COMENIUS UNIVERSITY IN BRATISLAVA
FACULTY OF MATHEMATICS, PHYSICS AND INFORMATICS

EFFECTS OF TRANSCRANIAL MAGNETIC STIMULATION PROTOCOLS ON THE NEUROPLASTICITY OF THE PRIMARY MOTOR CORTEX

DIPLOMA THESIS

2015 **Bc. Miroslav Heriban**

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Study programme:	Cognitive Science (Single degree study, master II. deg., full time form)
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Supervisor:	Jurij Bon, M.D., MSc

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Comenius University in Bratislava
Faculty of Mathematics, Physics and Informatics

THESIS ASSIGNMENT

Name and Surname:	Bc. Miroslav Heriban
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Title: Effects of transcranial magnetic stimulation protocols on the neuroplasticity of the primary motor cortex

Aim: To measure and compare the effects of TMS protocols on the primary motor cortex.

Annotation: The goal of this master thesis is to measure and compare the effects of cTBS (continuous theta burst stimulation) protocols, which are a specific subset of repetitive transcranial magnetic stimulation (rTMS) protocols, on the primary motor cortex and thus help to propose the most perspective alternative for the application of TMS as an alternative treatment of depression.

Keywords: transcranial magnetic stimulation, TMS, rTMS, theta burst stimulation, cTBS, primary motor cortex, depression

Supervisor:	Jurij Bon, M.D., MSc
Department:	FMFI.KAI - Department of Applied Informatics
Head of department:	prof. Ing. Igor Farkaš, PhD.
Assigned:	15.05.2015

Approved: 15.05.2015 prof. Ing. Igor Farkaš, PhD.
Guarantor of Study Programme

Student

Supervisor

Univerzita Komenského v Bratislave
Fakulta matematiky, fyziky a informatiky

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Názov: Effects of transcranial magnetic stimulation protocols on the neuroplasticity of the primary motor cortex
Efekty protokolov transkraniálnej magnetickej stimulácie na neuroplasticitu primárnej motorickej kôry

Cieľ: Zmerať a porovnať efekty protokolov TMS na primárnu motorickú kôru.

Anotácia: Cieľom tejto diplomovej práce je zmerať a porovnať účinky protokolov cTBS (kontinuálna dávkovaná stimulácia v téta (Θ) pásme), ktoré sú špecifickou podskupinou protokolov repetitívnej transkraniálnej magnetickej stimulácie (rTMS), na primárnu motorickú kôru a tým pomôcť navrhnúť najperspektívnejšiu alternatívu pre aplikáciu TMS v alternatívnej liečbe depresie.

Kľúčové slová: transkraniálna magnetická stimulácia, TMS, rTMS, theta burst stimulation, cTBS, primárna motorická kôra, depresia

Vedúci: Jurij Bon, M.D., MSc
Katedra: FMFI.KAI - Katedra aplikovanej informatiky
Vedúci katedry: prof. Ing. Igor Farkaš, PhD.

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prof. Ing. Igor Farkaš, PhD.
garant študijného programu

študent

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Abstract

Our study, as a part of an ongoing project concerned with transcranial magnetic stimulation (TMS) as an alternative treatment of depression, was focused on testing of the most perspective continuous theta burst stimulation (cTBS) protocols, which are a subset of repetitive transcranial magnetic stimulation (rTMS) protocols, in their efficiency of inducing neuromodulatory changes in the primary motor cortex, concretely inhibitory effects. This would indicate their potential efficiency in inhibiting the dorsolateral prefrontal cortex, which is the brain structure that is currently being stimulated with conventional rTMS protocols as an alternative treatment of depression, but with a lower efficiency than other alternative treatments such as electroconvulsive theraphy (ECT), which has notable disadvantages including cognitive impairment. After an extensive review of literature and studies conducted with cTBS, we chose the standard 50 Hz cTBS and a relatively novel, but promising 30 Hz cTBS as the most suitable candidates for inducing inhibition in the primary motor cortex. We conceptualized a within-subject experimental design to assess their effects on the primary motor cortex. Our results showed that both tested cTBS protocols were not statistically significantly different in their effects on the primary motor cortex, which were slightly facilitatory rather than inhibitory. Furthermore, we found that the effects were intrapersonally as well as interpersonally variable, unstable over time and generally inconsistent. The cumulative effects of cTBS were also incoherent. Our findings have important implications on the TMS-depression project as well as the broad scientific community in TMS research, since the cTBS is widely considered as inhibitory. The limitations of our study include the potentially problematic assumption of the generalization of the primary motor cortex response on other brain structures, our general inexperience with TMS, the inherent variability of TMS, the author's general inexperience and other practical issues related to TMS.

Key words: transcranial magnetic stimulation, TMS, rTMS, theta burst stimulation, cTBS, primary motor cortex, depression

Abstrakt

Naša štúdia, ako súčasť prebiehajúceho projektu zaoberajúceho sa transkraniálnou magnetickou stimuláciou ako alternatívnou liečbou depresie, bola zameraná na testovanie najperspektívnejších protokolov kontinuálnej dávkovanej stimulácie v téta pásme (cTBS), ktoré sú podskupinou protokolov repetitívnej transkraniálnej magnetickej stimulácie (rTMS) a ich efektivity indukcie neuromodulačných zmien v primárnej motorickej kôre, konkrétne inhibičných efektov. Toto by indikovalo ich potenciálnu efektivitu inhibície dorzolaterálnej prefrontálnej kôry, ktorá je mozgovou štruktúrou, ktorá býva v súčasnosti stimulovaná konvenčnými protokolmi rTMS v rámci alternatívnej liečby depresie, no pri nižšej efektivite ako iné alternatívne liečby ako elektrokonvulzívna terapia (ECT), ktorá má podstatné nevýhody zahŕňajúce zhoršenie kognitívnych funkcií. Po rozsiahlom štúdiu literatúry a štúdií realizovaných s cTBS sme vybrali štandardný 50 Hz cTBS a relatívne nový, no sľubný 30 Hz cTBS protokol ako najvhodnejších kandidátov pre indukciu inhibície v primárnej motorickej kôre. Skonceptualizovali sme vnútrosubjektový experimentálny dizajn pre zmeranie ich efektov na primárnu motorickú kôru. Naše výsledky ukázali, že oba testované protokoly neboli štatisticky signifikantne odlišné v ich efektoch na primárnu motorickú kôru, ktoré boli skôr mierne facilitačné ako inhibičné. Navyše sme zistili, že tieto efekty boli intrapersonálne ako i interpersonálne variabilné, nestabilné v čase a celkovo inkonzistentné. Kumulatívne efekty cTBS boli tiež inkoherentné. Naše zistenia majú podstatné implikácie pre projekt TMS-depresia ako aj pre širokú vedeckú komunitu výskumu TMS, keďže v súčasnosti sú cTBS protokoly všeobecne považované za inhibičné. Medzi limity našej štúdie patrí potenciálne problematický predpoklad generalizácie odozvy primárnej motorickej kôry na iné mozgové štruktúry, našu celkovú neskúsenosť s TMS, inherentnú variabilitu TMS, autorovu celkovú neskúsenosť a iné praktické problémy spojené s TMS.

Kľúčové slová: transkraniálna magnetická stimulácia, TMS, rTMS, theta burst stimulation, cTBS, primárna motorická kôra, depresia

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1. Introduction

1.1 Theoretical introduction

Historically, brain-related diseases, dysfunctions and lesions have been interpreted as and attributed to various mysterious causes and mechanisms, many of them not even taking into account the brain as a relatively independently functioning organ despite the already acquired knowledge about the gross anatomical structure of the brain from numerous post-mortem studies. This is understandable, considering the overall lack of detailed insight into the brain itself ranging from the structural (anatomical) to the functional aspect due to its principal inaccessibility in vivo in real time. Even methodologically correct and acceptable scientific inquiries were possible only indirectly, assuming certain (non-materialistic) mechanisms and totally omitting the issue of localization, without the possibility of reaching a definitive conclusion (keeping in mind the potential perils and threats of logical fallacies like double causation, etc.). Another very popular approach were (and remain until now) case studies conducted on subjects who have been inadvertently injured and consequently suffered from direct cognitive impairment, which was used as evidence for inductive reasoning in terms of localization of certain brain functions.

Broadly speaking, there are 2 main aspects of the study of the brain: the anatomical and the functional aspect. The anatomical structure of the brain has been explored by autopsies (as stated above) and more recently with the help of gradually emerging technologies (such as CT and MRI). Then there are 2 aspects of functional studies of the brain. First, there is the matter of localization of various brain functions. This has been made possible by the introduction of additional technologies (e. g. PET, fMRI), which measure or display certain processes (levels of sugar or oxygenation) with a relatively high spatial resolution, which are believed (the actual relations between those 2 phenomena are still a matter of debate) to indicate activity in the brain region where they occur, allowing indirect induction about the localization of brain functions based on the correlation of specific tasks (demanding specific brain functions) and the assumed activity of the brain.

The second approach to functional studies of the brain are through neuromodulatory interventional effects at various levels (physical, chemical). By administering a substance (e. g. neurotransmitters) or affecting the function of certain elements of the brain in a non-invasive manner we can observe the effects or consequences these interventions have on mechanisms of the brain in the given brain regions. One of these non-invasive neuromodulatory methods is transcranial magnetic stimulation (TMS), which uses the physical properties of magnetism and electricity to induce changes in the cortical areas of the brain.

As with all emerging technologies, TMS is also going through its own developmental cycle regarding its use in research, from the euphoric and mostly ad hoc applications since its introduction in the 2nd half of the 1980s to more systematic, incremental and goal-oriented studies of today. The gradual and cumulative nature of various implications of such studies gives rise to a growing knowledge base of mechanisms and suitability of applications of TMS.

The general deficiency of new and efficient treatment methods in psychiatry also recently enabled TMS to enter the clinical/therapeutic field. The novelty of TMS in this department limits its efficiency at least temporarily, with prospects of much room for optimization and improvement in both the methodological and procedural aspects of its use as well as the very basics of the physical features and properties of TMS itself.

