the corrected *P*-values).

*P*-values were corrected according to Greenhouse-Geisser when appropriate, and *post hoc* Newman-Keuls tests were applied in case of significant interactions to further detail differences between factor combinations. The nominal level of significance for behavioral data was set to $P<0.05$.

### 2.4.2 BOLD

Image pre-processing and statistical analyses were performed using Statistical Parametric Mapping (SPM5, Wellcome Department of Cognitive Neurology, London, UK; http://www.fil.ion.ucl.ac.uk). First, data from all experimental sessions were temporally slice-time corrected and resliced. Second, functional images for each series were realigned in order to correct for small movements during the session. Third, functional images acquired during the pre and post stimulation fMRI sessions of one experimental day were spatially aligned and co-registered to the individual T1 image. Fourth, all images were spatially normalized to a canonical T1 template in MNI (Montreal Neurological Institute) space. Finally, data were spatially smoothed with a 12 $mm^3$ full width at half maximum isotropic Gaussian kernel. Session separated regressors in the General Linear Model described the occurrences of the different motor responses of the right hand or left hand in form of delta functions at each onset of a button press which were convoluted with a canonical hemodynamical response function. Image time series were scaled to a grand mean of 100 over all voxels and volumes. Low frequency drifts were removed by a high pass filter with a cut-off of 128 s. Parameter estimation was corrected for serial correlations by use of a first-order autoregressive model.

An individual first-level analysis for each of the 14 different sessions including the baseline BOLD scans (7 control and 7 real iTBS sessions) was carried out. The difference of neural activity upon right minus left hand motor responses was estimated using a one-tailed t contrast. These 14 individual contrast images were transferred to a group analysis. A full factorial analysis of variance was set up with two factors: treatment (2 levels) and time (7 levels). Treatment effects were tested by means of an appropriate two-tailed *F*-test contrasting differential neural activity following control and real iTBS per each of the five BOLD scans that had followed the intervention phase. Treatment-effects were evaluated on statistical significance within a functional motor mask that had been computed before (see supplemental Figure 12 and Table 7). The level of significance to infer significant treatment effects was set to $P<0.05$ (family-wise error (FWE)- corrected).

### 2.4.3 CASL

Image pre-processing and statistical analyses of perfusion data were done using Statistical Parametric Mapping (SPM5, Wellcome Department of Cognitive Neurology, London, UK; http://www.fil.ion.ucl.ac.uk) in combination with software implemented in MATLAB (The Mathworks, Natick, MA) for use as a toolbox under SPM5. The toolbox code is based on a MATLAB script by H.Y. Rao and J.J. Wang from the Center for Functional Imaging at the University of Pennsylvania (http://cfn.upenn.edu/perfusion/software.htm) that implements a single compartment continuous arterial spin labeling perfusion model (Wang et al. 2005) for reconstructing raw perfusion images from EPI images by subtracting labeled and unlabeled control images, and for calculating images of quantified rCBF in units of mL/100 g tissue/min.

Images of each perfusion block were realigned to correct for head movements, and pairwise difference images between label and control images were converted into absolute rCBF images. Additionally, averaging across labelled and control images delivered one mean EPI per block. The mean EPIs from perfusion (P) blocks P2–P6 of the first experimental day and the mean EPIs from all perfusion blocks of the second experimental day were co-registered onto the mean EPI of block P1 of the first day. The transformation matrices for the mean EPI of one perfusion block were then applied to the rCBF images of the corresponding perfusion block, thus aligning all rCBF images across blocks and test days. The individual T1 image was then co-registered onto the individual mean EPI of the first perfusion block. The co-registered T1 image was then used to derive the normalization matrices with respect to a canonical T1 template in MNI (Montreal Neurological Institute) space. Resulting normalized rCBF images were spatially smoothed with a three-dimensional, 12 $mm^3$ full-width at half-maximum isotropic Gaussian kernel. General linear models were used for voxel-wise averaging of the rCBF data in each subject, including the volume mean over time as a covariate to reduce the effect of spatially coherent noise (Wang et al. 2003). RCBF images were scaled to a grand mean of 50.

For each subject the differential time course of rCBF changes between real iTBS and control was determined by pairwise subtraction of the mean rCBF images per perfusion block of either test day. Resulting individual mean difference images were transferred to a group random-effects analysis. The design comprised the factors *time* with 5 levels (5 perfusion blocks following either control or real iTBS) and *subjects* in order to further remove subjects' related variability. To infer significant treatment effects in terms of changing mean rCBF differences over time these differences were tested by means of an appropriate *F*-Test comprising all five perfusion blocks following the intervention testing on significant deviations from zero per each perfusion block. Local effects were considered statistically significant at voxel level of $P<0.01$ uncorrected, constituting clusters of at least 68 significant contiguous voxels.

# 3. RESULTS

## 3.1 Effects of iTBS on contralateral cMAPs amplitude

Magnetic stimulation was well tolerated by the most of participants. No subject reported severe side effects after iTBS. Two participants experienced headache which disappeared after some minutes without the intake of medication.

Repeated measures ANOVA (considering all subjects in one group) showed a significant time effect on cMAPs amplitude in the right APB muscle (in mV) evoked by single pulse TMS in all subjects ($F$=3.55; $P$=0.032; ε=0.48). Post hoc tests revealed a significant increase in the amplitude of cMAPs after iTBS up to a factor of 2.2 lasting for 42 min (interval of 14-20 min: $P$=0.011, 25-31 min: $P$=0.001 and 36-42 min; $P$=0.0003) but not at later intervals of 47-53 min and 58-64 min (Figure 8).

Considering two groups of subjects separated according to their BDNF polymorphism (Table 6), numerical differences were observed. Non-Val/Val carriers had higher motor thresholds, both at rest and active (Figure 9) and lower baseline cMAPs amplitude as compared with Val/Val (mean 34.07 $\pm$ 4.25 and 36.37 $\pm$7.01 for the RMT; mean 25.3 $\pm$4.87 and mean 26.12 $\pm$ 6.39 for the AMT; mean 0.67 $\pm$ 0.61 and 0.55 $\pm$ 0.36 for the baseline amplitude). However, one-way ANOVA revealed no significant differences in RMT ($F_{(1,15)}$= 2.4; $P$=0.1419), AMT ($F_{(1,15)}$= 1.59; $P$=0.2265) and cMAPs baseline amplitude ($F_{(1,15)}$= 0.228; $P$=0.639) between Val/Val and Non/Val carriers. ITBS did not evoke different effects in the two BDNF groups (Figure 10). Neither significant main effect *group* ($F_{(1,16)}$= 1.50; $P$= 0.238) nor significant *group-by-time* interactions were found ($F_{(5,75)}$= 1.39; $P$= 0.237).

**Table 6:** General data from volunteers

| Subject | BDNF Polymorphism | RMT | AMT | Hand Dominance score | Age |
|---|---|---|---|---|---|
| 1 | Val/Val | 36 | 28 | 0.66 | 24 |
| 2 | Val/Val | 34 | 23 | 1 | 27 |
| 3 | Non-Val/Val | 40 | 28 | 0.69 | 28 |
| 4 | Non-Val/Val | 26 | 17 | 1 | 28 |
| 5 | Val/Val | 41 | 30 | 0.83 | 25 |
| 6 | Val/Val | 31 | 21 | 0.91 | 28 |
| 7 | Non-Val/Val | 37 | 25 | 0.9 | 23 |
| 8 | Non-Val/Val | 40 | 30 | 1 | 28 |
| 9 | Val/Val | 34 | 28 | 0.91 | 26 |
| 10 | Val/Val | 32 | 24 | 0.71 | 28 |
| 11 | Val/Val | 32 | 21 | 1 | 28 |
| 12 | Non-Val/Val | 37 | 24 | 1 | 28 |
| 13 | Non-Val/Val | 50 | 40 | 1 | 33 |
| 14 | Non-Val/Val | 29 | 22 | 0.9 | 25 |
| 15 | Val/Val | 33 | 23 | 1 | 28 |
| 16 | Val/Val | 45 | 39 | 0.76 | 24 |
| 17 | Val/Val | 31 | 26 | 0.60 | 33 |
| **Mean** | | **36.06** | **26.44** | **0.87** | **27.29** |
| **S.D.** | | **6.10** | **6.22** | **0.14** | **2.76** |

**Note:** BDNF= Brain-derived neurotrophic factor; RMT= resting motor threshold; AMT= active motor threshold; SD= standard deviation; Val= Valine.

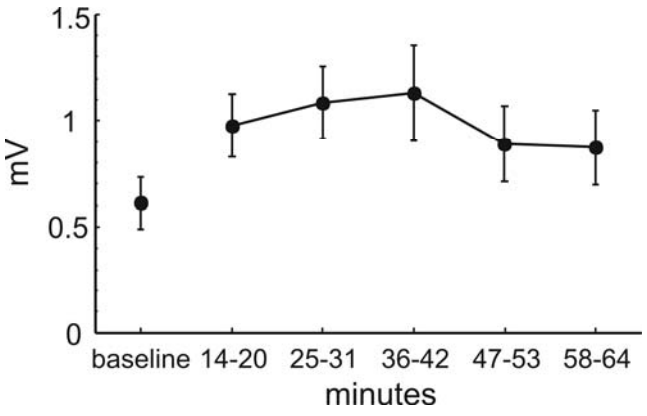



**Figure 8:** Compound muscle action potentials (cMAPS) amplitude of the right hand after intermittent theta-burst stimulation. Baseline amplitude of cMAPs was assessed during 10 min at rest. After application of intermittent theta-burst stimulation cMAPs were measured every minute for one hour and averaged in time-blocks corresponding to the time-line of the different blood oxygen level dependent scans. Amplitude was given in millivolts (mV). The increase in the amplitude of cMAPs was significant ($F_{(5,80)}$=3.54, $P$=0.006). Error bars depict standard error of the mean (SEM).

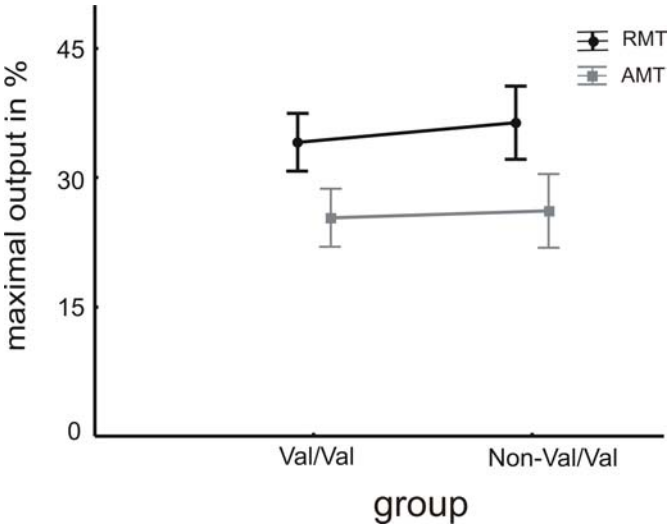



**Figure 9:** Motor thresholds in carriers of different brain-derived neurotrophic factor gene polymorphisms (Val/Val and Non-Val/Val). Values are given in percentage (%) of the maximal output of the stimulator. RMT= resting motor threshold; AMT= active motor threshold. No significant differences were observed. Error bars depict standard error of the mean (SEM).

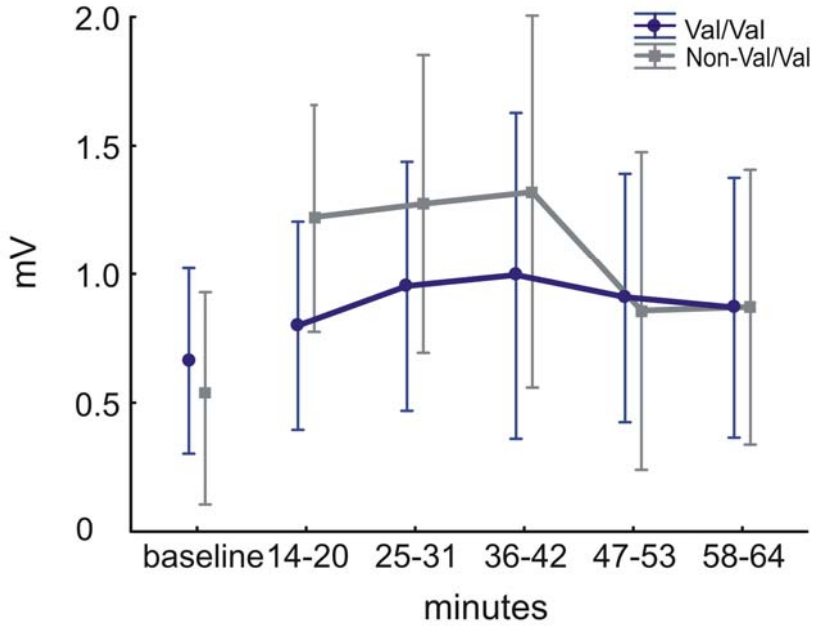



**Figure 10:** Compound muscle action potentials (cMAPs) amplitude of the right hand in carriers of different brain-derived neurotrophic factor gene polymorphisms (Val/Val and Non-Val/Val). Before and after the application of intermittent theta-burst stimulation cMAPs amplitude evoked by a single pulse of transcranial magnetic stimulation was assessed. cMAPs amplitude is showed in millivolts (mV). No significant differences were found between groups. Error bars depict standard error of the mean (SEM).

## 3.2 fMRI Measurements

### 3.2.1 Choice reaction time task during BOLD experiment

Mean reaction times (RTs) were slightly faster after iTBS for both hands (417 ± 8 ms, mean ± standard error of the mean (SEM)) compared to control (434 ± 9 ms). The three-factor ANOVA yielded a significant effect of factor *treatment* ($F_{(1,16)}$= 6,49, $P$= 0.021). Neither the factor *hand* nor the factor *time* reached significance ($F_{(1,16)}$=0.71; $P$=0.41 and $F_{(4,64)}$=2.02; $P$=0.101). Although no interactions were found group-averaged mean RTs data were depicted separately for the left and right hand (Figure 11) to ease graphical inspection of treatment effects of iTBS.

All but one subject made at least one error in the choice reaction time task. However, overall error rates per session and hand were very low (range 1 to 6, left control: 4.2 ± 1.6; left iTBS: 4.8 ± 1.3; right control: 2.4 ± 1.1; right iTBS: 2.0 ± 0.7; mean ± SD). Mean error rates did not significantly differ comparing control and iTBS sessions (right hand: $Z$=0.55, $P$=0.58, left hand: $Z$=0.67, $P$=0.50, Wilcoxon signed-rank test). No significant correlations between RTs and error rates were found.