1.2 Contextual framework of the project

The aim of this master thesis has been composed and conceptualized as a part of an ongoing project concerned with transcranial magnetic stimulation (TMS) as an alternative or adjunctive treatment for depression ("Influence of different transcranial magnetic stimulation protocols on biomarkers and symptoms of depression") at the Neurological clinic, which is an institution within the University Clinical Center (Univerzitetni Klinični Center) in Ljubljana, Slovenia. As such, this overlaying project is focused on the use of the available technology of TMS in the treatment of depression while monitoring the outcome of the treatment in terms of biomarkers of depression (blood levels of brain-derived neurotrophic factor (BDNF)), the clinical picture consisting of various depression

symptoms (HAM-D) and quality of life scales (SF-36) and functional brain activity (fMRI).

Our research, within the scope of this master thesis, was conducted on the premises of the Neurological clinic, namely in the TMS laboratory that holds all the relevant and critical equipment – TMS equipment suitable for both single, paired-pulse as well as repetitive TMS (rTMS) and electroencephalographic (EEG) and electromyographic (EMG) recording devices. The study has been approved by the national committee for medical ethics in Slovenia and thus was ethically sound.

The overlaying project (TMS and depression) acknowledges the severity, importance and problematic aspects of major depressive disorders (MDDs). MDDs and more profoundly, their more persistent subset of treatment-resistant depression (TRD) are a growing concern worldwide in terms of direct and indirect healthcare costs. TRD has no universal consensual definition and depending on the concrete definition accounts for between 20-30% and 60% (if defined as recurring MDD) of overall MDDs (Fekadu et al., 2009). TRD, which is a highly relapsing (higher readmission rate than general MDDs) and potentially chronic condition that is associated with increased mortality, disability and comorbidity, in turn generating high healthcare and other indirect costs, has been focused on only recently with the intent of searching for alternative non-medicational treatments (Fekadu et al., 2009).

This master thesis has been drafted as the first phase of the aforementioned TMS-depression project and was supposed to explore and test the properties of TMS protocols that would be chosen as the most promising alternatives to be later applied as treatment of depression in the following phases of the project.

1.3 Conceptual introduction

1.3.1 Transcranial magnetic stimulation

Transcranial magnetic stimulation (TMS) is a non-invasive neuromodulation technique that has been developed in 1985, albeit it uses the concepts of electric energy and magnetism, which were well-known and understood long before that. The reasons for the relatively late development of the TMS technology mostly

relate to the lagging implementational aspect of the hardware of TMS. Although the design of modern contemporary TMS equipment is rather complicated and complex and a matter of secret know-how of the companies producing these machines, its crude simplification for illustrative purposes will suffice: the basic layout includes a capacitor that is charged up inside the stimulator machine and upon activation of a switch, the accumulated electrical charge/current flows through a high-voltage cable to an auxiliary coil with windings inside it. The very simplified principal mechanism of action of TMS is the flow of a high electric current that induces a changing magnetic field inside the coil, which in turn induces a flow of electric current in the brain tissue near the surface of the scalp where the coil is placed.

Without getting into the subtle technicalities of the TMS technology and its physical mechanisms due to the limited extent of this text, depending on the exact flow of the electric current various types of pulses waveforms can be produced – in case the electric current flows only once from the capacitor to the coil and slowly dissipates there, the resulting TMS pulse is monophasic, while if the electric current flows from the capacitor to the coil, then again to the capacitor (gaining an opposite polarity) and back to the coil (in the opposite direction) in one cycle, the resulting waveform is biphasic (Wassermann et al., 2008). Theoretically, depending on the exact configuration and design of the TMS device, the production of more complex pulse waveforms are possible. However, in current practice, only monophasic and biphasic waveforms are used, each having its own specific and inherent merits and properties, which in turn manifest in practical differences (e. g. monophasic pulses generally yield a higher threshold than biphasic pulses).

Another hardware-related issue is the form of the TMS coil that is being placed on the scalp of participants. Initially, circular TMS coils have been used for stimulation, providing a good coverage of the stimulation area of the brain, but with the disadvantage of their lack of focality, which not only makes it crude in terms of localization and confinement of the stimulation to a smaller area of the brain, but also the precise location of the most intense stimulation spot on the coil is seldom exactly known. To allow for more precise stimulation of the cortical areas of the brain, the figure-8 coil form has been invented, with 2 windings inside its casing and with the most intense stimulation at a relatively small point where the 2 windings are closest (Walsh, Pascual-Leone, 2003). There are also other coil

forms we will not disclose concretely, because they are outside of our scope of application. As for the depth of the TMS, it is generally usable only for the cortical areas of the brain that are adjacent to the scalp.

The electric current induced in the brain practically causes hyperpolarization and depolarization of the neurons. The effects of the stimulation vary depending on the functionality of the area that is being stimulated. For instance, TMS can be used to generate “phosphenes” (flashes of light subjectively reported by participants) when applied over the visual cortex. When applied over the motor cortex of the brain (M1), short muscle twitches of muscles corresponding to their representation on the motor cortex of the brain follow, which can be recorded electrophysiologically as “motor evoked potentials” (MEPs (the value of a peak of electric potential of muscle activation measured with electrodes related to the stimulation by TMS) with electrodes placed on the muscles that are associated with the respective area in the brain that receives the TMS.

Without elaborating more on the physiological mechanisms of excitatory/facilitatory and inhibitory effects of TMS, we to have formulate their practical definitions. Both of these effects are related to a baseline state of excitability of the M1. Excitation/facilitation is an increased response of the M1 to the same stimulation intensity that has been used to test the baseline excitability. Practically, this means lower motor thresholds (a lower intensity is needed to evoke a measurable response) and increased MEPs (higher values of muscle activation at the same intensity compared to a previous state). Conversely, inhibition is a decreased response of the M1 to the same stimulation intensity that has been applied to the baseline. In practice, among the consequences are higher motor thresholds (a higher intensity is needed to evoke a measurable response) and decreased MEPs (lower values of muscle activation at a constant intensity).

Several TMS protocols have been investigated over the last three decades. A rough classification is to divide the protocols by the number of pulses delivered per instance over a short period of time. First, there are single pulse techniques which consist of only one pulse at a time. This technique is largely used to test for the general excitability of the M1 area. Single pulses can be also used to measure the cortical silent period (CSP), which is the short period of time of no muscle activity after a steadily contracted muscle has been stimulated with TMS on its

corresponding M1 area occurring due to the mechanism of neurons.

The second group covers paired-pulse techniques which use a sub-threshold conditioning stimulus (CS) and a supra-threshold test stimulus (TS), if not stated otherwise. These are being computed based on the previously assessed value of the resting motor threshold (RMT (which is the lowest stimulation intensity to evoke a measurable response of the resting corresponding muscle)). The active motor threshold (AMT) is the lowest stimulation intensity to evoke a measurable response when the muscle is being steadily contracted. AMT is lower than RMT, while there is generally a large interindividual, but low intraindividual and interhemispheric variability in both those measures (Hallet, Chokroverty, 2005). Different interstimulus intervals (ISIs) can test for various properties of the M1. Some of the most common measures tested with paired-pulse techniques, based on the range of the ISI, are: Short-interval Intracortical Inhibition (SICI): 2 – 10 ms; Long-interval Intracortical Inhibition (LICI): 50 – 200 ms, but with both stimuli being supra-threshold; and Intracortical Facilitation (ICF): 10 – 20 ms.

The third type of protocols (repetitive TMS) uses a number of pulses in a certain frequency or combination of frequencies (between <1 Hz and 50 Hz). Depending on the frequencies, one can distinguish between two sorts of repetitive TMS (rTMS): single pulses delivered regularly in a low or high frequency sequence (standard or conventional rTMS) or burst protocols where stimulation is delivered in bursts of a few pulses, while those bursts are delivered at a different (lower) frequency. Repetitive protocols are, in contrast to single- or paired-pulse protocols, not only used for diagnostic and research purposes but also have clinical and therapeutic applications.

1.3.2 Depression and TMS

Relatively recently, rTMS has been approved as a therapeutic tool and treatment method in cases of MDD (especially TRD). Since ECT, highly effective (50-60% of TRD, 80% of MDD (Allan, Ebmeier, 2011)), but associated with various adverse effects (cognitive impairment (e. g. retrograde amnesia)), is considered as a last-resort treatment, emphasis has been put on exploring TMS as a safer, less invasive, more comfortable, but on the other hand also (as of now) less effective treatment. One mechanism of action of TMS in MDD is the application of various (either high- or low-frequency) rTMS protocols on the left and/or right

dorsolateral prefrontal cortex (DLPFC), depending on the properties of the given rTMS protocol (Blumberger et al., 2013).

1.3.3 TMS and the Primary Motor Cortex

Since the response of the DLPFC to inhibitory TMS protocols cannot be assessed directly and can be measured only indirectly by monitoring the symptomatic aspect of the MDD, we decided to measure the effects of those protocols on the M1, which would indicate a similar response also of different brain structures. As of now, studies on the effects of TMS protocols on M1 are fairly common.

1.3.4 Theta burst stimulation (TBS)

Theta burst stimulation has been introduced as a more promising, effective and comfortable alternative to the conventional rTMS protocols and is considered to be generally of lower stimulation intensity, shorter application time per session and with longer-lasting after-effects of the stimulation compared to the latter alternative (Cárdenas-Morales et al., 2010). All standard TBS protocols consist of burst of 3 pulses delivered at 50 Hz frequency (interval between individual pulses of 20 ms) repeated in a theta rhythm interval range (4-7 Hz), usually at 5 Hz (every 200 ms). There are 3 main sub-categories: intermittent TBS (iTBS) which uses the aforementioned frequencies during 2 s long trains of pulses every 10 s (leaving an 8 s long pause in between); intermediate TBS (imTBS) with 5 s long trains repeated every 15 s (10 s pause); and continuous TBS (cTBS) with uninterrupted progression at the frequencies specified above. All three variants sum up to the total of 600 pulses delivered, each lasting for a time calculated by the stated parameters (iTBS: 190 s; imTBS: 110 s; cTBS: 40 s) (Huang et al., 2005). Sometimes, different versions of these protocols are applied with varying numbers of pulses totally delivered, which is usually stated explicitly (e. g. cTBS600 (the standard version) or cTBS (a short version with only 300 pulses in total)). Each of these versions have its practical merits: iTBS is considered as facilitatory (increasing MEP size), cTBS is inhibitory (Wu et al., 2012) and imTBS has no significant effect on MEPs. The general layout of these protocols is displayed below in Figure 1.