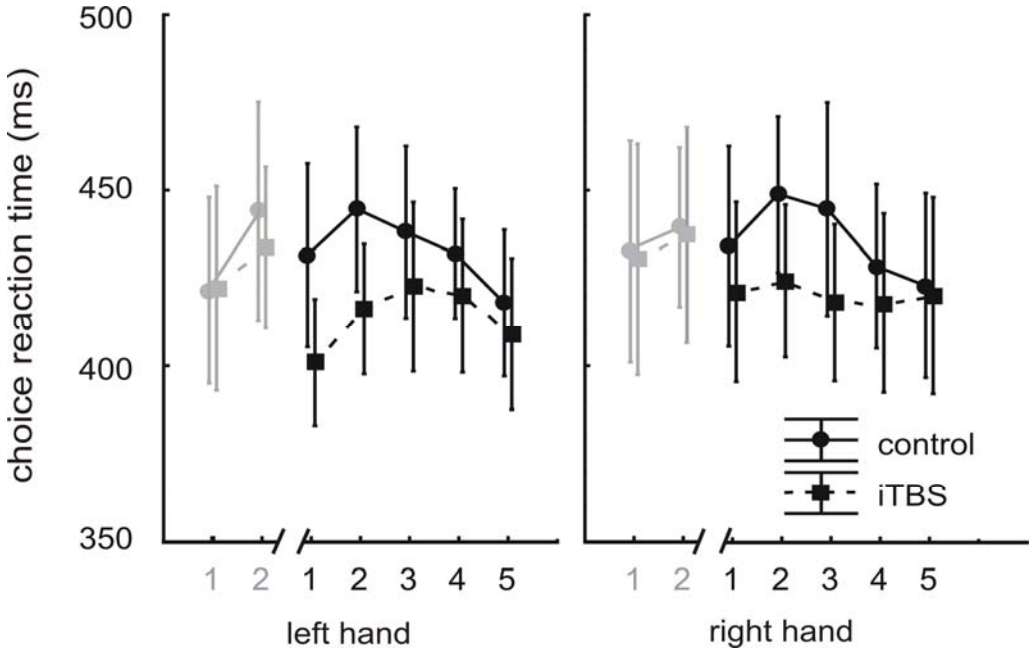



**Figure 11:** Results of choice reaction time task. The task was performed with the right and left hand (black lines) among the five blood oxygen level-dependent (BOLD) scans following intermittent theta-burst stimulation (iTBS) or control session. Mean reaction times (RTs) data are given in milliseconds (ms). RTs were depicted separately for the left and right hand. Lines in light gray depict RTs obtained during the two baseline BOLD scans but this data was not included in further analysis. It indicates that the treatment effect could be calculated in the presence of homogenous baselines. Error bars depict standard error of the mean (SEM).

### 3.2.2 Effects of iTBS on BOLD signal

Two functional regions of interest (ROIs) defining motor areas were determined using *t*-statistics contrasting neural activity upon right hand minus left hand motor responses, and vice versa. These *t*-contrasts were calculated for an average of the 2 x 2 BOLD scans at the beginning of the experiment prior to the intervention comprising control and real iTBS. For each t-test the level of significance was set to $P<0.05$ (FWE-corrected see Table 7). Both statistical parametric maps at this threshold were saved and later combined to one single image serving as a functional motor mask to assess treatment effects of iTBS (Figure 12). The cluster of significant differential activity on the right hemisphere stemming from a contrast of left hand minus right hand motor responses was larger as compared with its contralateral counterpart (dominant hand responses).

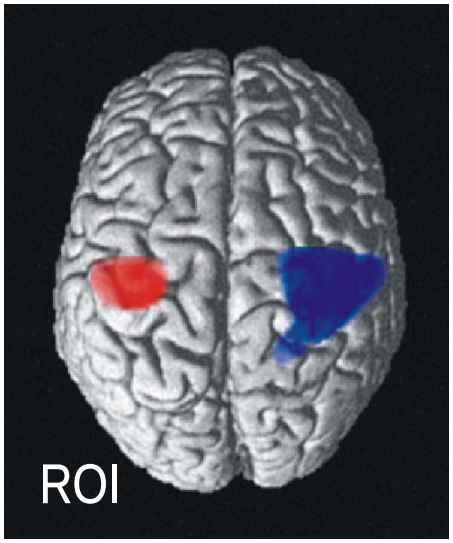



**Figure 12:** Functional region of interest (ROI) established as a result of the blood oxygen level-dependent (BOLD) signal during the choice reaction time task of the right hand (red) and the left hand (blue) motor responses.

**Table 7:** Blood oxygen level-dependent signal in the mask used as a functional region of interest

| *Brain region* | *BA* | *Talairach coordinates* | | | *Z-score* | *Number of significant voxels* |
|---|---|---|---|---|---|---|
| | | *X* | *Y* | *Z* | | |
| Left<br>S1<br>M1 | 3,1<br>4 | -42 | -20 | 66 | 7.37 | 1056 |
| Right<br>S1<br>S2<br>M1<br>SMA, PMA | 3,1,2<br>5,7<br>4<br>6 | 42 | -22 | 54 | 7.99 | 3238 |

***Note:*** BA: Brodmann area; S1: primary somatosensory cortex; S2: secondary somatosensory cortex; M1: primary motor cortex; SMA: supplementary motor area; PMA: pre-motor area.

Computing treatment effects in the two functional motor areas we observed a significant effect of an *F*-test in left and right precentral gyri when contrasting the control versus the real iTBS measurements across all time-points following the intervention. This significant result was due to the negative effect sizes from the second to the fifth BOLD scan after iTBS (25-64 min, Figure 13 A and B, Table 8) indicating that neural activations for right hand motor responses minus left hand motor responses decreased after iTBS when compared to control sessions.

To obtain further insight into the origin of the effect sizes' directions, individual BOLD responses upon left and right hand motor activity against baseline were averaged across all voxels in the left and right M1 (Figure 13 C) significant in the *F*-test above. These parameter estimates were propagated to a repeated-measures ANOVA with factors *hand* (2 levels), *treatment* (2 levels), and *time* (5 levels). This analysis yielded a significant main effect of *hand* ($F_{(1,16)}$=62.5; $P$=0.00001 and $F_{(1,16)}$=41.5; $P$=0.00001) for the left and right M1 respectively as well as a significant interaction of factors *treatment*-by-*hand* in both hemispheres (left M1: $F_{(1,16)}$=8.83; $P$=0.0089; right M1: $F_{(1,16)}$=24.8; $P$=0.0001). No other

significant main effects (*treatment*: $F_{(1,16)}$=0.002; *P*=0.96; *time*: $F_{(4,64)}$=0.377; *P*=0.82) were observed. Averaged over time-points, a post-hoc analysis (Newman-Keuls tests) of the significant *treatment*-by-*hand* interaction revealed that the BOLD signal on right hand movements significantly decreased after iTBS in both left M1 (*P*=0.047, Figure 13 C, red bars) and right M1 (*P*=0.0003, Fig. 5 C, blue bars) when compared against control sessions.

The slight increase of BOLD signals in both M1 regions upon left hand motor responses however, failed significance when comparing iTBS and control measurements averaged over time-points (*P*=0.056 for left and right M1, Figure 13 C, red bars). Since graphical inspection revealed that the direction of left hand signals changed from the first to the second session, we propagated data from session 2-5 to a repeated-measures ANOVA with factors *hand* (2 levels), *treatment* (2 levels) and *time* (4 levels). Results of the main effects and the interaction *treatment*-by-*hand* remained stable. However, post-hoc testing revealed that the increase of BOLD signal upon left hand motor responses were significant in both motor regions (left M1 *P*=0.012, right M1 *P*=0.008).

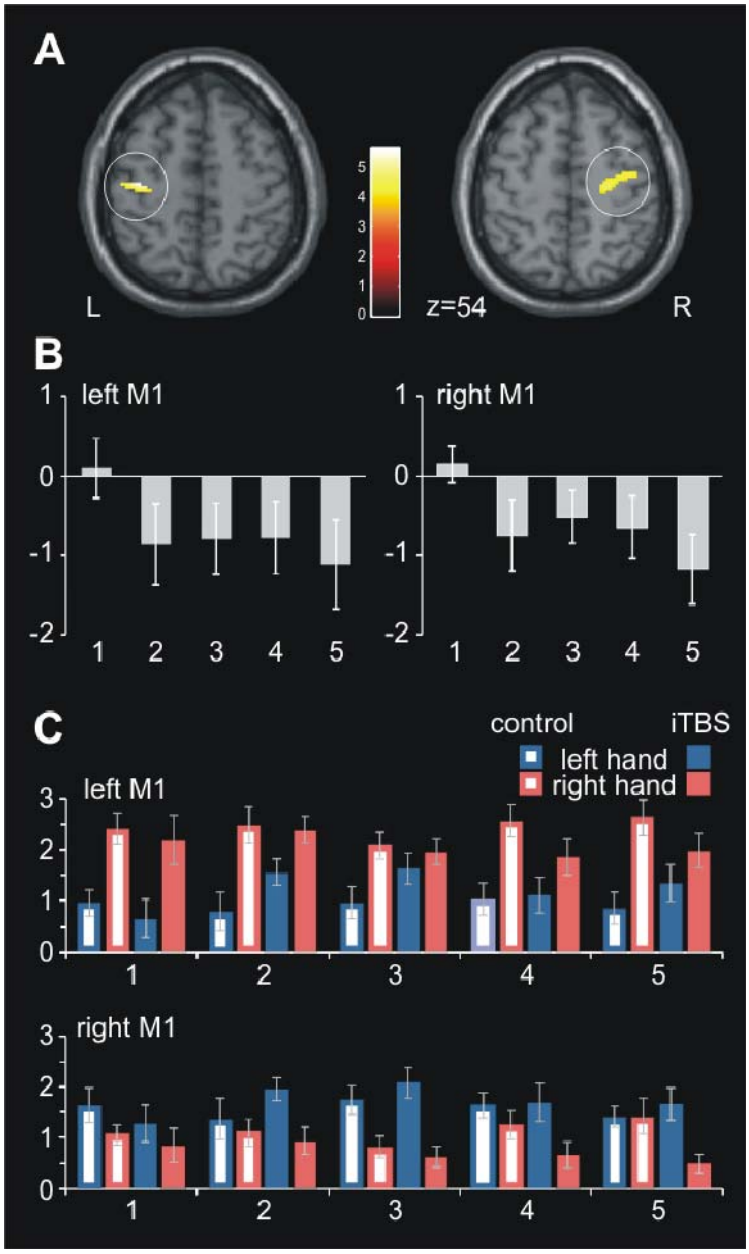



**Figure 13:** Significant treatment effects on blood oxygen level-dependent (BOLD) signal in left and right clusters of the primary motor cortex (M1). *A*, Results are displayed on an axial section of an standardized canonical T1-image at *P*<0.01 (false discovery rate-corrected). Left (L) M1: 94 voxel, z-score of peak voxel = 3.81, x, y, z-coordinates of the peakt voxel: -34 -16 56; Right (R) M1: 187 voxel, z-score = 4.59, x, y, z: 26 -18 48; *t*-values are color coded. *B*, Parameter estimates of treatment effects averaged over significant voxels in the clusters of left and right M1 shown in *A. C*, Mean neural magnitudes for each combination of factors treatment, *hand* and *time* are shown. Abscissa: Numbers refer to BOLD scans after treatment (14 – 64 min). Error bars depict standard error of the mean (SEM).

Assessing treatment effects ($P$<0.05, false discovery rate (FDR)-corrected) beyond the predefined functional motor masks the same pattern of results was additionally observed in some remote brain regions. The effects were included in premotor, somatosensory, cingulate and other areas (Figure 14 and Table 8). All these regions are involved in the execution of simple motor tasks as demonstrated in previous studies (Toni et al. 1999; Solodkin et al. 2001; Rounis et al. 2006), showing that the facilitatory effect of iTBS might be extended to the whole motor system.

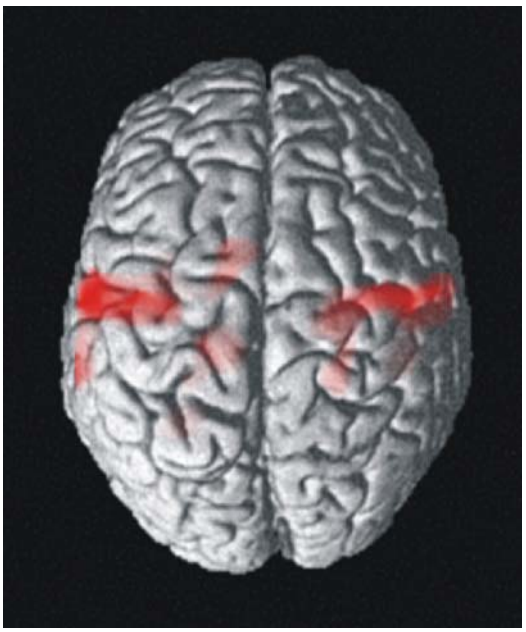


**Figure 14:** *Treatment* effects on blood oxygen level-dependent (BOLD) signal in a whole brain analysis. The same Fisher ($F$)-test as for the within-region of interest analysis was applied testing on significant differential (right hand minus left hand) signal changes by comparison of intermittent theta-burst stimulation versus control over the five time-points Significance was set at $P < 0.05$ (false discovery rate (FDR)-corrected).

**Table 8**: Treatment effects on blood oxygen level-dependent signal changes in a whole brain analysis after the application of intermittent theta-burst stimulation

| *Brain region* | *BA* | *Talairach coordinates* | | | *Z-score* | *Number of significant voxels* |
|---|---|---|---|---|---|---|
| | | *X* | *Y* | *Z* | | |
| Right | | | | | | |
| M1 | 4 | 26 | -18 | 48 | 4.59 | 2089 |
| SMA, PMA | 6 | 48 | -12 | 8 | 3.99 | |
| Superior temporal | 41 | 46 | -34 | 12 | 3.95 | |
| Left | | | | | | |
| Middle and posterior cingulate | 24/31 | -16 | -28 | 50 | 4.53 | 268 |
| S1 | 3 | -62 | -10 | 40 | 4.45 | 1746 |
| M1 | 4 | -36 | -6 | 32 | 4.17 | |
| SMA, PMA | 6 | -36 | -14 | 54 | 3.9 | |
| Angular gyrus | 39 | -28 | -54 | 24 | 4.36 | 168 |

*Note:* BA: Brodmann area; M1: primary motor cortex; S1: primary somatosensory cortex; PMA: premotor area. SMA supplemental motor area.