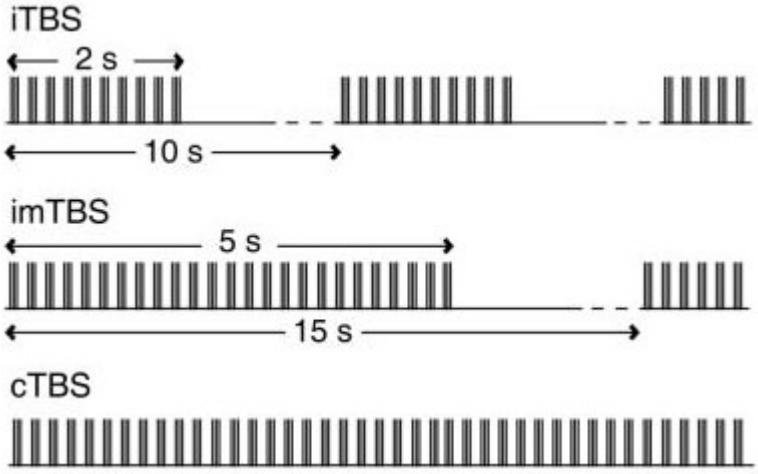


Figure 1. Illustration of various TBS protocols (Huang et al., 2005)

The 50 Hz cTBS inhibitory protocol, applied by Huang et al. (2005) on the human primary motor cortex, showed from the beginning profound effects on MEPs, decreasing them up to 50% of the baseline level up to 25 minutes after stimulation; and SICI, increasing it by 1/3 between 5-10 minutes after application and later returning to the pre-cTBS state at 20-25 minutes after the application of cTBS. Further experimentation with TBS in general (both cTBS and iTBS) led to the findings that their effect does not depend on the current direction or pulse configuration (Zafar et al., 2008). A comparison of various target structures stimulated by cTBS (cTBS-300 and cTBS-600) resulted in data suggesting that regardless of the lateralization of stimulation over M1, the contralateral muscle represented in the given M1 has its MEPs decreased, while the ipsilateral muscle's MEPs are facilitated in the relatively same extent and stimulation over the right dorsal premotor cortex and mid-occipital region did not modify MEP size (Stefan et al., 2008). A complementary study by Ortu et al. (2009) involving the left dorsal premotor cortex found that its stimulation by cTBS decreases MEP size and a pilot study preliminarily suggests that left SMA stimulation does not affect the contralateral MEP size.

Stagg et al. (2009) used magnetic resonance spectroscopy to measure the levels of GABA and G1x (glutamate/glutamine) in the cTBS-stimulated areas. The post-cTBS increase (relative to NAA) of GABA, but not Glx, supports the hypothesis that the primary mechanism of action of cTBS is realized through increased GABAergic activity.

Cárdenas-Morales et al. (2010) provide a good review of TBS research, with a focus on long-term potentiation (LTP) and long-term depression (LTD) as well as

GABA mechanisms of TBS and a comparison between conventional rTMS and TBS – effects of conventional rTMS protocols on SICI considerably vary between studies, but changes induced by TBS protocols appear to be more consistent. This fact may reflect the presence of GABAergic activity at the intracortical level when using TBS and could indicate at least a partial difference from conventional rTMS protocols. The authors conclude in an overview that TBS is advantageous over standard rTMS protocols because of a relatively lower stimulation intensity as well as stimulation time, but the mechanism of action of TBS is currently not understood (various theories suggest the involvement of NMDA (Huang et al., 2007), GABA receptors (Stagg et al., 2009) and even levels of BDNF).

Practically, the effect of cTBS is dependent not only on the total number of pulses (300 vs. 600), but also on the exact site of stimulation. Comparing the effect of the 30 Hz cTBS protocol on the primary somatosensory cortex (SI) and the M1, a study (Jacobs et al., 2014) showed increased MEPs over the SI (facilitatory effect) and decreased MEPs (inhibitory effect) as compared to both baselines, while there was no significant change in both conditions in SICI and only a significant decrease in ICF over the M1 (no change of ICF after the cTBS over the SI). These findings and their implications also contribute to the debate about what exact measure is the most representative for plasticity changes in the M1.

Studies of cTBS effects on depression are relatively rare at present. A case series with 7 TRD patients who received 2×600 pulses of iTBS at 80% RMT for 3 weeks to the left DLPFC achieved remission rates of 43% according to the HDRS and 49% to the BDI depression rating scales (Holzer et al., 2010). The methodologically problematic aspects of this study include the facts that not all of the patients were off medication (2 unmedicated, 5 medicated) and that the DLPFC was defined by the 5 cm anterior to the scalp position for optimum stimulation of the right abductor pollicis brevis muscle, a technique that has been criticized in other publications. Plewnia et al. (2014) conducted an experiment with 2 groups (experimental – iTBS over L-DLPFC + cTBS over R-DLPFC; control – bilateral sham stimulation (as an adjunctive treatment besides medication and psychotherapy)) of MDD patients carried out for the course of 6 weeks (30 sessions). There are measurable differences between both groups in terms of response and remission criteria-based counts according to various depression

assessments (MADRS, HAMD, BDI) and their mean values, albeit not statistically significant. However, the authors argue that given the pilot character of the study, the results have to be viewed upon as merely preliminary.

1.3.5 Preconditioning prior to the application of inhibitory rTMS

Findings of studies of cTBS stimulation effects have generally shown that the polarity of its effect cannot be considered universal and there are notable inter-individual differences (some subjects' M1 being inhibited while others' facilitated after the application of the standard 50 Hz cTBS protocol (Goldsworthy et al., 2012)). To eliminate this incoherence by trying to unify the polarity of the cTBS effect, we searched for a possibility of preconditioning the M1 so that it then would react to an inhibitory cTBS stimulation exclusively with inhibition.

Transcranial direct current stimulation (tDCS) may be used to influence or direct the polarization of the effect of TMS. Depending on preconditioning 1 Hz rTMS consisting of 900 pulses with either (facilitatory) anodal or (inhibitory) cathodal tDCS, the polarity of the MEP response (assessed by means of single-pulse and paired-pulse techniques) after the rTMS stimulation was reversed in comparison with the polarity of the MEPs directly after tDCS, while the overall level of MEPs remained unchanged in the sham-controlled condition (Siebner et al., 2004). The same principal fashion of results was acquired in a similar experiment with different parameters (5 Hz rTMS, 100 pulses, MEPs assessed by single pulses) by Lang et al. (2004).

Huang et al. (2007) focused mainly on the influence of memantine (acting on NMDA receptors). Memantine is known to block training-induced M1 plasticity. The study incorporated only 6 subjects and used iTBS600 (which is considered to be excitatory) and cTBS300 (an inhibitory protocol). During 4 sessions, combinations of those protocols and memantine/placebo have been applied to the participants, with measurements including RMT, AMT and MEPs. The results showed facilitated MEPs in the iTBS + placebo condition and suppressed MEPs in the cTBS + placebo condition, while after the application of both protocols together with memantine, the MEPs remained unaltered, which indicates that memantine is responsible for the suppression of after-effects in MEPs and this finding provides evidence that the after-effects produced by iTBS and cTBS are NMDA-dependent and hence are likely to involve plasticity-like changes at

synaptic connections in the M1. Memantine thus does not seem to be a suitable drug for the purpose of the support of cTBS effects, since we are interested in the opposite (reinforcing, not countering) modulatory effect of cTBS stimulation. Another proposition of a factor in the direction of TMS-induced plasticity (facilitatory vs. inhibitory effects) is the intracellular calcium concentration, which should be possible to influence with certain pattern of TMS (pulse intensity, repetitions, frequency) (Fung & Robinson, 2014).

A comprehensive review of drugs affecting various TMS measures shows that one of the most relevant drugs for the purpose of inhibitory modulation of TMS is Lorazepam (Ziemann, 2004). Lorazepam is a GABA-A agonist (a substance that binds to GABA-A receptors and activates them) which significantly reduces MEP size and increases SICI response, while it slightly increases the CSP, indicating that it indeed fosters an inhibitory effect. Lorazepam is a drug used for the treatment of anxiety disorders and since its adverse effects include depression or its intensification, it might not be a suitable drug for patients suffering from depression. A more recent review of pharmacologic effects on cortical excitability measures suggests that Lamotrigine (acting by blocking voltage-gated Na^+ channels) increases the motor threshold, but does not affects other TMS measures, Lorazepam reduces MEPs and increases SICI and Reboxetine (a norepinephrine agonist) increases MEPs and ICF (Paulus et al., 2008). However, the problem is that these proposed substances do not consistently alter all TMS measures in the inhibitory direction.

From these findings, we concluded that it was not feasible to precondition the M1 before TMS to strengthen and unify its effects, especially considering the possibility that adding various elements with potential side-effects to a study that intended to recruit healthy participants as subjects might have not been considered ethical and would have been more likely subject to disapproval on the part of the national committee for medical ethics.

1.4 Conceptual design of the experiment

Based on the extensive study of literature on previous research carried out on TMS, depression and M1, we decided to investigate the empirical differences in efficiency of 2 promising cTBS protocols in inhibiting the M1, which would

indicate their efficiency in inhibiting the DLPFC as well. We chose the standard cTBS protocol (50 Hz cTBS) and the promising modified 30 Hz cTBS (see figure 2 for parameters) (Goldsworthy et al., 2012).