### 3.2.3 Effects of iTBS on CASL perfusion

Similar to the analytic procedure for the BOLD data, treatment effects on perfusion data (CASL) were analyzed by an *F*-test voxel-wise contrasting mean perfusion rates between iTBS and control measurements across the five perfusion blocks following the TMS intervention. With this analysis confined to the inclusive functional motor masks no significant effects were observed at the site of stimulation (left M1) nor contralateral in right M1 ($P>0.1$ uncorrected). In non-motor regions related to the resting state brain some activation patterns were observed (Table 9; Figure 15).

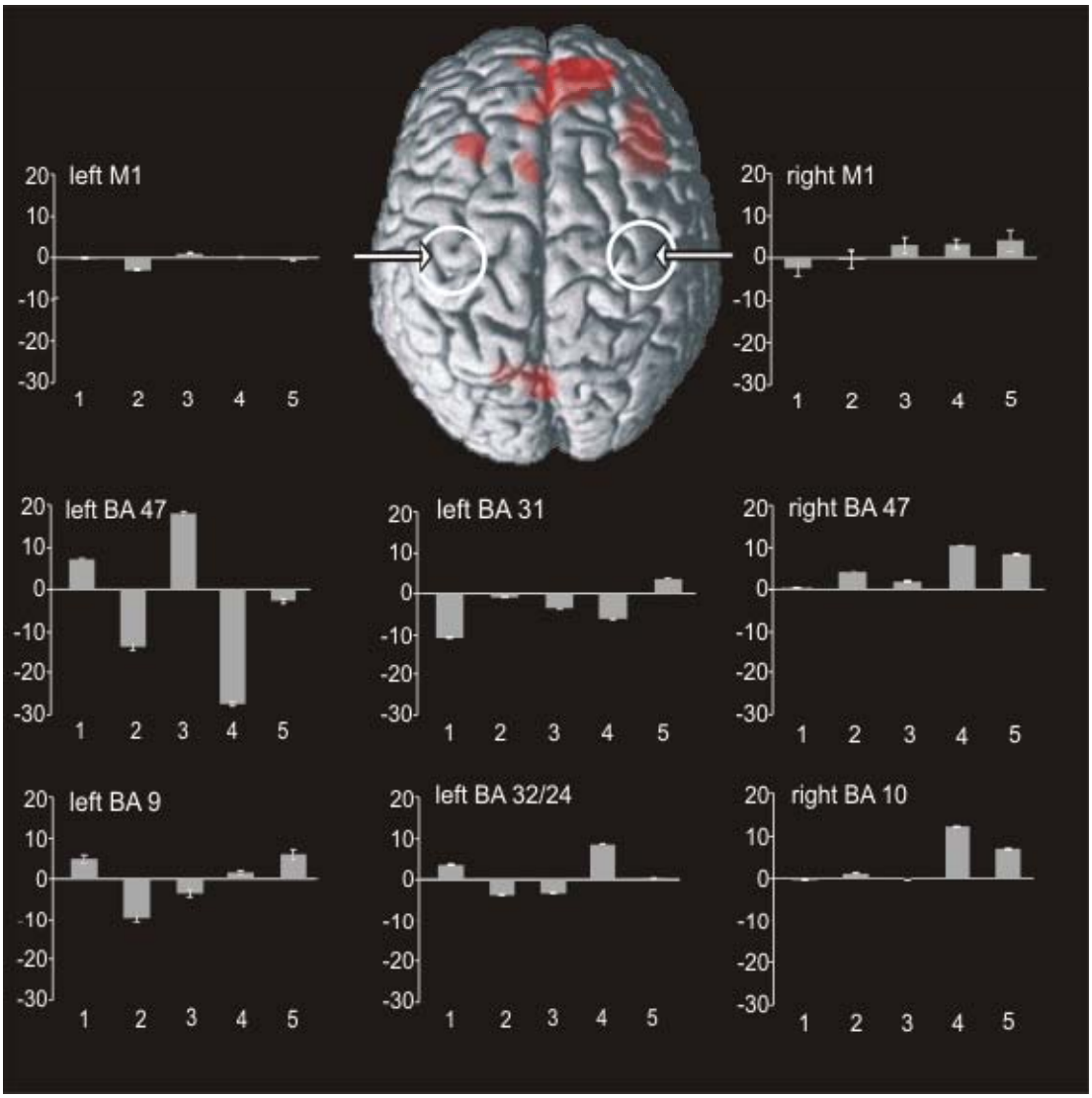



**Figure 15:** Regional cerebral blood flow (rCBF) changes in the whole brain after the application of real intermittent theta-burst stimulation (iTBS) over left primary motor cortex (M1). A contrast of *treatment* (real iTBS versus control session) pooled across all levels of time is shown on the rendered T1-weighted canonical template (extent threshold, $P<0.01$). No significant differences at the site of stimulation were found. BA= Brodmann area.

**Table 9:** Treatment effect on regional cerebral blood flow at rest after the application of intermittent theta-burst stimulation over the left primary motor cortex

| *Brain region* | *BA* | *Talairach coordinates* | | | *Z-value* | **Number of significant voxels** |
|---|---|---|---|---|---|---|
| | | *x* | *y* | *z* | | |
| Right | | | | | | |
| Inferior frontal | 47 | 38 | 28 | 2 | 3.71 | 528 |
| Middle frontal | 10 | 12 | 56 | 24 | 3.22 | 540 |
| Left | | | | | | |
| Cuneus | 31 | -2 | -74 | 22 | 3.3 | 227 |
| Inferior prefrontal | 47 | -30 | 26 | -20 | 3.22 | 105 |
| Anterior middle cingulate | 24/32 | -8 | 14 | 42 | 2.78 | 80 |
| Superior frontal | 9 | -32 | 24 | 40 | 2.73 | 88 |

***Note:*** BA= Brodmann area

# 4. DISCUSSION

The main aim of the present study was to explore effects of iTBS, a subthreshold rTMS protocol over left M1 on neuronal activity and rCBF. In line with previous electrophysiological studies iTBS increased the amplitude of cMAPs, thus replicating its excitatory profile. When subjects were divided according to their BDNF polymorphisms, no differences in cortical excitability were found. Furthermore, the minimum sample size required for studies comparing genetic factors in fMRI was not reached; therefore, we analyzed further fMRI data without considering genetic factors between subjects.

Behaviorally, iTBS was associated with reduced RTs obtained from a choice reaction-time task. This task served as the functional challenge during fMRI used to monitor changes in neural activity after iTBS in terms of BOLD signal changes. Compared to the control session, a significant decrease in BOLD activity on right hand motor responses was observed with iTBS. Interestingly, this decrease was evident in both, ipsi- and contralateral M1 regions with respect to the stimulation site. Less consistent, left hand motor responses after iTBS yielded a tendency to an increase in BOLD signal without reaching significance. MR-based perfusion imaging scanned by CASL did not indicate that the excitatory effects of iTBS involve a change in rCBF at rest.

### 4.1 EMG monitoring and choice reaction time task

The present results confirm and extend previous evidence of the facilitatory effect of iTBS on the amplitude of cMAPs (Huang et al. 2005; Huang et al. 2007). Moreover, increases in the amplitude of cMAPs after iTBS were stronger and lasted longer than those recently described (Huang et al. 2005; Cheeran et al.

2008). Very likely, this difference with previous reports may be due to the higher stimulation intensity used (90% AMT as compared to 80% AMT in Huang et al. 2005, Huang et al. 2007). Significance of iTBS differences of cMAPs amplitude vanished in time intervals after one hour with respect to the beginning of iTBS application. This supports the assumption that the reliable excitatory iTBS effects will presumably no longer exist beyond this time-point (at least with the given iTBS protocol). The higher, but still subthreshold stimulation intensity used in this study was within a safe stimulus parameter range; additionally, we observed no severe side effects, in particular no induced seizures.

An additional factor that could lead to the stronger effect on cMAPs amplitude was the voluntary muscle contraction that followed the stimulation. Previous state of the neuronal activity determines the degree of the future synaptic effects (metaplasticity, see Abraham and Bear 1996). Following this assumption, it has been shown that different rTMS protocols are affected by ongoing muscle activity (Gentner et al. 2008; Huang et al. 2008). It was demonstrated that a slight contraction after iTBS enhanced its facilitatory effect (Huang et al. 2008). Thus, in our data, the muscular contraction might interfere with the rebound of inhibitory processes permitting just the excitatory processes to evolve and thus, facilitating cMAPs amplitude for a longer time span.

Moreover, a slight voluntary muscular contraction alone reduced the amount of SICI and increased SICF (Ortu et al. 2008; Ridding et al. 1995), without changing cMAPs amplitude (Gentner et al. 2008; Huang et al. 2008). In contrast, iTBS was shown to facilitate cMAPs amplitude and to increase SICI, whereas it did not affect SICF (Huang et al. 2005, Huang et al. 2008). The contraction might reduce the inhibitory GABAergic inputs involved in SICI and/or could facilitate excitatory processes involved in SICF, modifying the original effects of iTBS. However, these speculations have to be tested experimentally.

A behavioral effect as a consequence of iTBS has not been observed in previous studies. Huang et al. (2005) did not find tapping frequency changes after iTBS, although they described a significant increase in tapping frequencies for the right

hand after cTBS stimulation of the left M1 region. As regards present data, the decrease in RTs observed for the right hand is in line with the facilitation obtained from electrophysiological data. The decrease in RTs observed for the left hand was unexpected, and lacks a satisfactory explanation. We only can speculate that acceleration of motor responses in the choice reaction time task evoked by iTBS could be conditioned either by a modulation of premotor regions not specific for the side of reaction, or by modulation of supramodal cortical regions involved in the attentional aspects of the task.

### 4.1 EMG recordings and BDNF

Out data showed no differences in RMT, AMT and baseline cMAPs amplitude between Val/Val and Non/Val carriers. These results are in line with other studies suggesting physiological consequences of "Met" BDNF polymorphism to be present after internal or external activation, but not manifested in the basal state (Kleim et al. 2006; Cheeran et al. 2008). This is also in line with the assumption that transfection of neurons with the BDNF "Met" allele does not affect constitutive BDNF secretion but does reduce BDNF secretion in response to neuronal stimulation (Egan et al. 2003; McHughen et al. 2009).

However, in contrast to the above mentioned studies, our data showed iTBS to have an excitatory after-effect in all participants independently of BDNF genotype. Previous studies have suggested that the physiological difference in Non-Val/Val carriers became evident in response to behaviorally driven increases in neural activity and during short-term learning (Kleim et al. 2006; McHughen et al. 2009). Furthermore, Cheeran et al. (2008) explored the effects of both TBS protocols on healthy subjects with different BDNF genotypes and found that Non-Val/Val subjects responded neither to iTBS nor to cTBS delivered at 80% AMT. They suggest that Non-Val/Val carriers are less susceptible to TBS than Val/Val maybe due to difficulties to induce plasticity (LTP and LTD) of certain neural circuits. According to this hypothesis, it could be that these “difficulties” depend on the threshold to induce plasticity. Thus, the deliveration of iTBS at 90% of the AMT

followed by a voluntary contraction might reach the LTP-threshold for the Non-Val/Val carriers by interfering with the inhibitory processes and permitting just excitation to evolve. A further difference between our protocol and the above mentioned study (Cheeran et al. 2008) was the voluntary contraction after iTBS. This contraction might have balanced the modulatory effect in both Val/Val and Non-Val/Val carriers.

Although some animal studies have attempted to elucidate the mechanisms by which BDNF promotes LTP, there are still several open questions regarding how BDNF acts on cells. First, it is not yet understood whether it acts at a pre-synaptic level (Gottschalk et al. 1998; Pozzo-Miller et al. 1999) or post-synaptic level (Aoki et al. 2000; Manabe 2002). Second, cellular responses to BDNF differed markedly depending on the application time of BDNF; hence, the effects of acute BDNF deliveration might be different to those observed after gradual concentrations of BDNF (Ji et al. 2010). Third, there is evidence for the influence of BDNF not only in excitatory activity but also in inhibitory modulation, since BDNF decreased $GABA_A$ receptor activity in the rat hippocampus, facilitating the induction of LTP (Tanaka et al. 1997; Manabe 2002; Cheng and Yeh 2003).

Taking altogether, it might be that "Met" allele difficulties to induce plasticity of neural circuits are related to one or more of these factors. Considering that surface EMG recordings are limited to give only indirect measurements of LTP and LTD-like processes, we can only speculate about the interplay between inhibitory and excitatory modulation involved in iTBS after-effects. Further studies especially in animal models are required to elucidate differences in molecular processes related to BDNF. The deliveration of acute and repetitive BDNF concentrations at a pre-synaptic and post-synaptic level could be helpful to reveal the role of BDNF in LTP in Val/Val and Non-Val/Val carriers.

## 4.2 Effects on fMRI

### 4.2.1 BOLD signal

Since our study represents the first experimental step towards an MR-based evaluation of a novel and specific subthreshold high-frequency rTMS protocol, it is rather difficult to align present data with previous results using a combined multimodal approach. These previous studies showed that high frequency (3-10 Hz) subthreshold rTMS evoked changes in BOLD signals during motor activity only in remote areas immediately after the stimulation (Bestmann et al. 2003; Bestmann et al. 2004) or as a persisting modulation in an off-line design (Yoo et al. 2008). By contrast, suprathreshold rTMS was shown to induce immediate changes at the stimulated area and some more distal regions (Bestmann et al. 2004; Moisa et al. 2010). From that pattern observed in previous results, our data of BOLD signal changes predominantly at the stimulated area as well as at several cortical motor pathways (e.g. contralateral M1, SMA, PMA, cingulate cortex) would rather align with conventional suprathreshold (Bestmann et al. 2004) than with subthreshold rTMS. However, on the one hand, the present iTBS protocol was applied with subthreshold intensities. On the other hand, it led to pronounced excitatory effect in cMAPs amplitude which is hardly reached with conventional suprathreshold short rTMS protocols (Pascual-Leone et al. 1994; Maeda et al. 2000). A few EEG studies revealed that the major impact of subthreshold inhibitory TBS occurs directly in the stimulated area itself and at lower level in remote areas (Sağlam et al. 2008; Schindler et al. 2008). Thus, it could be that TBS modulates neuronal activity in a different way compared to conventional rTMS. This could include modulation of the area close to the coil and other remote cortical regions, but not subcortical structures such as basal ganglia. Further evidence for this assumption is the difference in SICI between conventional rTMS protocols and TBS. Conventional subthreshold high-frequency rTMS protocols reduce SICI (Peinemann et al. 2000; Di Lazzaro et al. 2002) whereas iTBS increases it, suggesting that this pattern alters GABAergic pathways in a different way (Huang et al. 2005).