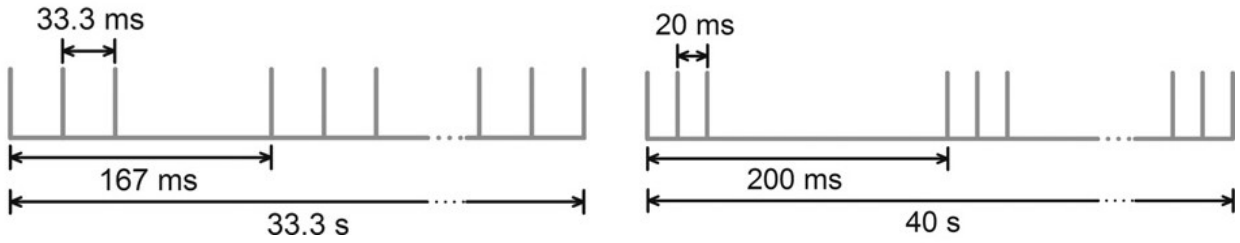


Figure 2. The parameters of 30 Hz (left) and 50 Hz (right) cTBS (Goldsworthy et al., 2012)

One aspect we learned that has not been explicitly studied yet in previous experiments involved the cumulative effects of a repeated application of inhibitory TMS protocols over an extended period of time (consecutive daily stimulation), which is one of two innovative additions we intended to incorporate into our study, because this knowledge is crucial for the efficiency of protocols as potential treatment of depression (the TMS treatment of depression is a long-term process). To measure the effects of these protocols on the M1 and their differences, we employed a within-subject experimental design to test our hypothesis in a serial manner (each participant underwent both experimental conditions and also served as the control condition by means of measures taken before the modulatory stimulation). This design allowed us to avoid the usually very variable interindividual differences in the relevant measures of TMS by focusing only on differences of each participant over time after being subjected to all experimental conditions. In each healthy subject we conducted cTBS sessions with one of the two protocols (randomly selected 30 Hz or 50 Hz) either one per protocol or the extended version with daily stimulation for a period of 5 days per protocol, repeated after at least 5 days of rest (to ensure that the 2 conditions/protocols would not interfere) with the same time course of the other protocol.

After each session, measures of M1 activation of a certain muscle by means of electromyographic (EMG) electrodes placed accordingly, have been elicited through the application of single or paired TMS pulses. The other significant innovative aspect of our study was the continuous recording of EEG data (TMS-EEG technique (with the exception of the duration of the cTBS protocols)), which on one hand served the purpose of observing potential changes in EEG oscillatory activity related to inhibitory cTBS changes (Barr et al., 2009), while also acting as a safety enhancement to monitor for any potential epileptogenic activity during the

stimulation (rare epileptic seizures are the only known serious side effect of cTBS, related mainly to protocols with high stimulation intensities, pulse frequencies and durations (Rossi et al., 2009)). The EMG data have been then analyzed to examine the differences between the experimental conditions, while the EEG data remain to be analyzed at the Neurological clinic and are not a part of this master thesis. We invited healthy subjects for the study and asked them to participate either only in the first session for both protocols (1+1 day for each subject) or to participate in the whole duration of the study (5+5 days for each subject). They did not receive any payment or benefits for participating and were of course able to withdraw from the study without any consequences anytime.

1.5 Formulation of the conceptual hypothesis

Based on the studied literature, we formulated and explicated our expectations of future findings of our study. Growing evidence suggested that the finally chosen different experimental conditions (in the form of the 30Hz cTBS and the 50 Hz cTBS) would differ in their effects on the M1 (Goldsworthy et al., 2012). We therefore postulated our conceptual hypothesis as follows:

H: Differences exist in the effects of the 30Hz cTBS and the 50Hz cTBS experimental condition on the neuroplasticity of the M1.

As a supplement, we also formulated a research question (aimed at exploration rather than verification as in case of the hypothesis) concerning the cumulative effects of cTBS stimulation (since there is insufficient literature and knowledge base on this topic):

What (if any) will be the differences in various consecutive daily stimulation sessions regarding the effects of the 30Hz cTBS and the 50Hz cTBS experimental condition on the neuroplasticity of the M1?

2. Methods

Generally, procedures in TMS-related research are a matter of know-how and expertise of the given TMS laboratory, because available publications seldom describe the exact procedural instructions. A recently founded TMS lab (like the one we were a part of at the Neurological clinic in Ljubljana) has to establish its own experimental designs and procedures based mostly on the study of literature, consultations with experts (inside and outside of the institution) and last but not least, by trial end error. This is a costly and long-term process that deals with very subtle details and postpones the realization of the intended studies themselves. During the pre-pilot (various testing of TMS and EEG/EMG recording equipment) and pilot phases of this study (and a preceding one concerned with intentions and their effects on TMS-induced muscle activation) we were forced to solve a variety of issues and problems hampering our attempts of recording data reliably and validly. In this section, we will describe some of those problems and specify our solutions so that it is explicit and understandable how we worked on the implementation of our experiment.

2.1 TMS equipment

For the purposes of our research, we used a Magstim BiStim^2 TMS device for delivering single pulses of TMS, Magstim BiStim^2 in dual mode for paired pulses both with a 70 mm figure-8 shaped Alpha coil and Magstim Rapid^2 with a 70 mm figure-8 air-cooled coil (with the vacuum cooling turned off) for delivering the cTBS. Our recording equipment comprised of Brain Products BrainCap MR 64-channel EEG cap with only half of the active electrodes (32 channels) plugged into the 32-channel BrainAmp MR+ amplifier, connected to a PC. For the EMG measurements, disposable electrodes of bely-tendon montage were used, plugged into a Digitimer D360 8-channel amplifier that was connected to a CED (Cambridge Electronics Design Limited) Micro3 1401 analog-to-digital converter and then to a PC through a USB port. The EEG recording software was the BrainVision recorder with a setting of the sampling rate at 5000 Hz. The EMG signal was recorded by the CED Signal 5.11 software, which controlled also the stimulation timing and parameters and filtered the EMG signal with a 20 Hz low-cut and a 2000 Hz high-cut filter at a sampling rate of 5000 Hz. The paradigm that displayed hints for experimenters on respective blocks of measurements and a

fixation cross for the participants, was run in Psychtoolbox-3 (Matlab 2013a).

2.2 Site of stimulation

Similar to other studies, we assessed the M1 response by recording the EMG signal of the first dorsal interosseous muscle (FDI) on the right hand (R-FDI) (Wu et al., 2012). There are at least three good reasons for using this particular muscle: first, choosing an identical site to other studies gave us the opportunity to compare the results later against those of these other studies; the FDI is relatively well-represented on the M1, which simplifies the procedure of searching for the hotspot; and finally, the FDI is a relatively large muscle on the hand, which makes the electrodes easier to place correctly. By determining the hotspot corresponding to the R-FDI in the M1 (which means stimulating the M1 over the left hemisphere) and the resting motor threshold (RMT), we acquired baseline stimulation intensities which were used later to calculate the stimulation intensities for both the single/paired pulse stimulation needed for the various TMS measures (MEP, RC, SICI, etc.), as well as for the intensity setting for the inhibitory cTBS stimulation itself – 80% of RMT in 50 Hz cTBS (Huang et al., 2005) and 80% of RMT in 30 Hz cTBS (Goldsworthy et al., 2012).

2.3 Selection of dependent variables (TMS measures)

There is no consensus among studies of TMS effects on the measure that would best represent the excitatory/facilitatory or inhibitory properties/characteristics of those effects. The simplest measure of general activation of the M1 are MEPs. They are also convenient in terms of requirements towards the TMS equipment (single-pulse TMS devices are the cheapest and least complicated kind), total energy and number of pulses delivered to the M1 and their values are robust in the statistical sense, because they are being used in relatively long sequences (blocks) that provide enough trials to present a sufficient sample per subject. The problem with MEPs is that they are intrapersonally variable over extended periods (days).

However, other measures should not be ignored even though they are employed less frequently in research. We incorporated the SICI, ICF and CSP into

the 1st and last sessions of the full-scale 5 consecutive daily sessions version of the experiment, because they exhibit various properties of the M1. We excluded other very rarely used measures such as LICI, mostly because of their lacking database we could later compare it to.

2.4 Hotspot search

The so-called hotspot is the exact localization on the M1 where the response of the corresponding muscle to a TMS pulse over the M1 is the most profound and highest in terms of muscle activation measured by EMG electrodes mounted on the target muscle. The most important premise in facilitating a simplified hotspot search is choosing a muscle that is universally very useful and exercised and well-represented on the M1. There are cases when certain minor muscles are so weakly represented on the M1 that even the correctly chosen hotspot for that muscle causes activation rather in adjacent muscles than in the target muscle and in some instances, the activation of a certain muscle might be impossible to differentiate from the activation of another muscle (Criswell, 2011). What needs to be taken into account are also inter-individual differences of the participants' brains that manifest in different structural forms of the M1 and in some special cases (e. g. hotspot located in a sulcus, which increases the distance between the TMS coil and the target tissue of the M1) it may even be practically inaccessible, deeming that particular participant useless for the purposes of the study.

The practical procedure (or its exact step-by-step implementation) of determining the hotspot is also a matter of debate rather than consensus among TMS researchers (Awiszus, in press). Studies carried out with TMS do not generally explicitly disclose the exact process of the hotspot search other than just vaguely reporting that it has been accomplished (creating incompatibility issues between those studies with respect to the comparability of their results based on different procedures). To make our hotspot search as precise and transparent (multiple researchers took turns in handling and placement of the TMS coil, so the procedure had to be explicitly formulated) as possible, we employed a fabric grid with dimensions of 5×5 cm with squares measuring 1×1 cm mounted and fixated on the EEG cap so that it safely covered the area where the hotspot could be located. Single TMS pulses at an initial intensity of 60% (after a gradual increase to this level for the participant to adapt himself) of the maximum stimulating intensity

output of the Magstim BiStim^2 TMS device were then delivered to different, relatively distant (5 cm) ad hoc starting points on the M1 (at least 2-3 on each spot, because the first pulse has to be discarded/ignored given the fact that the first pulse after relocation usually induces a very high amplitude in the EMG signal) to determine the approximate location of the hotspot and to place the grid there. The most promising point was then chosen and tested with the coil relocated to its neighboring points, typically with 5 pulses per point. Fine-tuning was possible in the quadrant between the best candidate points and finally one definitive point was chosen (in case of doubts, the experimenter might have extended the number of pulses and if 2 or more equally suitable points were found, the one with the most consistent muscle activation was preferred) with a recommended limit of 50 pulses in total.

The hotspot search procedure should be generally as fast as possible, not primarily due to timing constraints, but to limit the total number of delivered pulses (see also section 2.6), which could on their own (without further modulatory stimulation) cause undesirable changes in the neuroplasticity of the M1 and could interact with the modulatory stimulation and distort the overall effect (by adding another independent variable to the experimental one).