A second explanation for the differences in BOLD-signal pattern in our work as compared with previous studies would be a putative sensitivity difference between our experimental approach and previous studies. The choice-reaction time task we used could be more sensitive for mapping the modulatory effects on M1 as compared to tapping or sequential finger tasks (Bestmann et al. 2003; Bestmann et al. 2004; Yoo et al. 2008). The latter tasks which are suitable for block design yield a continuous motor activity whereas the choice reaction-time task results in distinct motor actions interleaved with long periods of rest. These distinct motor actions might be more sensitive for network modulation compared to continuous motor activity modulation.

### 4.2.2 Direction of BOLD modulation and possible mechanisms

The main iTBS treatment effect was conditioned by an overall significant decrease in BOLD signals in bilateral M1 regions upon right hand motor responses in a choice reaction-time task. To a slightly lesser extent this treatment effect further depended on an increase of BOLD-signal associated with left hand motor responses.

While an increase in BOLD signal is most likely to reflect an increase in neural activity, it is much more difficult to assign a decrease in BOLD signal to a particular change in neural activity (Logothetis 2008). Across multiple sessions of a simple motor task, a decrease in BOLD signal was observed in healthy subjects over time suggesting habituation or attentional modulation to take place (Loubinoux et al. 2001; Goodyear and Douglas 2009). Moreover, decreased activity in prefrontal, anterior cingulate, parietal, premotor and motor cortex and reduced GABA concentrations at least in the primary sensorimotor cortex have been found over scans during short-term motor learning (Floyer-Lea and Matthews 2005; Floyer-Lea et al. 2006).

Our study design, however, excludes the presence of a mere habituation process. We included a control condition incorporating the same task which therefore

controlled for any putative habituation or adaptation phenomena that might have biased inference of treatment effects. An alternative explanation to account for the BOLD decreases suggests that after iTBS the motor network requires less neuronal activity to perform the same task. The altered cortical excitability induced by rTMS might change the efficacy of neural signal transmission resulting in reduced post-synaptic field potentials and therefore in reduced BOLD signal similarly to the decrease observed during short-term motor learning. Although the mechanisms by which iTBS exerts these effects are not completely understood, it is quite plausible that facilitation after iTBS results from a change in the balance between inhibitory and excitatory forces (Huang et al. 2005; Thickbroom 2007; Stagg et al. 2009). Recently, changes in the activity of cortical neurons have been demonstrated in rats. Specifically, iTBS reduced the number of inhibitory cells expressing PV immediately after stimulation (Benalli et al. 2009) supporting the assumption that an important part of modulation occurs within the inhibitory pathway. In line with this concept is the observation that a pharmacological block of GABA induces LTP after electrical TBS in the rat motor cortex (Castro-Alamancos et al. 1995). Furthermore, it is plausible that the combination of iTBS with the voluntary muscle contraction decreased the amount of SICI; thus, resulting in less inhibition that could be reflected as a decreased in the BOLD-signal.

In PET studies an rCBF increase associated with inhibitory subthreshold 1 Hz rTMS during motor activity was observed (Chouinard et al. 2003; Lee et al. 2003). Lee et al. (2003) proposed an attenuation of the motor network efficacy as a condition for rCBF increase. This is in line with the spectroscopic demonstration of an increase in GABA concentration following cTBS in the absence of glutamate/glutamine (Glx) level changes (Stagg et al. 2009). Our observations are in favor of this assumption but with the opposite direction; i.e. excitatory iTBS induces facilitation of motor network efficacy during motor activity via decrease of inhibitory activity, therefore decreasing the overall BOLD signal.

Following this framework, the increase in BOLD signals related to left hand motor activity might then reflect a tendency to inhibition within the motor network. This

would align with previous electrophysiological data observing that after TBS, the amplitude of cMAPs in the non-stimulated hemisphere was affected in the opposite direction to that in the stimulated hemisphere probably mediated by transcallosal connections (Stefan et al. 2008; Suppa et al. 2008). Moreover, after iTBS an increase in SICI in the non-stimulated hemisphere was observed (Suppa et al. 2008). Notably, however, we also observed an acceleration in reaction times for left hand motor responses which does not easily integrate into the framework ipsilateral facilitation and contralateral inhibition. At present we are not in the position to give a conclusive account for this observation. Previous motor studies (e.g. Dassonville et al. 1997; Zeng et al. 2007), however, demonstrated that the motor network controlling for movements of the left, non-dominant hand is not just a mirror of the dominant right hand motor network and an explanation for this conflicting result might be covered in this phenomenon.

### 4.2.3 Functional imaging at rest

Cerebral perfusion in the motor ROIs at rest did not change with iTBS confirming previous observations on rTMS/PET measurements at rest (Conchou et al. 2008). This indicates that the BOLD deactivation is not caused by a global change in brain perfusion within the network and supports the idea that the facilitating effect of iTBS is activity-dependent. In contrast, previous PET/TMS studies observed that 1 Hz and 5 Hz subthreshold rTMS induced increases in rCBF not only during motor activity but even at rest in bilateral M1, SMA and right premotor cortex (Lee et al. 2003, Rounis et al. 2005). Up to now there is no conclusive explanation for the observed differences in rTMS-induced rCBF changes at rest. The number, intensity, grouping of applied stimuli and differences in mechanisms between TBS pattern and conventional rTMS protocols might be responsible for such discrepancies. Furtheremore, it could be that the after-effects of iTBS at rest can be only observed during within few minutes after stimulation. We started fMRI perfusion 20 after the beginning of iTBS and therefore, it might be plausible that the effects at rest were diminished within this period.

However, we found an increase in rCBF in remote regions including the right inferior frontal cortex and cingulate. Decreases in middle frontal regions were also found in our study. It is well known that in the absence of goal-directed tasks or external input, neural activity is increased in a specific network of brain regions (the default state network). This network involves activation of the medial and posterior cingulate, medial frontal cortex, inferior frontal cortex and superior temporal cortex (Raichle et al. 2001; Damoiseaux et al. 2006; Buckner et al. 2008; Detre et al. 2009). The modulations observed in our study after iTBS seem not to follow a trend. They could just be due to the variability in the resting state activity. Although ASL perfusion at rest has shown to be consistent over time (Hermes et al. 2007), some fluctuations in CBF in prefrontal, cingulated, and temporo-parietal cortex over sessions have been observed suggesting a position-to-position variance (Viviani et al. 2009). Further investigations are required to clarify systematic modulations in the default network.

### 4.3 Limitations and future prospects

BOLD and CASL fMRI are good tools to measure neuronal activity, one has to keep in mind however, that they asses indirectly this activity. Thus, it could be that rTMS affects neuronal activity which is not possible to measure with functional imaging techniques. We did not use in vivo magnetic resonance spectroscopy to measure changes in GABA concentrations and therefore, we can only transpolate previous findings to our data. Future work needs to characterize changes in GABA and Glu to elucidate if both neurotransmitters are involved in the iTBS after-effects.

The combination of TBS with neuroimaging appears as a useful approach to monitor treatment effects for example in the rehabilitation of cortical motor deficits following brain strokes. The additional involvement of ipsi- or contralaterally lesioned brain regions might also be helpful in a further understanding of motor network processing. Further studies are required to elucidate whether the combination of TBS and muscular voluntary contraction has therapeutical

applications, i.e. in the recovery of motor function after stroke, motor conversion disorder or in case of focal dystonia. Additionally, investigations comparing different motor tasks, i.e. sequential finger movements might reveal further potential in plasticity modulation. Up to now, the effects of repeated application of iTBS in a daily scheme have not been explored. They might induce a longer lasting modulation effect. Our study only included a single sessions of iTBS and did not explore the chronic application of this novel pattern. Further experiments with a range of iTBS intensities and different number of trains would be required to optimize the after-effects of this rTMS protocol.

A limitation of our study was the sample size, which did not allow us to analyze differences to undergo neuronal plasticity induced by rTMS between Val/Val and Non-Val/Val carriers. Moreover, it remains unclear whether the deliberation of TBS enhances the BDNF secretion in response to behaviorally driven increases in neural activity (i.e. motor training) in met carriers. Considering on the one hand the findings of Egan (2003) that transfection of neurons with the BDNF met allele reduced BDNF secretion in response to neuronal stimulation and on the other hand that rTMS had an impact on the long-term expression of BDNF (Angelucci et al. 2004; Müller et al. 2000), it would have clinical implications particularly in studies utilizing these protocols as a therapeutic intervention i.e. in stroke rehabilitation or depression.

Finally, the approach to monitor changes of network activity induced by iTBS using fMRI BOLD-signal might be transferred to non-motor regions. This would be helpful especially in regions where no electrophysiological access is available non-invasively.

## 4.4 Conclusion

In the present study we provide evidence for the facilitating effect of iTBS on bilateral M1 regions. This effect was reflected by a significant decrease in BOLD signal on right hand motor responses similar to the decrease observed during short-term motor learning. The facilitation was corroborated by increased cMAPs derived from electrophysiological monitoring. Modulation of neuronal activity induced by iTBS might differ at least partially from conventional subthreshold rTMS protocols, since the main effect was observed at site of stimulation. MR-based perfusion imaging (CASL) did not show any evidence for rCBF changes at rest in both motor areas. As regards future studies in this field, the combination of TBS with neuroimaging appears as a useful approach to induce and monitor treatment effects for example in the rehabilitation of cortical motor deficits following brain strokes. Besides a putative clinical benefit the additional involvement of ipsi- or contralaterally lesioned brain regions might also be helpful in a further understanding of motor network processing.

# 5. SUMMARY

Theta-burst stimulation (TBS) is a novel protocol of subthreshold repetitive transcranial magnetic stimulation (rTMS) inducing inhibitory or facilitatory effects in cortical excitability. So far, effects of TBS on motor cortex excitability have mainly been characterized by electrophysiological measurements of motor output. From functional imaging studies with conventional subthreshold rTMS protocols it remains unclear whether only remote or directly stimulated brain areas are modulated, and what type of modulation occurs (direction and dependency to neural activity. The main aim of the present study was to explore the after-effects of an excitatory TBS protocol called intermittent TBS (iTBS) over left M1 on neuronal activity and regional cerebral blood flow (rCBF).

In a within-subjects (17 healthy participants) repeated measurement design with control session we examined activation pattern in a choice reaction task with the two index fingers after application of iTBS. Intensity of iTBS was set to 90% of active motor threshold over the left motor cortex (M1). Neuronal activity was monitored by five consecutive event-related BOLD-signal fMRI scans during a choice reaction time task. Furthermore, rCBF at rest using MR-based perfusion imaging was interleaved with BOLD signal measurement. The complete scan lasted for one hour. For purpose of control the same protocol was measured in absence of iTBS on a separate day. In a third session, compound muscle action potentials (cMAPs) amplitude of the right hand at rest evoked by single pulse TMS was measured after iTBS for one hour.

Compared to control session a significant decrease in BOLD-signal due to right hand motor activity during the choice reaction task was observed mainly in the

stimulated M1 and motor related remote areas after stimulation. By contrast left hand motor responses after iTBS yielded a tendency to increase BOLD signal. CMAPs amplitude increased up to 42 minutes and reaction times during the motor task were accelerated. No changes in rCBF were detected.

The data demonstrate that subthreshold iTBS targets both the stimulated region and remote areas. The BOLD-signal decrease seems to represent a facilitating effect, reflecting reduced metabolic demand to generate efficient motor responses similar to changes observed in short-term motor learning. This facilitation was confirmed by cMAPs amplitude increase and reduced reaction times during the motor task. On the other hand, the increase observed during the left hand motor responses may indicate inhibition of the network and thus, more effort to generate adequate responses. The absence of rCBF changes at rest indicates a quite specific network modulation. The combination of TBS with neuroimaging appears as a useful approach to monitor treatment effects for example in the rehabilitation of cortical motor deficits following brain strokes.

# 6. REFERENCES


1. Abraham WC, Bear MF: Metaplasticity: the plasticity of synaptic plasticity. Trends Neurosci 19: 126-130 (1996)

2. Abraham WC, Williams JM: Properties and mechanisms of LTP maintenance. Neuroscientist 9: 463-474 (2003)

3. Ahmed Z, Wieraszko A: Modulation of learning and hippocampal, neuronal plasticity by repetitive transcranial magnetic stimulation (rTMS). Bioelectromagnetics 27: 288-294 (2006)

4. Allen SJ, Dawbarn D: Clinical relevance of the neurotrophins and their receptors. Clin Sci 110: 175-191 (2006)

5. Angelucci F, Oliviero A, Pilato F, Saturno E, Dileone M, Versace V, Musumeci G, Batocchi AP, Tonali PA, Di Lazzaro V: Transcranial magnetic stimulation and BDNF plasma levels in amyotrophic lateral sclerosis. Neuroreport 15: 717-720 (2004)

6. Aoki C, Wu K, Elste A, Len G, Lin S, McAuliffe G, Black IB: Localization of brain-derived neurotrophic factor and TrkB receptors to postsynaptic densities of adult rat cerebral cortex. J Neurosci Res 59: 454-463 (2000)

7. Artola A, Singer W: Long-term depression of excitatory synaptic transmission and its relationship to long-term potentiation. Trends Neurosci 16: 480-487 (1993)

8. Aydin-Abidin S, Trippe J, Funke K, Eysel UT, Benali A: High- and low-frequency repetitive transcranial magnetic stimulation differentially activates c-Fos and zif268 protein expression in the rat brain. Exp Brain Res 188: 249-261 (2008)

9. Baquet ZC, Gorski JA, Jones KR: Early striatal dendrite deficits followed by neuron loss with advanced age in the absence of anterograde cortical brain-derived neurotrophic factor. J Neurosci 24: 4250-4258 (2004)

10. Barker AT, Jalinous R, Freeston IL: Non-invasive magnetic stimulation of human motor cortex. Lancet 1: 1106-1107 (1985)

11. Bartsch AJ, Homola G, Biller A, Solymosi L, Bendszus M: Diagnostic functional MRI: illustrated clinical applications and decision-making. J Magn Reson Imaging 23: 921-932 (2006)

12. Benali A, Mix A, Trippe J, Eysel UT, Funke K: High and low frequency transcranial magnetic stimulation modulate the inhibitory system in the rat cortex. Soc Neurosci Abstr, Chicago: 719.712 (2009)

13. Berardelli A, Inghilleri M, Rothwell JC, Romeo S, Currà A, Gilio F, Modugno N, Manfredi M: Facilitation of muscle evoked responses after repetitive cortical stimulation in man. Exp Brain Res 122: 79-84 (1998)

14. Bestmann S, Baudewig J, Siebner HR, Rothwell JC, Frahm J: Subthreshold high-frequency TMS of human primary motor cortex modulates interconnected frontal motor areas as detected by interleaved fMRI-TMS. Neuroimage 20: 1685-1696 (2003)

15. Bestmann S, Baudewig J, Siebner HR, Rothwell JC, Frahm J: Functional MRI of the immediate impact of transcranial magnetic stimulation on cortical and subcortical motor circuits. Eur J Neurosci 19: 1950-1962 (2004)

16. Bestmann S, Baudewig J, Siebner HR, Rothwell JC, Frahm J: BOLD MRI responses to repetitive TMS over human dorsal premotor cortex. Neuroimage 28: 22-29 (2005)

17. Bestmann S, Ruff CC, Blankenburg F, Weiskopf N, Driver J, Rothwell JC: Mapping causal interregional influences with concurrent TMS-fMRI. Exp Brain Res 191: 383-402 (2008)

18. Bohning DE, Pecheny AP, Epstein CM, Speer AM, Vincent DJ, Dannels W, George MS: Mapping transcranial magnetic stimulation (TMS) fields in vivo with MRI. Neuroreport 8: 2535-2538 (1997)

19. Bohning DE, Shastri A, McConnell KA, Nahas Z, Lorberbaum JP, Roberts DR, Teneback C, Vincent DJ, George MS: A combined TMS/fMRI study of intensity-dependent TMS over motor cortex. Biol Psychiatry 45: 385-394 (1999)

20. Buckner RL, Andrews-Hanna JR, Schacter DL: The Brain's Default Network anatomy, function, and relevance to disease. Ann NY Acad Sci 1124: 1-38 (2008)

21. Buxton RB, Wong EC, Frank LR: Dynamics of blood flow and oxygenation changes during brain activation: the balloon model. Magn Reson Med 39: 855-864 (1998)

22. Canolty RT, Edwards E, Dalal SS, Soltani M, Nagarajan SS, Kirsch HE, Berger MS, Barbaro NM, Knight RT: High gamma power is phase-locked to theta oscillations in human neocortex. Science 313: 1626-1628 (2006)

23. Cárdenas-Morales L, Nowak DA, Kammer T, Wolf RC, Schönfeldt-Lecuona C: Mechanisms and Applications of Theta-burst rTMS on the Human Motor Cortex. Brain Topogr. 22: 294-306 (2010)

24. Castro-Alamancos MA, Donoghue JP, Connors BW: Different forms of synaptic plasticity in somatosensory and motor areas of the neocortex. J Neurosci 15: 5324-5333 (1995)

25. Cheeran B, Talelli P, Mori F, Koch G, Suppa A, Edwards MJ, Houlden H, Bhatia K, Greenwood R, Rothwell JC: A common polymorphism in the brain derived neurotrophic factor gene (BDNF) modulates human cortical plasticity and the response to rTMS. J Physiol 586: 5717-5725 (2008)

26. Chen R, Classen J, Gerloff C, Celnik P, Wassermann EM, Hallett M, Cohen LG: Depression of motor cortex excitability by low-frequency transcranial magnetic stimulation. Neurology 48: 1398-1403 (1997)

27. Cheng Q, Yeh HH: Brain-derived neurotrophic factor attenuates mouse cerebellar granule cell GABA(A) receptor-mediated responses via postsynaptic mechanisms. J Physiol 548: 711-721 (2003)

28. Chouinard PA, Van Der Werf YD, Leonard G, Paus T: Modulating neural networks with transcranial magnetic stimulation applied over the dorsal premotor and primary motor cortices. J Neurophysiol 90: 1071-1083 (2003)

29. Classen J, Witte OW, Schlaug G, Seitz RJ, Holthausen H, Benecke R: Epileptic seizures triggered directly by focal transcranial magnetic stimulation. Electroencephalogr Clin Neurophysiol 94: 19-25 (1995)

30. Conchou F, Loubinoux I, Castel-Lacanal E, Le Tinnier A, Gerdelat-Mas A, Faure-Marie N, Gros H, Thalamas C, Calvas F, Berry I, Chollet F, Simonetta Moreau M: Neural substrates of low-frequency repetitive transcranial magnetic stimulation during movement in healthy subjects and acute stroke patients. A PET study. Hum Brain Mapp 30: 2542-2557 (2008)

31. Damoiseaux JS, Rombouts SA, Barkhof F, Scheltens P, Stam CJ, Smith SM, Beckmann CF: Consistent resting-state networks across healthy subjects. Proc Natl Acad Sci USA 103: 13848-13853 (2006)

32. Daskalakis ZJ, Möller B, Christensen BK, Fitzgerald PB, Gunraj C, Chen R: The effects of repetitive transcranial magnetic stimulation on cortical inhibition in healthy human subjects. Exp Brain Res 174: 403-412 (2006)

33. Dassonville P, Zhu XH, Urgurbil K, Kim SG, Ashe J: Functional activation in motor cortex reflects the direction and the degree of handedness. Proc Natl Acad Sci USA 94: 14015-14018 (1997)

34. Davies CH, Starkey SJ, Pozza MF, Collingridge GL: GABA autoreceptors regulate the induction of LTP. Nature 349: 609-611 (1991)

35. Day BL, Rothwell JC, Thompson PD, Dick JP, Cowan JM, Berardelli A, Marsden CD: Motor cortex stimulation in intact man: Multiple descending volleys. Brain 110: 1191-1209 (1987)

36. Detre JA, Leigh JS, Williams DS, Koretsky AP: Perfusion imaging. Magn Reson Med 23: 37-45 (1992)

37. Detre JA, Wang J: Technical aspects and utility of fMRI using BOLD and ASL. Clin Neurophysiol 113: 621-634 (2002)

38. Detre JA, Wang J, Wang Z, Rao H: Arterial spin-labeled perfusion MRI in basic and clinical neuroscience. Curr Opin Neurol 22: 348-355 (2009)

39. Di Lazzaro V, Oliviero A, Mazzone P, Pilato F, Saturno E, Dileone M, Insola A, Tonali P, Rothwell J: Short-term reduction of intracortical inhibition in the human motor cortex induced by repetitive transcranial magnetic stimulation Exp Brain Res 147: 108-113 (2002)

40. Di Lazzaro V, Dileone M, Pilato F, Profice P, Ranieri F, Musumeci G, Angelucci F, Sabatelli M, Tonali PA: Repetitive transcranial magnetic stimulation for ALS - A preliminary controlled study. Neurosci Lett 408: 135-140 (2006)

41. Di Lazzaro V, Pilato F, Dileone M, Profice P, Capone F, Ranieri F, Musumeci G, Cianfoni A, Pasqualetti P, Tonali PA: Modulating cortical excitability in acute stroke: a repetitive TMS study. Clin Neurophysiol 119: 715-723 (2008a)

42. Di Lazzaro V, Pilato F, Dileone M, Profice P, Oliviero A, Mazzone P, Insola A, Ranieri F, Meglio M, Tonali PA, Rothwell JC: The physiological basis of the effects of intermittent theta burst stimulation of the human motor cortex. J Physiol 586: 3871-3879 (2008b)

43. Di Lazzaro V, Pilato F, Dileone M, Profice P, Oliviero A, Mazzone P, Insola A, Ranieri F, Tonali P, Rothwell J: Low-frequency repetitive transcranial magnetic stimulation suppresses specific excitatory circuits in the human motor cortex. J Physiol 586: 4481-4487 (2008c)

44. Egan MF, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, Bertolino A, Zaitsev E, Gold B, Goldman D, Dean M, Lu B, Weinberger DR: The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell 112: 257-269 (2003)

46. Eisenegger C, Treyer V, Fehr E, Knoch D: Time-course of "off-line" prefrontal rTMS effects--a PET study. Neuroimage 42: 379-384 (2008)

47. Fitzgerald PB, Brown TL, Daskalakis ZJ: The application of transcranial magnetic stimulation in psychiatry and neurosciences research. Acta Psychiatr Scand 105: 324-340 (2002)

48. Fitzgerald PB, Fountain S, Daskalakis ZJ: A comprehensive review of the effects of rTMS on motor cortical excitability and inhibition. Clin Neurophysiol 117: 2584-2596 (2006)

49. Floyer-Lea A, Matthews PM: Distinguishable brain activation networks for short- and long-term motor skill learning. J Neurophysiol 94: 512-518 (2005)

50. Floyer-Lea A, Wylezinska M, Kincses T, Matthews PM: Rapid modulation of GABA concentration in human sensorimotor cortex during motor learning. J Neurophysiol 95: 1639-1644 (2006)

51. Franca M, Koch G, Mochizuki H, Huang YZ, Rothwell JC: Effects of theta burst stimulation protocols on phosphene threshold. Clin Neurophysiol 117: 1808-1813 (2006)

52. Freund TF: Interneuron Diversity series: Rhythm and mood in perisomatic inhibition. Trends Neurosci 26: 489-495 (2003)

53. Gentner R, Wankerl K, Reinsberger C, Zeller D, Classen J: Depression of human corticospinal excitability induced by magnetic theta-burst stimulation: Evidence of rapid polarity-reversing metaplasticity. Cereb Cortex 18: 2046-2053 (2008)

54. George MS, Wassermann EM, Williams WA, Callahan A, Ketter TA, Basser P, Hallett M, Post RM: Daily repetitive transcranial magnetic stimulation (rTMS) improves mood in depression. Neuroreport 6: 1853-1856 (1995)

55. Gerschlager W, Siebner HR, Rothwell JC: Decreased corticospinal excitability after subthreshold 1 Hz rTMS over lateral premotor cortex. Neurology 57: 449-455 (2001)

56. Goodyear BG, Douglas EA: Decreasing task-related brain activity over repeated functional MRI scans and sessions with no change in performance: implications for serial investigations. Exp Brain Res 192: 231-239 (2009)

57. Gottschalk W, Pozzo-Miller LD, Figurov A, Lu B: Presynaptic modulation of synaptic transmission and plasticity by brain-derived neurotrophic factor in the developing hippocampus. J Neurosci18: 6830-6839 (1998)

58. Grossheinrich N, Rau A, Pogarell O, Hennig-Fast K, Reinl M K, S. , Dieler A, Leicht G, Mulert C, Sterr A, Padberg F: Theta Burst Stimulation of the Prefrontal Cortex: Safety and Impact on Cognition, Mood, and Resting Electroencephalogram. Biol Psychiatry 65: 778-784 (2009)

59. Hariri AR, Goldberg TE, Mattay VS, Kolachana BS, Callicott JH, Egan MF, Weinberger DR: Brain-derived neurotrophic factor val66met polymorphism affects human memory-related hippocampal activity and predicts memory performance. J Neurosci 23: 6690-6694 (2003)

60. Heide G, Witte OW, Ziemann U: Physiology of modulation of motor cortex excitability by low-frequency suprathreshold repetitive transcranial magnetic stimulation. Exp Brain Res 171: 26-34 (2006)

61. Hermes M, Hagemann D, Britz P, Lieser S, Rock J, Naumann E, Walter C: Reproducibility of continuous arterial spin labeling perfusion MRI after 7 weeks. MAGMA 20: 103-115 (2007)

62. Hess G, Aizenman CD, Donoghue JP: Conditions for the induction of long-term potentiation in layer II/III horizontal connections of the rat motor cortex. J Neurophysiol 75: 1765-1778 (1996)

63. Hrabetova S, Sacktor TC: Long-term potentiation and long-term depression are induced through pharmacologically distinct NMDA receptors. Neurosci Lett 226: 107-110 (1997)

64. Huang YZ, Edwards MJ, Rounis E, Bhatia KP, Rothwell JC: Theta burst stimulation of the human motor cortex. Neuron 45: 201-206 (2005)

65. Huang YZ, Chen RS, Rothwell JC, Wen HY: The after-effect of human theta burst stimulation is NMDA receptor dependent. Clin Neurophysiol 118: 1028-1032 (2007)

66. Huang YZ, Rothwell JC, Edwards MJ, Chen RS: Effect of physiological activity on an NMDA-dependent form of cortical plasticity in human. Cereb Cortex 18: 563-570 (2008)

67. Hubl D, Nyffeler T, Wurtz P, Chaves S, Pflugshaupt T, Lüthi M, von Wartburg R, Wiest R, Dierks T, Strik WK, Hess CW, Müri RM: Time course of blood oxygenation level-dependent signal response after theta burst transcranial magnetic stimulation of the frontal eye field. Neurosci 151: 921-928 (2008)

68. Ishikawa S, Matsunaga K, Nakanishi R, Kawahira K, Murayama N, Tsuji S, Huang YZ, Rothwell JC: Effect of theta burst stimulation over the human sensorimotor cortex on motor and somatosensory evoked potentials. Clin Neurophysiol 118: 1033-1043 (2007)

69. Isomura Y, Harukuni R, Takekawa T, Aizawa H, Fukai T: Microcircuitry coordination of cortical motor information in self-initiation of voluntary movements. Nat Neurosci 12: 1586-1593 (2009)

70. Izquierdo I, Bevilaqua LR, Rossato JI, Bonini JS, Medina JH, Cammarota M: Different molecular cascades in different sites of the brain control memory consolidation. Trends Neurosci 29: 496-505 (2006)

71. Ji RR, Schlaepfer TE, Aizenman CD, Epstein CM, Qiu D, Huang JC, Rupp F: Repetitive transcranial magnetic stimulation activates specific regions in rat brain. Proc Natl Acad Sci USA 95: 15635-15640 (1998)

72. Ji Y, Lu Y, Yang F, Shen W, Tang T, Feng L, Duan S, Lu B: Acute and gradual increases in BDNF concentration elicit distinct signaling and functions in neurons. Nat Neurosci 13: 302-309 (2010)

73. Kahana MJ: The cognitive correlates of human brain oscillations. J Neurosci 26: 1669-1672 (2006)

74. Kammer T, Vorwerg M, Herrnberger B: Anisotropy in the visual cortex investigated by neuronavigated transcranial magnetic stimulation. NeuroImage 36: 313-321 (2007)