2.5 Coil holding mechanisms/methods

In the very beginning of the pre-pilot stage of the experiment we were aware of the importance of maintaining a constant position towards the participant's head after the hotspot has been established. During a previous study, we were forced to use a mechanical holding arm that was mounted on the chair the participants were seated in to hold the TMS coil, mainly due to relatively long blocks of stimuli (8 minutes) which eliminated the possibility of holding the heavy coil manually without exhaustion and without unintentionally changing the coil's position on the participant's scalp, which is undesirable because of the increased variability and decreased reliability and validity of the results it produces. The position of the coil has to be kept constant on all 3 axes (dimensions), which not only means maintaining the correct hotspot location on the grid (the point is graphically labeled by then), but also at a 45° angle of the cable of the coil in the lateral-posterior direction and the inclination of the vertical axis of the coil itself towards the scalp. The 45° angle can be maintained by aligning the vertical axis of the coil

(parallel to the cable that is mounted to the coil) to a supportive line painted on the grid beginning in the hotspot. The last dimension (the inclination of the vertical axis of the coil) is the most difficult to maintain manually considering the obstructed view (especially if the experimenter is rather short) and tiring of the muscles of the experimenter's hand that naturally causes the coil holder to lower the far end of the coil where the handle of the coil is located.

The aforementioned issues can be eliminated by using a mechanical device to fixate the coil in the desired position. The concrete implementation we applied during a previous experiment as was stated above increased the stability of the coil's position, but was not sensitive to other practical placement problems. The first problem was the initial fixation of the arm that had to be done by tightening a screw of the arm's joints by hand, displacing the coil in that particular moment. The stabilized arm also excluded the possibility of adjusting the coil's position in real time for the duration of the block. Participants tended to lower their heads position by sinking/slipping into the chair with elapsing time during the blocks of stimuli, thus avoiding contact with the coil and increasing the distance between the coil and the scalp, which in turn diminished the overall stimulation intensity, again distorting the results by compromising the hotspot.

Based on these experiences, we decided to combine the 2 methods to benefit from the advantages of both of them. For the blocks of stimuli for the MEPs, RC, SICI, ICF and CSF, we held the coil by hand, which is considered a standard in TMS research and was practically feasible given the relatively short duration of those blocks (the duration of the MEP block slightly exceeded 2 minutes (20 pulses at an average interval of 7 seconds, while the other measurements used a 5 s interval, which decreased the durations of those blocks even further). For the duration of the cTBS protocol, mostly due to the heaviness of the air-cooled TMS coil, we used the same mechanical holding arm as described above, but we did not fully tighten the fixation screw so that it was still possible to compensate for the movement of the participant, but the coil was relatively steady, eliminating the strain on the experimenter's hand. Following the complaints of subjects during the pre-pilot phase claiming it was too tiring to intentionally maintain the position of their heads, we chose to hold their heads manually by pressing them very slightly against the fixated coil, which proved to be acceptable for both parties and essentially solved most of the problems related to coil positioning.

2.6 Total number of pulses delivered (and its effects)

The analysis of data from the pre-pilot phase gave rise to the issue of relevance of the total amount of pulses delivered to a participant during one session and its own effect on top of the studied potential effects of the cTBS protocols. It seemed that a too precise and thorough hotspot search (with too many pulses used to find the hotspot) combined with too long blocks of MEPs (including too many pulses again) elicited some meta-plasticity effect that would inhibit the M1 response regardless of the cTBS on its own. To eliminate this potential source of undesirable bias, we reduced the number of pulses per MEP block and formulated a quicker, but still precise hotspot search method. Furthermore, from the practical aspect, reducing the overall duration of the experimental session would have the benefit of being more attractive to potential participants, supporting the recruitment process.

2.7 Pulse timing variation (jitter)

Since the brain is very flexible in adapting itself to various frequently repeating patterns of stimuli from the environment, we were aware of the possibility that the M1 could be modulated just by the frequent repetition of pulses at constant intensities (which is what MEP blocks essentially are). After consultations with TMS experts at the Neurological clinic and also one of the authors of a study we used as a starting point (Goldsworthy et al., 2012), we introduced a slight variation (jitter) of the interval between the pulses of constant intensities in the MEP blocks, namely 7 s ± 10%. For the other measures (RC, SICI, ICF, CSP) we left a constant interval of 5 s, because there are fewer repetitions of pulses at one frequency which would not leave enough time and iterations for the M1 to adjust to this pattern (e. g. in the RC, intensities of 90, 100, 140 and 150% of the RMT are used with 5 pulses each).

2.8 Inherent variability of MEPs

During our pre-pilot testing, we found out that intra- as well as inter-block

(among baseline blocks) variability of MEPs are consistently considerably higher (intra-block variability reaching up to a maximum SD of 40% of MEP average of the respective block; inter-block variability reaching up to a max. 25% difference of the MEP average of all baseline blocks from the MEP average of an individual block) than in other studies that are being published (cf. Goldsworthy et al., 2012).

We first speculated the coil instability to be a reason for this discrepancy. Nonetheless, after extensive revision of the coil-holding method and the particular attention we paid to this crucial aspect of maintaining constant conditions within the experiment, we came to the conclusion that with realistic expectations no significant further improvement in this regard is possible. Moreover, the commonly employed method of holding of the TMS coil is by hand, which is fairly limited by the physiological parameters of the experimenters and which applies universally to all studies and consequently to their results.

Our concerns that variability is, partially due to the applied coil-holding method and perhaps also due to the nature of the TMS mechanism of action itself, inherent and inevitable in all TMS research, have been confirmed after a consultation with a TMS expert. Further inquiries (namely contacting the authors of one of the study presenting low variability (Goldsworthy et al., 2012)) revealed that variability is indeed a general issue that is being suppressed by pre-processing and filtering of the raw data before conducting the definitive data analysis (excluding outlier MEPs, choosing only an approximate 1mV intensity, etc.).

2.9 Cumulative effects extent/number of sessions/days

Cumulative effects of cTBS stimulation were one of the 2 major innovative aspects this study was focused on. Since the beginning of the conceptualization of the study when this aspect has been established we were aware of the fact that the practical realization of such an ambitious experimental design would be extremely fragile in terms of voluntary participation of subjects. To boost the viability of the study design, we ended up creating 2 potential scenarios of possible participation. The minimal variant would only make use of one participant for each condition once (2 sessions in total), allowing a direct comparison of the effects of the 2 different cTBS protocols, but omitting the cumulative effects. The full-scale variant would require participation for 7 consecutive daily session for each of the

2 protocols for the exploration of cumulative effects of cTBS. During the pilot testing stage, we decided to decrease the number of sessions on consecutive days per condition from the originally considered 7 sessions to 5, with the intention of making the experiment less time-consuming for the experimenters as well as potential participants. Each potential participant was approached with the offer of either full-scale participation or the option of participating at least in the minimal version of the experiment. The lack of means of motivation of the potential subjects (the guidelines at the Neurological clinic and the Faculty of medicine prohibited the award of credits for the completion of our experiment) other than their own interest in the topic and field of study made the acquisition of participants a complicated long-term process.

2.10 cTBS effect on EEG vs. non-EEG cap

Another issue we encountered once we decided to include the EEG component into our study was the practical incompatibility and interference of older EEG and TMS equipment (lately, the manufacturers of EEG equipment introduced special TMS-compatible EEG caps and amplifiers to the market, which we did not have at our disposal). This interference occurs possibly in both directions (EEG equipment affecting the TMS equipment and vice versa).

During a previous experiment when we used the Digitimer D360 amplifier (which we now used only for the recording of the EMG signal) with 4 passive EEG electrodes (2 channels) placed on the scalp near the stimulation site, the TMS pulse induced a very high spike in the EEG signal saturating the amplifier for a few seconds and rendering the recorded values of the signal invalid, especially in the time-frame (tens and hundreds of ms after the pulse) that is most relevant for the EEG results (incorporating event-related components like N200, P300, etc.). To circumvent and effectively eliminate this problem, we employed a “clamp” triggered by the PC where the experimental paradigm was run which paused the recording of the EEG signal by the amplifier for a brief period of time around the delivered TMS pulse (a few ms before the pulse to a few tens of ms after the pulse) so that the amplifier would not have picked up the TMS induced spike in the EEG signal and would have remained unaffected by it during the rest of the time when it was recording. This way we would have lost the EEG data during the brief period of the activated clamp, but preserved the rest of the recording including the

aforementioned important and relevant event-related components. In practice however, the clamping device proved to be unreliable and its functionality could not have been clearly verified.

During the preparatory stage of this study, we gained access to a qualitatively improved version of EEG recording equipment – the Brain Products BrainCap MR 64-channel cap with active EEG electrodes and a 32-channel BrainAmp MR+ amplifier that was declared to be suitable for TMS applications. This principally eliminated the problem of potential saturation of the amplifier due to TMS-induced power spikes/surges in the electrodes, but practically we experienced unexplained long and cumulative drifts of the EEG signal amplitudes resembling slow gradual saturation of the amplifier, which necessitated real-time observation of the EEG signal during the recording sessions and occasionally resetting the amplifier when the signal went off-scale on too many electrodes (the reset function adjusted the signal values to zero and the signal became (at least temporarily) recordable again).

The other type of influence (the influence of the EEG equipment on the TMS equipment) was a subject of more intensive investigation. In the previous study we used only 4 electrodes (2 channels) of EEG, mainly because the overall thickness (height) of the EEG cap with the passive electrodes and the rings they are mounted into evenly distributed on the surface of the cap (that means also above the stimulation site) would have created a huge distance (cca. 1 cm) between the participant's scalp and the TMS coil, creating an inevitable obstacle and making effective stimulation impossible. The only electrodes fitted on the cap were fCZ, CZ and ground and all the rings on the left hemisphere (which was the stimulation site) have been removed so that the only object between the scalp and the TMS coil was the negligibly thin fabric of the cap itself.

With the newly available EEG recording equipment for this study, recording became possible over the whole surface of the scalp, since the active electrodes together with their rings created a distance between the scalp and the TMS coil of no more than 0,5 cm. Theoretically, the increased distance should have been compensated for by the increased stimulation intensity based on the elevated threshold. Nevertheless, after extensive debate of whether this distance would have had a significant impact on the stimulation effect, we decided to compare the 2

conditions empirically: cTBS stimulation with the previously used electrode- and ring-less cap vs. cTBS stimulation with the "active" cap with all 64 active electrodes mounted. The pre-pilot measurements showed slight and measurable, but insignificant differences (namely a decreased stimulatory effect of single TMS pulses and a less profound cTBS after-effect although with the same polarity in the condition with the increased distance). Based on this evidence, we chose the option of recording the EEG with the fully fitted cap, since this also was one of the crucial and major innovative aspects of our study.

2.11 Monophasic vs. biphasic pulses

The previously mentioned (see section 1.3.1) different layout of monophasic and biphasic pulses add a slight, but important element into the experimental design. Since the RMT is different for both of these types of pulses, it had to be measured two or three times depending on the version of the experiment (short or long) and the session number (the 1st and last session of the cumulative effects version of the experiment incorporated more TMS measures with the additional use of paired-pulse techniques), given the differences in the TMS equipment (the single- or paired-pulse machine Magstim BiStim^2 delivers monophasic pulses with a different maximum stimulation output depending on whether it is in single or dual mode, while the repetitive pulse device Magstim Rapid^2 (which was used for the administration of the cTBS protocols) generates biphasic pulses).

2.12 Safety of the experiment (especially cTBS)

The potential adverse effects of single- and paired-pulse TMS stimulation are virtually statistically negligible. This in not the case of rTMS in general, which has been found to be slightly more risky. The cTBS we were planning on using in our study is considered to be generally relatively safe. Since the introduction of cTBS at least 49 studies with 741 participants have been conducted without any notable incidents (Rossi et al., 2009). However, there has been a single reported case of one TBS-related seizure in a healthy participant without any prior risk of epilepsy, where the authors speculated that it may have been linked to the unusually high stimulation intensity they used (Oberman, Pascual-Leone, 2009). We did not intend to use high-intensity stimulation and stuck to the default and

usually applied ratio of the cTBS intensity to the RMT. Considering the innovative design of our study, we were confident that the potentially acquired data would outweigh the potential risks associated with the employed methods, which consist mainly of possible mild skin discomfort on the scalp or mild headaches according to safety data collected in other studies with cTBS so far. Our evaluation of the proportions of the potential benefits and hazards of the study has been confirmed by the national committee for medical ethics in Slovenia, which allowed us to perform the experiment.

To further minimize the risk of potential side-effects of the cTBS on our sample of healthy participants, we asked them to fill out a questionnaire screening for various medical conditions they may have that would elevate the risk substantially (see Appendix A). Among them were epileptic seizures experienced in the past, head injuries, unexplained unconsciousness, brain surgery, metal pieces inside the skull and various life-supporting electrical devices (e. g. pacemakers, which could have been damaged by TMS pulses). A positive answer to any of those conditions generally excluded the potential participant from entering the empirical phase of our study. The inclusion of participants who have been taking medication at the time of the study was evaluated individually by a doctor depending on the exact type of medication.

2.13 Formulation of a working hypothesis

Following the study of other experiments conducted on the field of TMS, M1 and neuromodulation and the evaluation of various TMS measures, we specified exact variables for the reformulation of the conceptual hypothesis into a working hypothesis:

Statistically significant differences exist in the effects of the 30Hz cTBS and the 50Hz cTBS experimental condition on the neuroplasticity of the M1 measured mainly by means of MEPs and RC, with other measurements (SICI, ICF, CSP) acting as complementary elements.

The supplementary research question can be operationalized as follows:

What (if any) will be the differences in various consecutive daily stimulation

sessions regarding the effects of the 30Hz cTBS and the 50Hz cTBS experimental condition on the neuroplasticity of the M1, measured mainly by means of MEPs and RC (with the rest of the measurements acting as complementary elements)?

2.14 Final experimental design

We formulated detailed experimental procedure instructions for our own internal use (Appendix B). Essentially, the course of the experiment was this: upon a voluntary participation declaration on the part of the participant, the time of the first session has been agreed on and the participant was invited to the TMS laboratory. There he needed to read, fill out and sign the consent form with basic information about the experiment on a need-to-know basis and the TMS safety questionnaire. If the participant met the inclusion criteria (see section 2.12 and Appendix A), he was seated in a chair and started being set up with all the recording equipment (EEG, EMG). After the equipment was set up completely (including reaching acceptable levels of impedance of all electrodes on EEG and EMG), the experiment itself started.

The very first task was to find the hotspot on the M1 that would correspond to the chosen muscle (R-FDI) (see also section 2.4 hotspot search). Upon successful localization of the hotspot, the experimental paradigm (a program with instructions/hints for both the experimenters and the participant that also displayed a fixation cross the participant was tasked with staring at to avoid artifacts in the EEG signal related to potential eye movement) has been initiated. Then the stimulation intensities for the RMT and 1 mV MEPs (the intensity which was later used to record the MEP blocks) have been determined (either on the Magstim BiStim^2 in single-pulse mode or in the dual-mode (both modes yield different maximum stimulation intensities output), depending on which variant of the session it was (see next sentence)). For the full-scale version of the experiment, the 1st and last sessions for each of the conditions were longer, incorporating also the additional measures beside MEPs and RC: SICI, ICF and CSP (with all their respective preceding procedures such as reaching the maximum voluntary contraction of the muscle and using 20% of it as background contraction during the measurement of CSP).

From this point onwards, the rest of the session was common for both

variants of the sessions. It included recording of the recruitment curve (RC (a. k. a. input/output curve (which consists of MEPs at stimulation intensities of 90, 100, 140 and 150% of the RMT with 5 pulses per intensity = 20 in total))), determining the 1 mV intensity with the single-pulse mode of the Magstim BiStim^2 device and recording one baseline block of 1 mV intensity MEPs (20 pulses). These two blocks were labeled as pre-TBS and were used as baseline measures which have been compared later during the analysis against the post-TBS measures. The next step consisted of determining the RMT with the Magstim Rapid^2 rTMS machine (as already mentioned, the RMT of monophasic (Magstim BiStim^2) and biphasic (Magstim Rapid^2) pulses is different, following the distinct effects of those types of pulses on the M1) and the application of the cTBS modulatory protocol (experimental condition).

Immediately after the application of the cTBS protocol, post-TBS measures have been initiated, starting with a RC at time 0 (due to practical constraints of changing the equipment from the repetitive Magstim Rapid^2 back to the single-pulse Magstim BiStim^2, correctly placing the TMS coil, etc., a slight delay occurred, but for the sake of simplicity and illustration of the fact that the measure has been taken as soon as possible after the cTBS, we kept labeling this time point as 0). A complete RC was taken also at 30 and 60 minutes after cTBS, the latter measure demarcating the end of the experimental session. In between, blocks of MEPs (20 pulses each) were recorded at 5, 15, 25, 35, 45 and 55 minutes after cTBS.

2.15 Sample characteristics

The practical realization of this study and data acquisition and recording has been conducted during a prolonged period between October 2014 and May 2015. 12 subjects voluntarily participated in the study, 6 of them being male and 6 female, with a mean age of 25.6667 (SD±1.1547). All of the participants were university students at the time of their participation in this study, all were right-handed according to the Edinburgh Handedness Inventory and all of them were deemed suitable to enter the study based on their responses to the questionnaire assessing elevated risks of participating in TMS research due to various medical conditions. Only one subject (participant no. 5) had to be canceled at the very beginning (during the setup and testing of the EMG electrodes and signal) of the experiment

due to his/her inability to relax the given muscle, which yielded constant activation and was not suitable for the assessment of MEPs and other measures. Out of the 12 participants, 9 participated in the short version of the experiment (one day/session/stimulation per condition) and 3 in the full-scale version (one of those 3 subjects participated only in 4 consecutive sessions per condition instead of 5 due to some declared time constraints).

3. Results

The analysis of results in a study using electrophysiological measures (EEG, EMG, etc.) necessitates the extraction of relevant information from the large body of the acquired data. For instance, considering that one MEP block consisted of 20 pulses with an average (due to the aforementioned jitter in section 2.7) interval of 7 s, the 19 intervals between the pulses demarcated the minimal duration of the block (in reality, the recording extent for one block was slightly longer due to the start of the recording before the delivery of the 1st pulse and the last recording frame (EMG data have been recorded in multiple frames of a certain duration) lasting a few seconds after the last delivered TMS pulse of that block), which would be 133 s. Since the sampling rate of both the EEG and EMG equipment was set to 5000 Hz, in case of the EMG data we ended up with at least 665 000 values per one MEP block. The multiplication of this number by the number of MEP blocks per session (7), number of sessions (2 (for each condition one, in the case of the short version of the experiment)) and number of participants (12 (incorporating also the participants who chose the full-scale variant of the experiment, because their 1st sessions for each condition has been included in the analysis of the short experimental version)) would produce a large number around 110 millions of values in total (for all participants in the short version of the experiment). This number is even higher for the EEG data, since they have been recorded (with the exception of the duration of the cTBS protocol) continuously during the whole experiment.

However, most of these values were measures we were principally not interested in, because they did not hold any valuable information (e. g. the EMG signal showing background muscle contraction in between the TMS pulses). As was already mentioned, this master thesis only analyzed the EMG data. That meant that to work with the data, they had to be first exported from the recording software

(Signal 5.11) format to a Matlab file, from which the peak to peak amplitudes of the muscle activation following the TMS pulses (which were the relevant measures) were extracted into XLS tables. The result of this downscaling process provided us with the single values of muscle activation for each TMS pulse (20 values per block, since there were 20 TMS pulses per block). From there on, statistical tests have been conducted using the PSPP/SPSS software packages and graphs and tables have been created in LibreOffice Calc.

Since the values were mostly not normally distributed (I. e. not resembling a Gaussian distribution) within the MEP blocks (according to the Kolmogorov-Smirnov test with a Lilliefors significance correction (K-S/LSC) only 165/322 and with the Shapiro-Wilk (S-W) test only 99/322 MEP block data followed a normal distribution), we transformed the data using the natural logarithm (LN) function to compensate for the skewness of the data and for more MEP block to be normally distributed, which would allow the use parametric statistical tests (cf. Meenakshi, Schleper & Wassermann, 2003 and Wassermann, 2002). After the transformation, the majority of the values within MEP blocks became normally distributed (K-S/LSC: 266/322 and S-W: 241/322 MEP blocks normally distributed), which enabled the use of parametric statistical tests to test for the significance of the differences between groups (in the sense of various values/parameters of within-subject variables, not samples of subjects). Also, the first MEP from each MEP block was excluded from further analysis due to producing very frequently very large peak amplitudes (resulting in each MEP block consisting of 19 pulses for the purposes of the analysis), which is also a common practice in TMS research.