75. Karege F, Perret G, Bondolfi G, Schwald M, Bertschy G, Aubry JM: Decreased serum brain-derived neurotrophic factor levels in major depressed patients. Psychiatry Res 109: 143-148 (2002)

76. Khedr EM, Rothwell JC, Ahmed MA, Shawky OA, Farouk M: Modulation of motor cortical excitability following rapid-rate transcranial magnetic stimulation. Clin Neurophysiol 118: 140-145 (2007)

77. Kim SG, Ashe J, Hendrich K, Ellermann JM, Merkle H, Ugurbil K, Georgopoulos AP: Functional magnetic resonance imaging of motor cortex: hemispheric asymmetry and handedness. Science 261: 615-617 (1993)

78. Kim YH, You SH, Ko MH, Park JW, Lee KH, Jang SH, Yoo WK, Hallett M: Repetitive transcranial magnetic stimulation-induced corticomotor excitability and associated motor skill acquisition in chronic stroke. Stroke 37: 1471-1476 (2006)

79. Kleim JA, Chan S, Pringle E, Schallert K, Procaccio V, Jimenez R, Cramer SC: BDNF val66met polymorphism is associated with modified experience-dependent plasticity in human motor cortex. Nat Neurosci 9: 735-737 (2006)

80. Klintsova AY, Dickson E, Yoshida R, Greenough WT: Altered expression of BDNF and its high-affinity receptor TrkB in response to complex motor learning and moderate exercise. Brain Res 1028: 92-104 (2004)

81. Kujirai T, Caramia MD, Rothwell JC, Day BL, Thompson PD, Ferbert A, Wroe S, Asselman P, Marsden CD: Corticocortical inhibition in human motor cortex. J Physiol 471: 501-519 (1993)

82. Larson J, Lynch G: Induction of synaptic potentiation in hippocampus by patterned stimulation involves two events. Science 232: 985-988 (1986)

83. Laske C, Eschweiler GW: Brain-derived neurotrophic factor: from nerve growth factor to modulator of brain plasticity in cognitive processes and psychiatric diseases. Nervenarzt 77: 523-537 (2006)

84. Lee L, Siebner HR, Rowe JB, Rizzo V, Rothwell JC, Frackowiak RS, Friston KJ: Acute remapping within the motor system induced by low-frequency repetitive transcranial magnetic stimulation. J Neurosci 23: 5308-5318 (2003)

85. Lee SH, Kim W, Chung YC, Jung KH, Bahk WM, Jun TY, Kim KS, George MS, Chae JH: A double blind study showing that two weeks of daily repetitive TMS over the left or right temporoparietal cortex reduces symptoms in patients with schizophrenia who are having treatment-refractory auditory hallucinations. Neurosci Lett 376: 177-181 (2005)

86. Lisman JE, Idiart MA: Storage of 7 +/- 2 short-term memories in oscillatory subcycles. Science 267: 1512-1515 (1995)

87. Logothetis NK, Pauls J, Augath M, Trinath T, Oeltermann A: Neurophysiological investigation of the basis of the fMRI signal. Nature 412: 128-130 (2001)

88. Logothetis NK: What we can do and what we cannot do with fMRI. Nature 453: 869-878 (2008)

89. Loubinoux I, Carel C, Alary F, Boulanouar K, Viallard G, Manelfe C, Rascol O, Celsis P, Chollet F: Within-session and between-session reproducibility of cerebral sensorimotor activation: a test--retest effect evidenced with functional magnetic resonance imaging. J Cereb Blood Flow Metab 21: 592-607 (2001)

90. Maeda F, Keenan JP, Tormos JM, Topka H, Pascual-Leone A: Interindividual variability of the modulatory effects of repetitive transcranial magnetic stimulation on cortical excitability. Exp Brain Res 133: 125-130 (2000)

91. Magistretti PJ, Pellerin L: Cellular mechanisms of brain energy metabolism and their relevance to functional brain imaging. Philos Trans R Soc Lond B Biol Sci 354: 1155-1163 (1999)

92. Malenka RC, Bear MF: LTP and LTD: an embarrassment of riches. Neuron 44: 5-21 (2004)

93. Manabe T: Does BDNF have pre- or postsynaptic targets? Science 295: 1651-1653 (2002)

94. Mansur CG, Fregni F, Boggio PS, Riberto M, Gallucci-Neto J, Santos CM, Wagner T, Rigonatti SP, Marcolin MA, Pascual-Leone A: A sham stimulation-controlled trial of rTMS of the unaffected hemisphere in stroke patients. Neurology 64: 1802-1804 (2005)

95. Markram H, Toledo-Rodriguez M, Wang Y, Gupta A, Silberberg G, Wu C: Interneurons of the neocortical inhibitory system. Nat Neurosci 5: 793–807 (2004)

96. McHughen S, Rodriguez PF, Kleim JA, Kleim ED, Crespo LM, Procaccio V, Cramer S: BDNF Val66Met Polymorphism Influences Motor System Function in the Human Brain. Cereb Cortex 5:1254-1262 (2009)

97. Mima T, Sadato N, Yazawa S, Hanakawa T, Fukuyama H, Yonekura Y, Shibasaki H: Brain structures related to active and passive finger movements in man. Brain 122: 1989-1997 (1999)

98. Moisa M, Pohmann R, Uludag K, Thielsher A: Interleaved TMS/CASL: A motor cortex study. in Third international conference on TMS and tDCS October 1-4, Göttingen, Germany (Ed. George M S). Brain Stimulation pp. 290 (2008).

99. Moisa M, Pohmann R, Ewald L, Thielscher A: New coil positioning method for interleaved transcranial magnetic stimulation (TMS)/functional MRI (fMRI) and its validation in a motor cortex study. J Magn Reson Imaging 29: 189-197 (2009)

100. Moisa M, Pohmann R, Uludag K, Thielscher A: Interleaved TMS/CASL: Comparison of different rTMS protocols. NeuroImage 49: 612-620 (2010)

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

102. Mulkey RM, Malenka RC: Mechanisms underlying induction of homosynaptic long-term depression in area CA1 of the hippocampus. Neuron 5: 967-975 (1992)

103. Müller MB, Toschi N, Kresse AE, Post A, Keck ME: Long-term repetitive transcranial magnetic stimulation increases the expression of brain-derived neurotrophic factor and cholecystokinin mRNA, but not neuropeptide tyrosine mRNA in specific areas of rat brain. Neuropsychopharmacol 23: 205-215 (2000)

104. Nair DG: About being BOLD. Brain Res 50: 229-243 (2005)

105. Nowak D, Berner J, Herrnberger B, Kammer T, Grön G, Schönfeldt-Lecuona C: Continuous theta-burst stimulation over the dorsal premotor

cortex interferes with associative learning during object lifting. Cortex 45: 473-482 (2008)

106. Nowak DA, Hoffmann U, Connemann BJ, Schönfeldt-Lecuona C: Epileptic seizure following 1 Hz repetitive transcranial magnetic stimulation. Clin Neurophysiol 117: 1631-1633 (2006)

107. Nyffeler T, Wurtz P, Lüscher H, Hess C, Senn W, Pflugshaupt T, von Wartburg R, Lüthi M, Müri R: Extending lifetime of plastic changes in the human brain. Eur J Neurosci 24: 2961-2966 (2006)

108. O'Reardon JP, Solvason HB, Janicak PG, Sampson S, Isenberg KE, Nahas Z, McDonald W, Avery D, Fitzgerald PB, Loo C, Demitrack MA, George MS, Sackeim HA: Efficacy and safety of transcranial magnetic stimulation in the acute treatment of major depression: a multisite randomized controlled trial. Biol Psychiatry 62: 1208-1216 (2007)

109. Ogawa S, Lee TM, Kay AR, Tank DW: Brain magnetic resonance imaging with contrast dependent on blood oxygenation. Proc Natl Acad Sci USA 87: 9868-9872 (1990)

110. Ogiue-Ikeda M, Kawato S, Ueno S: Acquisition of ischemic tolerance by repetitive transcranial magnetic stimulation in the rat hippocampus. Brain Res 1037: 7-11 (2005)

111. Oldfield RC: The assessment and analysis of handedness: The Edinburgh inventory. Neuropsychologia 9: 97-113 (1971)

112. Ortu E, Deriu F, Suppa A, Tolu E, Rothwell JC: Effects of volitional contraction on intracortical inhibition and facilitation in the human motor cortex. J Physiol 586: 5147-5159 (2008)

113. Ouyang Y, Rosenstein A, Kreiman G, Schuman EM, Kennedy MB: Tetanic stimulation leads to increased accumulation of Ca(2+)/calmodulin-dependent protein kinase II via dendritic protein synthesis in hippocampal neurons. J Neurosci 19: 519-527 (1999)

114. Pascual-Leone A, Valls-Sole J, Brasil-Neto JP, Cohen LG, Hallet M: Seizure induction and transcranial magnetic stimulation. Lancet 339: 997 (1992)

115. Pascual-Leone A, Houser CM, Reese K, Shotland LI, Grafman J, Sato S, Valls-Solé J, Brasil-Neto JP, Wassermann EM, Cohen LG: Safety of rapid-rate transcranial magnetic stimulation in normal volunteers. Electroencephalogr Clin Neurophysiol 89: 120-130 (1993)

116. Pascual-Leone A, Valls-Solé J, Wassermann EM, Hallett M: Responses to rapid-rate transcranial magnetic stimulation of the human motor cortex. Brain 117: 847-858 (1994)

117. Pascual-Leone A, Tormos JM, Keenan J, Tarazona F, Cañete C, Catalá MD: Study and modulation of human cortical excitability with transcranial magnetic stimulation. J Clin Neurophysiol 15: 333-343 (1998)

118. Peinemann A, Lehner C, Mentschel C, Conrad B, Siebner HR: Subthreshold 5-Hz repetitive transcranial magnetic stimulation of the human primary motor cortex reduces intracortical paired-pulse inhibition. Neurosci Lett 15: 21-24 (2000)

119. Peinemann A, Lehner C, Mentschel C, Munchau A, Conrad B, Siebner HR: Subthreshold 5-Hz repetitive transcranial magnetic stimulation of the human primary motor cortex reduces intracortical paired-pulse inhibition. Neurosci Lett 296: 21-24 (2000)

120. Pezawas L, Verchinski BA, Mattay VS, Callicott JH, Kolachana BS, Straub RE, Egan MF, Meyer-Lindenberg A, Weinberger DR: The brain-derived neurotrophic factor val66met polymorphism and variation in human cortical morphology. J Neurosci 24: 10099-100102 (2004)

121. Pozzo-Miller LD, Gottschalk W, Zhang L, McDermott K, Du J, Gopalakrishnan R, Oho C, Sheng ZH, Lu B: Impairments in high-frequency transmission, synaptic vesicle docking, and synaptic protein distribution in the hippocampus of BDNF knockout mice. J Neurosci 19: 4972-4983 (1999)

122. Raichle ME: Behind the scenes of functional brain imaging: a historical and physiological perspective. Proc Natl Acad Sci USA 95: 765-772 (1998)

123. Raichle ME, MacLeod AM, Snyder AZ, Powers WJ, Gusnard DA, Shulman GL: A default mode of brain function. Proc Natl Acad Sci USA 98: 676-682 (2001)

124. Rao SM, Binder JR, Hammeke TA, Bandettini PA, Bobholz JA, Frost JA, Myklebust BM, Jacobson RD, Hyde JS: Somatotopic mapping of the human primary motor cortex with functional magnetic resonance imaging. Neurology 45: 919-924 (1995)

125. Reis J, Swayne OB, Vandermeeren Y, Camus M, Dimyan MA, Harris-Love M, Perez MA, Ragert P, Rothwell JC, Cohen LG: Contribution of transcranial magnetic stimulation to the understanding of cortical mechanisms involved in motor control. J Physiol 586: 325-351 (2008)

126. Ridding MC, Taylor JL, Rothwell JC: The effect of voluntary contraction on cortico-cortical inhibition in human motor cortex. J Physiol 487: 541-548 (1995)

127. Ridding MC, Rothwell JC: Perspectives - Opinion - Is there a future for therapeutic use of transcranial magnetic stimulation? Nat Rev Neurosci 8: 559-567 (2007)

128. Rossini PM, Barket AT, Berardelli A, Caramia MD, Caruso G, Cracco RQ, Dimitrijevic MR, Hallett M, Katayama Y, Lücking CH: Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. Electroenceph Clin Neurophysiol Suppl 91: 79-92 (1994)

129. Rothwell JC, Thompson PD, Day BL, Boyd S, Marsden CD: Stimulation of the human motor cortex through the scalp. Exp Physiol 76: 159–200 (1991)

130. Rounis E, Lee L, Siebner HR, Rowe JB, Friston KJ, Rothwell JC, Frackowiak RS: Frequency specific changes in regional cerebral blood flow and motor system connectivity following rTMS to the primary motor cortex. NeuroImage 26: 164-176 (2005)

131. Rounis E, Stephan KE, Lee L, Siebner HR, Pesenti A, Friston KJ, Rothwell JC, Frackowiak RS: Acute changes in frontoparietal activity after repetitive transcranial magnetic stimulation over the dorsolateral prefrontal cortex in a cued reaction time task. J Neurosci 26: 9629-9638 (2006)

132. Sağlam M, Matsunaga K, Murayama N, Hayashida Y, Huang YZ, Nakanishi R: Parallel inhibition of cortico-muscular synchronization and cortico-spinal excitability by theta burst TMS in humans. Clin Neurophysiol 119: 2829-2838 (2008)

133. Schindler K, Nyffeler T, Wiest R, Hauf M, Mathis J, Hess C, Müri R: Theta burst transcranial magnetic stimulation is associated with increased EEG synchronization in the stimulated relative to unstimulated cerebral hemisphere. Nuerosci Lett 436: 31-34 (2008)

134. Schwenkreis P, Witscher K, Pleger B, Malin JP, tegenthoff M: The NMDA antagonist memantine affects training induced motor cortex plasticity-a study using transcranial magnetic stimulation. BMC Neurosci 6:35 (2005)

135 Siebner HR, Peller M, Willoch F, Minoshima S, Boecker H, Auer C, Drzezga A, Conrad B, Bartenstein P: Lasting cortical activation after repetitive TMS of the motor cortex: a glucose metabolic study. Neurology 54: 956-963 (2000)

136. Siebner HR, Takano B, Peinemann A, Schwaiger M, Conrad B, Drzezga A: Continuous transcranial magnetic stimulation during positron emission tomography: a suitable tool for imaging regional excitability of the human cortex. NeuroImage 14: 883-890 (2001)

137. Siebner HR, Lang N, Rizzo V, Nitsche MA, Paulus W, Lemon RN, Rothwell JC: Preconditioning of low-frequency repetitive transcranial magnetic stimulation with transcranial direct current stimulation: evidence for homeostatic plasticity in the human motor cortex. J Neurosci 24: 3379-3385 (2004)

138. Solodkin A, Hlustik P, Noll DC, Small SL: Lateralization of motor circuits and handedness during finger movements. Eur J Neurol 8: 425-434 (2001)

139. Stagg CJ, Wylezinska M, Matthews PM, Johansen-Berg H, Jezzard P, Rothwell JC, Bestmann S: Neurochemical effects of theta burst stimulation as assessed by magnetic resonance spectroscopy. J Neurophysiol 101: 2872-2877 (2009)

140 Stefan K, Gentner R, Zeller D, Dang S, Classen J: Theta-burst stimulation: remote physiological and local behavioral after-effects. NeuroImage 40: 265-274 (2008)

141. Suppa A, Ortu E, Zafar N, Deriu F, Paulus W, Berardelli A, Rothwell JC: Theta burst stimulation induces after-effects on contralateral primary motor cortex excitability in humans. J Physiol 586: 4489-4500 (2008)

142. Talelli P, Cheeran BJ, Teo JTH, Rothwell JC: Pattern-specific role of the current orientation used to deliver Theta Burst Stimulation. Clin Neurophysiol 118: 1815-1823 (2007)

143. Tanaka T, Saito H, Matsuki N: Inhibition of GABAA synaptic responses by brain-derived neurotrophic factor (BDNF) in rat hippocampus. J Neurosci 17: 2959-2966 (1997)

144. Teo JTH, Swayne OB, Rothwell JC: Further evidence for NMDA-dependence of the after-effects of human theta burst stimulation. Clin Neurophysiol 118: 1649-1651 (2007)

145. Tergau F, Naumann U, Paulus W, Steinhoff BJ: Low-frequency repetitive transcranial magnetic stimulation improves intractable epilepsy. Lancet 353: 2209 (1999)

146. Thickbroom GW: Transcranial magnetic stimulation and synaptic plasticity: experimental framework and human models. Exp Brain Res 180: 583-593 (2007)

147. Tokay T, Holl N, Kirschstein T, Zschorlich V, Köhling R: High-frequency magnetic stimulation induces long-term potentiation in rat hippocampal slices. Neurosci Lett 461: 150-154 (2009)

148. Toni I, Schluter ND, Josephs O, Friston K, Passingham RE: Signal-, set- and movement-related activity in the human brain: an event-related fMRI study. Cereb Cortex 9: 35-49 (1999)

149. Touge T, Gerschlager W, Brown P, Rothwell JC: Are the after-effects of low-frequency rTMS on motor cortex excitability due to changes in the efficacy of cortical synapses? . Clin Neurophysiol 112: 2138–2145 (2001)

150. Trippe J, Mix A, Aydin-Abidin S, Funke K, Benali A: Theta burst and conventional low-frequency rTMS differentially affect GABAergic neurotransmission in the rat cortex. Exp Brain Res (In press)

151. Turrigiano GG, Leslie KR, Desai NS, Rutherford LC, Nelson SB: Activity-dependent scaling of quantal amplitude in neocortical neurons. Nature 391: 845-846 (1998)

152. Viviani R, Sim EJ, Lo H, Richter S, Haffer S, Osterfeld N, Thöne J, Beschoner P: Components of variance in brain perfusion and the design of studies of individual differences: the baseline study. Neuroimage 15: 12-22 (2009)

153. Wang J, Aguirre GK, Kimberg DY, Detre JA: Empirical analyses of nullhypothesis perfusion FMRI data at 1.5 and 4 T. NeuroImage 19: 1449-1462 (2003)

154. Wang Z, Wang J, Connick TJ, Wetmore GS, Detre JA: Continuous ASL (CASL) perfusion MRI with an array coil and parallel imaging at 3T. Magn Reson Med 54: 732-737 (2005)

155. Wassermann E, Grafman J, Berry C, Hollnagel C, Wild K, Clark K, Hallett M: Use and safety of a new repetitive transcranial magnetic stimulator. Electroenceph Clin Neurophysiol 101: 412-417 (1996)

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

157. Wu T, Sommer M, Tergau F, Paulus W: Lasting influence of repetitive transcranial magnetic stimulation on intracortical excitability in human subjects. Neurosci Lett 287: 37-40 (2000)

158. Yoo WK, You SH, Ko MH, Tae Kim S, Park CH, Park JW, Hoon Ohn S, Hallett M, Kim YH: High frequency rTMS modulation of the sensorimotor networks: behavioral changes and fMRI correlates. NeuroImage 39: 1886-1895 (2008)

159. Zafar N, Paulus W, Sommer M: Comparative assessment of best conventional with best theta burst repetitive transcranial magnetic stimulation protocols on human motor cortex excitability. Clin Neurophysiol 119: 1393-1399 (2008)

160. Zeng L, Chen H, Ouyang L, Yao D, Gao JH: Quantitative analysis of asymmetrical cortical activity in motor areas during sequential finger movement. Magn Reson Imaging 25: 1370-1375 (2007)

161. Ziemann U, Lönnecker S, Steinhoff BJ, Paulus W: Effects of antiepileptic drugs on motor cortex excitability in humans: a transcranial magnetic stimulation study. Ann Neurol 40: 344-345 (1996)

162. Ziemann U: Intracortical inhibition and facilitation in the conventional paired TMS paradigm. Electroenceph Clin Neurophysiol Suppl 51: 127-136 (1999)

163. Ziemann U: TMS induced plasticity in human cortex. Rev Neurosci 15: 253-266 (2004)

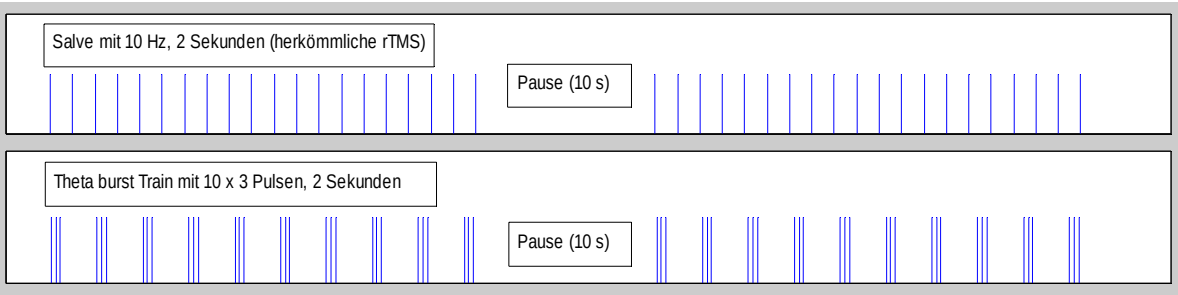
# APPENDIX

## Appendix A: Written information to participants about the study.

Sehr geehrte Probandin, sehr geehrter Proband,

Sie interessieren sich für unsere oben genannte Studie. Die transkranielle Magnetstimulation (TMS) ist ein Verfahren, in dem mittels einer an den Kopf des Probanden gehaltenen Spule ein Magnetfeld erzeugt wird, welches in dem Teil der Hirnrinde, der sich unter der Spule befindet, Nervenzellen erregen kann. Repetitive Stimulation bedeutet, dass wiederholt Magnetimpulse gegeben werden. Repetitive Stimulation kann zu einer vorübergehende Veränderung der Erregbarkeit der stimulierten Hirnrinde führen. Diese Veränderung hält in der Regel etwa eine Stunde an. Sie wird seit über 10 Jahren systematisch untersucht und experimentell auch zu therapeutischen Zwecken in der Psychiatrie eingesetzt.

In unserer Studie soll das so genannte Theta burst-Verfahren zur Anwendung kommen. Es unterscheidet sich von den bisher verwendeten repetitiven Verfahren nur durch scheinbar kleine Details. Während bisher 20-40 Magnetimpulse regelmäßig alle 50-100 Millisekunden wiederholt wurden (sogenannte Salven mit 10 bis 20 Hz, Dauer 2 sec), werden beim Theta burst-Verfahren drei Magnetimpulse mit einer Frequenz von 50 Hz appliziert. Diese Einheit dauert 40 Millisekunden und wird innerhalb von 2 sec 10 mal wiederholt (mit 5 Hz, sogenannte Theta-Frequenz), Den Block von 10 x 3 Magnetimpulsen bezeichnen wir als Train. Ein Train wird über eine Gesamtzeit von 190 sec alle 10 sec wiederholt, so dass insgesamt 600 Magnetimpulse während einer Stimulationssitzung gegeben werden (intermittierende Theta burst Stimulation, iTBS). In einer kürzlich veröffentlichten Studie zeigte sich, dass das Theta burst-Verfahren eine stärkere Veränderung der Erregbarkeit auslöst im Vergleich zu den herkömmlichen repetitiven Verfahren, obwohl die verwendete Stimulationsstärke deutlich niedriger war.




Ziel dieser Untersuchung ist es, die Effekte des Theta burst-Verfahrens (iTBS) auf die motorische Hirnrinde genauer zu charakterisieren. In der im Jahre 2005 veröffentlichten Studie wurde die Modulation der Erregbarkeit direkt mit TMS ermittelt. Einzelne Test-Stimuli wurden appliziert und die motorische Reaktion wurde aufgezeichnet. Nach der iTBS waren die Antworten auf die Test-Stimuli stärker geworden. Dieser Effekt hielt etwa eine Stunde an. Danach unterschieden sich die Antworten nicht von den ursprünglich gemessenen.

Wir wollen diesen Effekt replizieren. Zusätzlich möchten wir die vorübergehenden Veränderungen der motorischen Hirnrinde mit Hilfe von funktioneller Bildgebung erfassen. Dies erfolgt mit Hilfe der Kernspintomographie. Sie liegen in der Messröhre und müssen nach Anweisung über die Videobrille (Pfeil) und den Kopfhörer (Piepston )mit Ihrer linken oder rechten Hand so schnell wie möglich einen Knopf drücken. Die Hirnfunktion in Ruhe wird ebenfalls gemessen. Die Untersuchung zielt zunächst auf Fragen der Grundlagenforschung. Wir erhoffen uns von den Ergebnissen jedoch auch, die Magnetstimulation gezielter zu therapeutischen Zwecken bei Patienten einsetzen zu können. Die Untersuchungen finden an 3 aufeinanderfolgenden Tagen zur jeweils gleichen Zeit statt. Jede Untersuchung dauert etwa 2.5 Stunden.

Ablauf:

| Tag | 1 | 2 | 3 |
|---|---|---|---|
| Intervention | Theta busrt | keine | Theta burst |
| Messung | TMS | Kernspin | Kernspin |

Vorbereitung an Tag 1 ausserhalb der Kernspintomographie

- Montage der Messelektroden an der rechten Hand
- Aufsuchen des optimalen Stimulationsortes über der linken Hirnrinde
- Messung der Motorschwelle (15 min)
- Messung der Antwort des Handmuskels auf TMS-Pulse (10 min)
- Erste Umlagerung in die Kernspintomographie (Tag 2 und 3)
- Reaktion der Hirnrinde auf Bewegung als Basiswert (20 Minuten)

- Lagerung ausserhalb der Kernspintomographie
- Intervention: Stimulation mit Theta bursts (3 min 10 sec)
- Zweite Umlagerung in die Kernspintomographie (Tag 2 und 3)
- Reaktion der Hirnrinde auf Bewegung nach der Intervention (60 Minuten)

An den Tagen 2 und 3 wird die Kernspintomographie nicht verwendet, vor und nach der Intervention wird kontinuierlich die Antwort des Handmuskels auf TMS-Pulse aufgezeichnet (Dauer 60 Minuten nach der Intervention). Gesamtdauer des Experimentes: ca. 8 Stunden

Die TMS-Methode gilt als sicher und nebenwirkungsarm. Die häufigste Nebenwirkung ist die als unangenehm oder schmerzhaft empfundene lokale Muskelanspannung in der Kopfhaut unter der Spule. Diese ist völlig harmlos, aber gewöhnungsbedürftig. Selten können Kopfschmerzen auftreten, diese sprechen auf normale Kopfschmerzmittel an. Weiterhin kann der beim Aufbau des Magnetfeldes entstehende Knall störend sein. Daher müssen Sie einen Gehörschutz (Oropax) tragen.

Eine sehr seltene, aber prinzipiell mögliche Komplikation bei repetitiver Stimulation ist die Auslösung eines Krampfanfalles. Dies ist unter Berücksichtigung der gültigen Sicherheitsvorschriften bei den normalen repetitiven Protokollen nicht aufgetreten. Für das Theta burst-Verfahren gibt es noch keine Sicherheitsvorschriften. Es wurde bisher nur von einer Arbeitsgruppe in London erfolgreich und sicher eingesetzt. Ein entscheidender Parameter ist die Stimulationsintensität. Sie liegt beim Theta Burst-Verfahren deutlich unter den üblichen Intensitäten der normalen Protokolle (80% im Vergleich zu 110-120% der Motorschwelle). Die Wahrscheinlichkeit für das Auftreten eines Krampfanfalles lässt sich etwa vergleichen mit der Wahrscheinlichkeit, in einer Diskothek bei flackerndem Licht einen Krampfanfall zu erleiden. Falls wider Erwarten doch ein Krampfanfall auftreten sollte, haben wir alle medizinischen Vorkehrungen getroffen. Bleibende Schäden sind in einem Zeitraum von 20 Jahren nicht beobachtet worden.

Bei subjektiver Unverträglichkeit gegenüber der Magnetstimulation, z.B. dem Auftreten von Kopfschmerzen, kann die Untersuchung jederzeit abgebrochen werden. Starke Kopfschmerzen können Ihre Verkehrstüchtigkeit beeinträchtigen. Das Experiment selbst verursacht keine Beeinträchtigung der Verkehrstüchtigkeit. Die Bilderserien der Kernspintomographie ergeben keine diagnostische Untersuchung, bei der eine Erkrankung des Gehirns ausgeschlossen werden kann. Dennoch kann es sein, dass Hinweise auf eine nicht normal ausgeprägte Anatomie oder auf krankhafte Befunde festgestellt werden. Die Teilnahme an dieser Studie setzt voraus, dass Sie einverstanden sind, diese Befunde zu erfahren.