We divided the data analysis in 2 main sections: first, we present the individual analysis of various MEP blocks (time-points) for one session per condition (incorporating all 12 participants in our study) in a gradual manner, starting with differences between blocks (measures conducted at various times before (only one baseline block) and after (6 blocks) the cTBS stimulation), then between conditions (30 Hz and 50 Hz cTBS protocol) and finally we pooled the participants together to see what the prevailing effect of both the experimental conditions might have been to compare them and evaluate their applicability in future research.

In the 2nd major section, we concentrated on the cumulative effects of cTBS

in consecutive daily sessions, which is an innovative aspect of the study of cTBS this project intended to introduce since the very beginning. Unfortunately, due to the time-consuming schedule of the full-scale version of the experiment and the very low number of participants in that variant (only 3 subjects, one of them completing only 4 session per condition), the findings cannot be considered much more than just a preliminary exploratory probe.

For the purposes of the analysis, MEP blocks were labeled with their order in the sequence within the session, with MEP1 being the baseline (pre-TBS) MEP block of that session and MEP2-MEP7 being the post-TBS MEP blocks (measured at 5 (MEP2), 15, 25, 35, 45 and 55 (MEP7) minutes after cTBS). Also, any cited absolute values are transformed natural logarithms. The graphs contain mean values of MEP blocks (if not stated otherwise) normalized to the baseline of the actual session and since the mean and normalization have been performed after the transformation of the data to natural logarithm values, the resulting values relate to the baseline (which is 0) with positive values indicating excitation/facilitation and negative values indicating inhibition.

Given the tight time-frame (the last session/recording took place in May 2015, leaving us with less than a month to analyze and interpret the data), timing constraints and continually emerging issues we had to address during the data analysis, we managed to process only the MEP data (excluding the RC, CSP, SICI and ICF data from the extent of this thesis), which is suboptimal, but still containing the most valuable and meritory information for our conclusions as the MEPs are by far the most numerous and frequent measure and the additional measures have been considered only complementary from the beginning of this study (and they will be analyzed later).

3.1 Short-term effects of cTBS

3.1.1 Individual findings of effects of condition and time

The first measure being determined after finding the hotspot was the RMT (see also section 2.14 and Appendix B). It is a value that is related to the maximum stimulation output (MSO) of a given TMS device. The absolute MSO is a characteristic of the machine that is different for the single-pulse Magstim

BiStim^2, for the dual-mode of Magstim BiStim^2 (connecting 2 Magstim 200 devices together to allow paired-pulse stimulation) and for the repetitive Magstim Rapid^2. In addition to that, the single-pulse and dual-mode of Magstim BiStim^2 produces monophasic pulses, while the repetitive Magstim Rapid^2 produces pulses in a biphasic waveform and these 2 pulse forms yield a different RMT. Consequently, the RMT assessed by the single-pulse and repetitive machine cannot be directly compared. The RMT determined with the single-pulse TMS device was used to set the stimulation intensities for the RC measurements (the paired-pulse techniques (SICI, ICF) used the dual-mode RMT as the reference for the intensities of their stimuli) and the repetitive RMT was used for the calculation of the cTBS intensity (80% of the RMT). Since the RMTs have been determined only on 2 occasions (once for each session/condition), the inference possibilities from these values are fairly limited. However, it is reasonable to expect similar values of those 2 instances in each individual participant (see Table 1. below).

Exp. Condition	Device type	Participant number											
		1	2	3	4	6	7	8	9	10	11	12	13
30 Hz cTBS	Single pulse	47	41	46	51	54	49	48	51	43	53	42	50
	Repetitive	61	55	74	72	61	72	69	71	66	71	58	73
50 Hz cTBS	Single pulse	47	43	52	49	50	50	48	46	50	42	40	51
	Repetitive	65	68	70	70	61	68	67	65	74	61	57	76

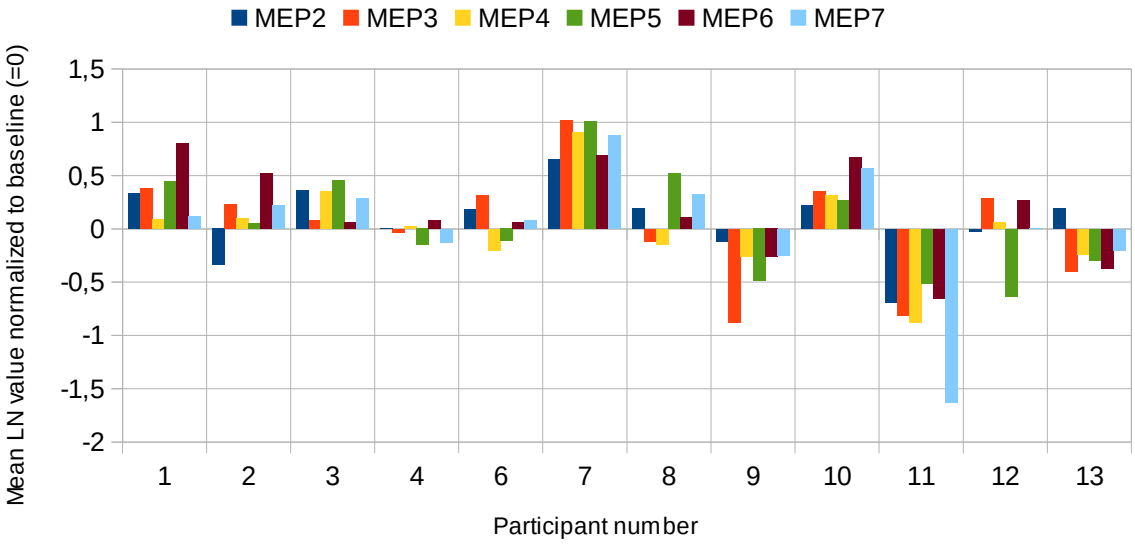
Table 1. Percentage of max. stimulation output of TMS device at RMT

From the table we can see that the pre-TBS MSO values for the single-pulse device are similar for both conditions in each participant except for subjects number 3, 9, 10 and 11. Since we ensured that both the experimental conditions would be sufficiently apart in time not to interfere with each other, those discrepancies could not be explained by our experimental design and the variables we controlled, but would have to be attributed to some inherent changes in cortical excitability (some form of meta-plasticity) over time, which are presently not understood, neither on the physiological/chemical level of brain function, nor in relation with TMS research.

Next, we report the statistically significant differences in individual subjects regarding the various time-points of the MEP blocks and the experimental condition determined by the application of the repeated measures ANOVA test with post hoc follow-up tests using the Bonferroni correction for repeated measures (see

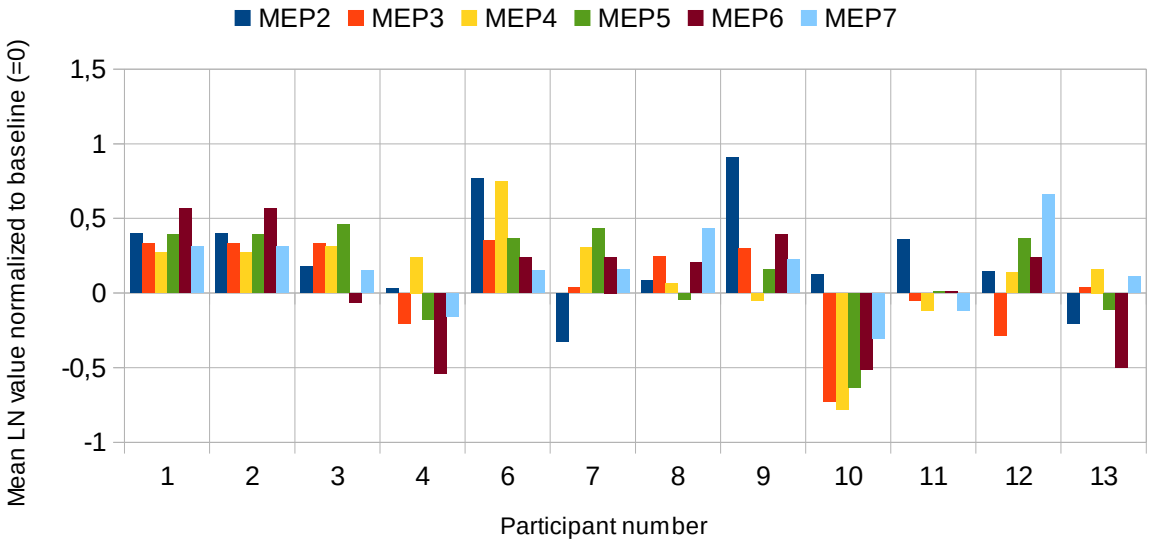
Table 2 below the graphs 1 and 2 for a summary of statistically significant differences). The graphs 1 (30 Hz cTBS condition) and 2 (50 Hz cTBS condition) display the mean values of each MEP block normalized to the corresponding baseline MEP block (note that MEP block number 1 from Table 2 is the baseline MEP block for each participant and condition in graphs 1 and 2, so it is not displayed, because it would have been located on the X axis (Y=0)) for each participant.

Graph 1. Mean values of MEP blocks normalized to baseline for 30 Hz cTBS



We can clearly observe from Graph 1 that in the 30 Hz cTBS condition, there have been more MEP blocks with facilitation rather than inhibition (possible interpersonal invalidity of the expected effect), which is undesirable considering the fact that 30 Hz cTBS protocol is supposed to be an improved version of the already inhibitory 50 Hz cTBS protocol. The extent of variability is also unsatisfactory. Moreover, although this graph is primarily concerned with the visualization of intrapersonal differentiation of the effects of cTBS over time, it is evident that the interpersonal variability is notable too (ranging from the exclusively facilitated subject number 7 to the exclusively inhibited participant number 11 (interpersonal unreliability)). Of particular interest is also the inconsistency of effects during the time-course of 1 hour after cTBS. The graph shows oscillations in practically all participants with multiple drifts between slight inhibition and facilitation in participants who were relatively unaffected by the cTBS, while even in exclusively inhibited or facilitated participants the effect varies over time, indicating intrapersonal unreliability of the effect of cTBS.