Es ist möglich, dass die Wirkung des Theta-burst Protokolls abhängt von einer genetischen Disposition (BDNF Val66Met). Jeder Mensch hat eine von drei möglichen Kombinationen im Gen. Wir möchten daher bei Ihnen zwei kleine Röhrchen Blut abnehmen, um Ihre Disposition sowie die Konzentration im Serum analysieren zu können. Wenn Sie möchten, können wir Ihnen nach Beendigung der Studie Ihr Ergebnis mitteilen. Es gibt erste Hinweise auf Zusammenhänge

zwischen BDNF-Genetik und neuropsychiatrischen Erkrankungen. Diese sind aber vage und haben keine Bedeutung in der Diagnostik. Die Blutzellen sollen für eventuelle weitere genetische Untersuchungen aufgehoben werden. Hierzu bitten wir Sie, den Übereignungsvertrag zu unterschreiben. Bestimmte Personen können wir nicht untersuchen, daher beantworten Sie uns bitte folgende Fragen:

| | | **Ja** | **Nein** |
|---|---|---|---|
| 1. | Hatten Sie jemals einen epileptischen Anfall? | | |
| 2. | Leiden Sie unter Migräne oder Spannungskopfschmerz? | | |
| 3. | Sind Sie Träger eines Herzschrittmachers? | | |
| 4. | Wurden bei Ihnen Operationen am Herz oder Kopf durchgeführt? | | |
| 5. | Sind Sie Träger eines Hydrocephalus-Shunts? | | |
| 6. | Befinden sich in Ihrem Körper Metallimplantate oder Splitter? | | |
| 7. | Ist Ihre Haut großflächig tätowiert? | | |
| 8. | Leiden Sie an Enge-Angst (Klaustrophobie)? | | |
| 9. | Leiden Sie aktuell unter den Nachwirkungen eines Schädel-Hirn-Traumas? | | |
| 10. | Leiden Sie aktuell an einer neurologischen oder psychiatrischen Erkrankung | | |
| 11. | Leiden Sie an einer schweren oder chronischen körperlichen Erkrankung? | | |
| 12. | Leiden Sie aktuell an einer Erkrankung (z.B. grippaler Infekt)? | | |
| 13. | Sind Sie Rechtshänder? | | |
| 14. | Sind Sie einverstanden, auffällige Befunde aus der Kernspintomographie | | |
| | mitgeteilt zu bekommen? | | |
| **Für Frauen:** | | | |
| 15. | Sind Sie schwanger? | | |
| 16. | Tragen Sie eine Spirale? | | |

**FREIWILLIGKEIT:**
An diesem Forschungsprojekt nehmen Sie freiwillig teil. Ihr Einverständnis können Sie jederzeit und ohne Angabe von Gründen widerrufen. Alle bis dahin erhobenen Daten und Proben werden vernichtet.

**ERREICHBARKEIT DES PROJEKTLEITERS:**
Sollten während des Verlaufes des Forschungsprojektes Fragen auftauchen, so können Sie jederzeit folgende Ansprechpartner unter der Telefonnummer erreichen:
Dr. Thomas Kammer: 0731 500-61544, Prof. Dr. Georg Grön: 0731 500-61422

**VERSICHERUNG:**
Während der Teilnahme an dem Forschungsprojekt genießen Sie Versicherungsschutz. Es gelten die allgemeinen Haftungsbedingungen.

Einen Schaden, der Ihrer Meinung nach auf die Untersuchung zurückzuführen ist, melden Sie bitte unverzüglich dem Projektleiter.

**SCHWEIGEPFLICHT/DATENSCHUTZ:**
Alle Personen, welche Sie im Rahmen dieses Projektes betreuen, unterliegen der Schweigepflicht und sind auf das Datengeheimnis verpflichtet.
Die studienbezogenen Untersuchungsergebnisse sollen in anonymisierter Form in wissenschaftlichen Veröffentlichungen verwendet werden.
Soweit es zur Kontrolle der korrekten Datenerhebung erforderlich ist, dürfen autorisierte Personen (z.B.: des Auftraggebers, der Universität) Einsicht in Aufzeichnungen nehmen.
Sofern zur Einsichtnahme autorisierte Personen nicht der obengenannten ärztlichen Schweigepflicht unterliegen, stellen personenbezogene Daten, von denen sie bei der Kontrolle Kenntnis erlangen, Betriebsgeheimnisse dar, die geheim zu halten sind.

Datum Name des/der aufklärenden Arztes/Ärztin

## Appendix B: Information and Consent (Einwilligungserklärung zum Datenschutz)

Inhalt, Vorgehensweise, Risiken und Ziel des obengenannten Forschungsprojektes sowie die Befugnis zur Einsichtnahme in die erhobenen Daten hat mir Dr. Thomas kammer ausreichend erklärt.

Ich hatte Gelegenheit Fragen zu stellen und habe hierauf Antwort erhalten.
Ich hatte ausreichend Zeit, mich für oder gegen die Teilnahme am Projekt zu entscheiden.

Eine Kopie der Probandeninformation und Einwilligungserklärung habe ich erhalten.

Ich willige in die Teilnahme am Forschungsprojekt ein.

.....
(Name des Probanden)

Ulm, ..... .....
(Datum) (Unterschrift des Probanden)

## Information und Einwilligungserklärung zum Datenschutz

Bei wissenschaftlichen Studien werden persönliche Daten und medizinische Befunde über Sie erhoben.

Die Speicherung, Auswertung und Weitergabe dieser studienbezogenen Daten erfolgt nach gesetzlichen

Bestimmungen und setzt vor Teilnahme an der Studie folgende freiwillige Einwilligung voraus:

1. Ich erkläre mich damit einverstanden, dass im Rahmen dieser Studie erhobene Daten / Krankheitsdaten auf Fragebögen und elektronischen Datenträgern aufgezeichnet und ohne Namensnennung verarbeitet werden

2) Außerdem erkläre ich mich damit einverstanden, dass eine autorisierte und zur Verschwiegenheit verpflichtete Person (z.B.: des Auftraggebers, der Universität) in meine erhobenen personenbezogenen Daten Einsicht nimmt, soweit dies für die Überprüfung des Projektes notwendig ist. Für diese Maßnahme entbinde ich den Arzt von der ärztlichen Schweigepflicht.

.....
(Unterschrift des Probanden)

## Appendix C: Edinburgh inventory for handness (Oldfield, 1971)

NAME:

GEBURTSDATUM:

BERUFSTÄTIGKEIT:

VERSUCHSNUMMER:

Vor Ihnen liegt eine Liste mit Tätigkeiten. Bitte geben Sie an, welche Hand Sie für diese Tätigkeit bevorzugen, indem Sie ein Kreuz (x) in die entsprechende Spalte machen. Wenn Sie in einem Fall keine Hand bevorzugen, tragen Sie bitte ein Kreuz in beide Spalten ein.
Versuchen Sie bitte, alle Fragen zu beantworten. Lassen Sie nur dann eine Lücke,

Wenn Sie mit einer Aufgabe überhaupt keine Erfahrung haben.

| | links | rechts |
|---|---|---|
| 1. schreiben | | |
| 2. zeichnen | | |
| 3. werfen | | |
| 4. schneiden | | |
| 5. Zahnbürste | | |
| 6. Messer (ohne Gabel) | | |
| 7. Löffel | | |
| 8. Besen (obere Hand) | | |
| 9. Streichholz anzünden (Streichholz) | | |
| 10. Schachtel aufmachen (Deckel) | | |
| 11. Welchen Fuß bevorzugen Sie zum Kicken? | | |
| 12. Welches Auge bevorzugen Sie, wenn sie photographieren? | | |

13. Besitzen Sie linkshändige Angehörige und in welchem Verwandtschaftsgrad?

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I want to thank my supervisor, Dr. Thomas Kammer, for his guidance, support, empathy and patience along this work (and also with my german). I also owe many thanks to Prof. Dr. Georg Grön for his great help and for always being willing to discuss with us several topics regarding this work.

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Finally, I want to thank all volunteers for participating in this study. I know that it was a difficult task.

# Curriculum Vitae

**Lizbeth Karina Cárdenas-Morales**
Born in Morelia, Mexico 30.06.1976
Bessererstr 16/2 • 89073 Ulm, Germany

## Research Interests

Clinical research, non-invasive brain stimulation, neuropsychiatric disorders.

## Education

2007 - DATO **Ph.D. student (Human Biology)**
Faculty of Medicine, University of Ulm, Germany
Concentrations:
- Repetitive transcranial magnetic stimulation (rTMS)
- Choice reaction time task/Functional magnetic resonance imaging (fMRI)
- Surface Electromyography (EMG)

*Dissertation:* Effects of theta-burst stimulation on the human primary motor cortex: functional imaging study.
*Advisor:* Dr. Thomas Kammer

2004-2006 **Master of Science Neurobiology** (9.2 from 10 maximal)
National University of México (U.N.A.M.), Mexico City
Concentrations:
- rTMS in epilepsy
- Electroencephalogram and surface EMG in epilepsy

*Thesis:* Effects of repetitive transcranial magnetic stimulation on the frequency of EEG paroxysmal activity and seizures in epileptic patients.
*Advisor:* Dr. Efrain Santiago-Rodríguez

1995-2001 **Licenciate (Bsc/MSc) in Psychology** (9.6 from 10 maximal with honors)
U.N.A.M., Mexico City
Concentrations:
- Stress management by means of biofeedback methods (clinical psychology).
- Antibodies levels as stress markers (IgA, IgG)

*Thesis:* Psychophysiological assessment of stress in patients with asthma.
- *Advisor:* Dr. Benjamin Domínguez, General Hospital of Mexico City and Faculty of Psychology, U.N.A.M.

1992-1994 National High School: "Antonio Caso", Mexico City
U.N.A.M.

## Work Experience

2009-DATO **Research assistant (HiWi)**
Psychiatry Hospital, University of Ulm, Germany
Concentrations:

- Collaboration in clinical research projects by using repetitive magnetic stimulation for measuring pain perception (emotional and sensorial factors) and for the treatment of depression and motor conversion.
- Elaboration of scientific papers and posters·

2002 – 2004 **Teaching activities (undergraduate programme of Psychology)**
National Polytechnic Institute, Faculty of Psychology, Mexico City
- Motivation and Emotion (second semester)
- Tutorials in learning and memory (third semester)

2002 – 2004 **Academic Director**
Bertrand Russell High School, Mexico City
Concentrations:
- Representing the Institution as the maximal academic authority
- Supervising the work of all teachers

## Fellowships

2007- DATO German Academic Exchange Service (DAAD)
Ph.D. fellowship

2004-2006 National Council of Sciences and Technology (CONACyT), Mexico
Master fellowship

06-09.2006 National University of Mexico (U.N.A.M.)
Summer Internship at the Faculty of Medicine, University of Ulm, Germany

## Languages

- Spanish: (Mother tongue)
- English: fluent written and spoken (C1-Level)
- German: intermediate (B2- Level)

## Computer skills

- MS Office Word, power point and excel (good)
- Corel draw (basic level)
- STATISTICA 8 (mainly analysis of variance, t- Student , and Spearman correlations)
- SPM5 (basic level)

## Interests

- Music, arts, traveling, cooking, cultural diversity, dogs, friends and films.

## List of Publications

**Cárdenas-Morales L,** Grön G, Kammer T. Exploring the effects of thetaburst stimulation on the human motor cortex: an fMRI study. Hum Brain Mapp *(in press).*

**Lizbeth Cárdenas-Morales**, Anne-K Fladung, Thomas Kammer, Christian Schmahl, Paul L Plener, Bernhard J Connemann, Carlos Schönfeldt-Lecuona. Exploring the use of repetitive peripheral magnetic stimulation for studying pain perception in borderline personality disorder. Psychiatry Res (*in press*).

2010 Herwig U, **Cárdenas-Morales** L, Connemann BJ, Kammer T, Schönfeldt-Lecuona C. Sham or real-Post hoc estimation of stimulation condition in a randomized transcranial magnetic stimulation trial. Neurosci Lett, 471:30-33.

**Cárdenas-Morales L**, Nowak DA, Kammer T, Wolf RC, Schönfeldt-Lecuona C. Mechanisms and Applications of Theta-burst rTMS on the Human Motor Cortex. Brain Topogr; 22:294-306.

C. Schönfeldt-Lecuona, J.-P. Lefaucheur, **L. Cárdenas-Morales**, R.C. Wolf, T. Kammer, U. Herwig. The value of neuronavigated rMS for the treatment of depression. Neurophysiologie Cliniq/ClinNeurophysiol. In press.

2008 Santiago-Rodríguez E, **Cárdenas-Morales L**, Harmony T, Fernández-Bouzas A, Hernández A. Repetitive transcranial magnetic stimulation decreases the number of seizures in patients with focal neocortical epilepsy. Seizure 17:677-683.

2006 Santiago-Rodriguez E, Alonso-Vanegas M, **Cárdenas-Morales L**, Harmony T, Carabias J & Bernardino M (2006). 'Effects of Two Different Cycles of Vagus Nerve Stimulation on Interictal Epileptiform Discharges'. Seizure 15:615-20.

2002 Traue H, Domínguez B and **Cárdenas L.** Biological, Psychological and Social Symptoms in Victims of Torture. In National Comision of Human Rights México (Ed) Memoria del Foro Sobre la Tortura, Mexico City, pp 163-179.

## Congresses

2010 *16th Meeting for Human brain mapping, Barcelona 6-10 June*. **Cárdenas-Morales**, Grön G and Kammer T. Poster presentation: Theta-burst stimulation effects over the human motor cortex: a functional imaging study.

2008 *Third International Conference in Transcranial Magnetic Stimulation, Göttingen, Germany*. **Cárdenas-Morales**, Grön G and Kammer T. Poster presentation: Exploring the effects of theta-burst stimulation: An off-line combination of fMRI and TMS.

2006 *XXII. International Congress of Clinical Neurophysiology, Edinburgh, Scotland.* Oral presentation: "Effects of rTMS on seizure frequency and epileptiform discharges in patients with partial Epilepsy".

2005 *XXVI International Congress on Epilepsy, Paris, France.* **Cárdenas-Morales L**, Santiago Rodríguez E.Poster presentation 'Effects of vagus nerve stimulation on the EEG in patients with epilepsy'.

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dieser Dissertationsschrift