Graph 2. Mean values of MEP blocks normalized to baseline for 50 cTBS



For the 50 Hz cTBS protocol in graph 2, the data follow a similar pattern as for 30 Hz cTBS. Again we can see predominant facilitation rather than inhibition (MEP blocks with facilitation are more numerous than blocks indicating inhibition). Compared to the 30 Hz cTBS, 50 Hz cTBS seems more facilitatory in general (which we will be able to directly compare in the next section). However, drifts and oscillations are present also in this condition with characteristics similar to the already described 30 Hz cTBS effects. A slight difference of the 50 Hz protocol is that there is not a single case of exclusive inhibition (across all MEP blocks/time-points). In Table 2 below we list the statistically significant differences (along with their effect sizes for the statistically significant differences of the conditions) of conditions and MEP blocks (MEP block number 1 is the baseline block) without elaborating more in detail about the discovered findings (as this would be too fine-grained for any general conclusions).

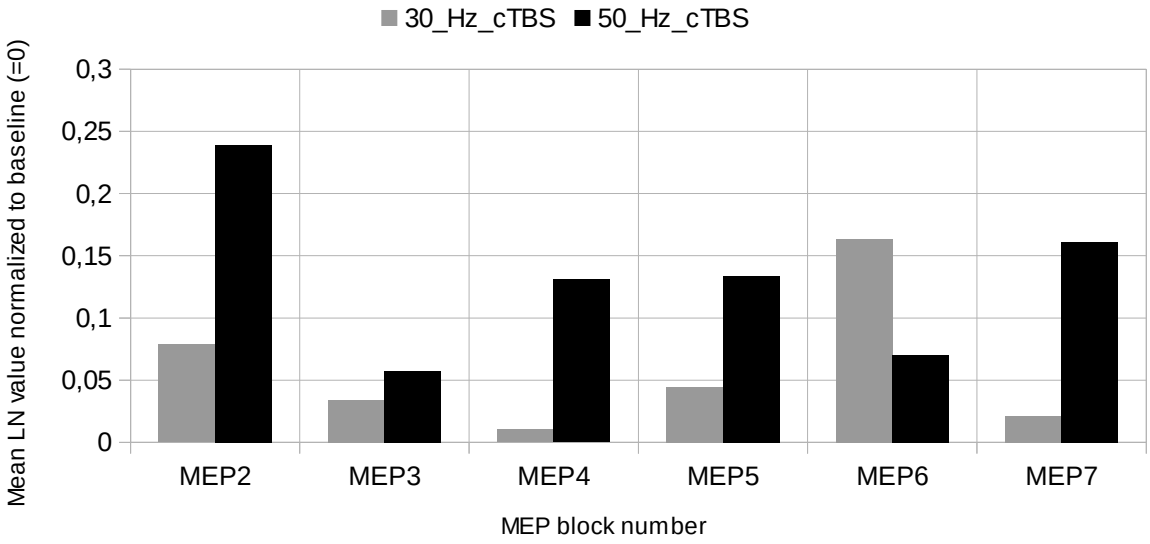
Participant number	Exp. Condition	Statistically significant differences between	
		Conditions	MEP blocks within condition
1	30 Hz cTBS	Yes (part. η^2=0,828)	1-6; 4-6
	50 Hz cTBS		None
2	30 Hz cTBS	No	None
	50 Hz cTBS		None
3	30 Hz cTBS	No	1-5; 2-6; 5-6
	50 Hz cTBS		1-5; 5-6
4	30 Hz cTBS	Yes (part. η^2=0,57)	2-7; 5-6; 6-7
	50 Hz cTBS		4-6
6	30 Hz cTBS	Yes (part. η^2=0,383)	None
	50 Hz cTBS		None
7	30 Hz cTBS	Yes (part. η^2=0,679)	1-all
	50 Hz cTBS		2-4,5,6
8	30 Hz cTBS	No	4-5
	50 Hz cTBS		None
9	30 Hz cTBS	Yes (part. η^2=0,435)	3-1,2,6,7
	50 Hz cTBS		2-1,4,5,7
10	30 Hz cTBS	Yes (part. η^2=0,216)	1-6,7
	50 Hz cTBS		1-5; 2-4
11	30 Hz cTBS	Yes (part. η^2=0,892)	1-2,4,5,6,7; 7-2,5,6
	50 Hz cTBS		2-1,3,4,5,7
12	30 Hz cTBS	No	5-all
	50 Hz cTBS		3-7
13	30 Hz cTBS	No	None
	50 Hz cTBS		6-1,4,7

Table 2. Statistically significant differences found for individual analysis

3.1.2 Aggregate analysis (the overall effect of condition and time)

Since the previously presented detailed findings in individual participants show us the intraindividual differences, but fail to expose the overall trends in the data (at least with respect to statistical tests and significance) we are most interested in (because they are meritory for the evaluation of our hypothesis), we have to change the perspective and analyze all subjects at once. For that we present graph 3 (below) where normalized mean values of all participants per MEP block and condition are shown.

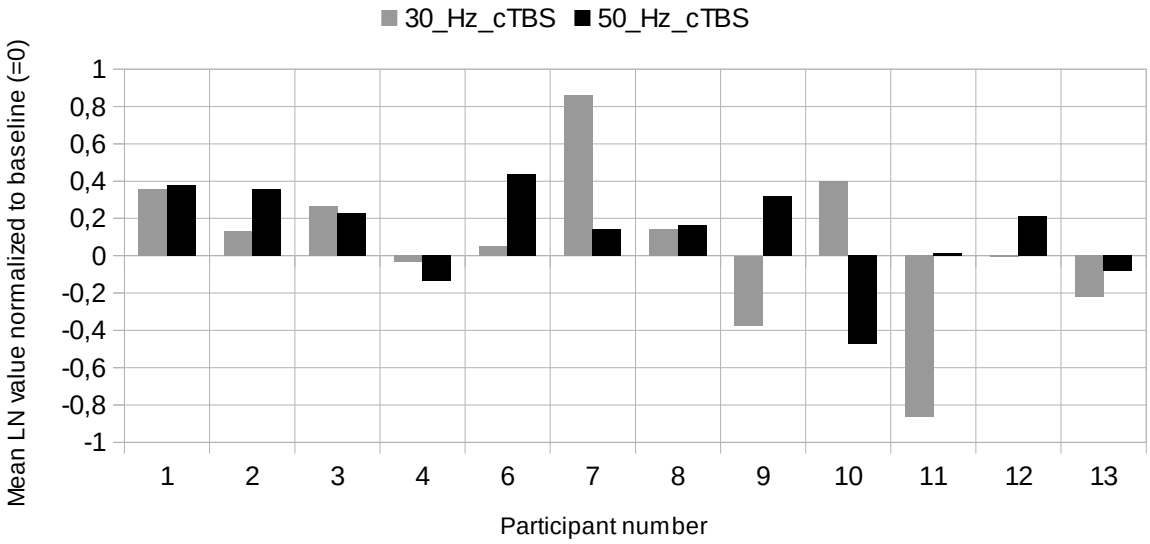
Graph 3. Mean values of MEP blocks normalized to baseline



The repeated measures ANOVA (factors: condition and MEP block number) shows that various MEP blocks are not significantly different from each other in the 30 Hz cTBS condition ($F(6; 1290)=1,85$; $p=0,086$). In the 50 Hz cTBS condition, MEP blocks as a whole were significantly different among each other ($F(6;1290)=2,625$; $p=0,016$; partial $\eta^2=0,012$). Post hoc tests with a Bonferroni correction discovered through pairwise comparisons that the only significantly different blocks were the baseline block (on Graph 3 represented as 0 on the Y axis) and block number 2 (MEP2) with a mean difference of -,237. We also compared the corresponding (matching with regard to time after cTBS/order within the session) pairs of MEP blocks to determine whether there are differences between the 2 experimental conditions: only MEP blocks number 6 (taken 45 minutes after stimulation) in each condition (30 Hz: $M=0,26$; $SD=0,772$; 50 Hz: $M=0,465$; $SD=0,768$) were statistically significantly different from each other ($F(1;215)=9,708$; $p=0,002$; partial $\eta^2=0,043$).

Additionally, we include graph 4 to illustrate the interindividual differences of the effects of cTBS protocols on individual participants. It confirms the tendencies we already observed in the individual analysis, namely the large interindividual variability, the predominantly facilitatory effects (but moderate in their extent) of both cTBS protocols (based on the frequency of facilitation when compared to instances of inhibitory MEP blocks) and two apparent outliers (subject 7 (facilitation) and subject 11 (inhibition) in the 30 Hz cTBS condition).

Graph 4. Individual mean values of MEP blocks normalized to baseline



3.1.3 Evaluation of our working hypothesis

The central question of our study, postulated in the form of the working hypothesis in section 2.13 (that there would be statistically significant differences between the 30 Hz cTBS and the 50 Hz cTBS conditions based mainly on MEP data) could have been only evaluated by a gross analysis of all the MEP data of all subjects. Therefore we compared the data between the 30 Hz cTBS (M=0,139; SEM=0,033) and the 50 Hz (M=0,093; SEM=0,025) cTBS conditions with a two-way repeated measures ANOVA test (factors: condition and MEP block), which showed that the 2 conditions are not statistically significantly different ($F(1;215)=1,461$; $p=0,228$), thereby disproving our hypothesis. There was also no statistically significant interaction between the 2 factors (condition and MEP block number)

3.2 Cumulative effects of cTBS

In this section we describe the overall results (we did not analyze individual participants) of the analysis of cumulative effects of cTBS over consecutive daily sessions of stimulation (up to 5 days/sessions per condition) which incorporated the processing of data from only 3 participants who were willing to participate in the full-scale version of the experiment. Since we already discovered that there were in some instances significant differences between MEP blocks within one session/day and that generally the mean MEP block values within one session were quite variable and oscillations and drifts were visible from the individual short-

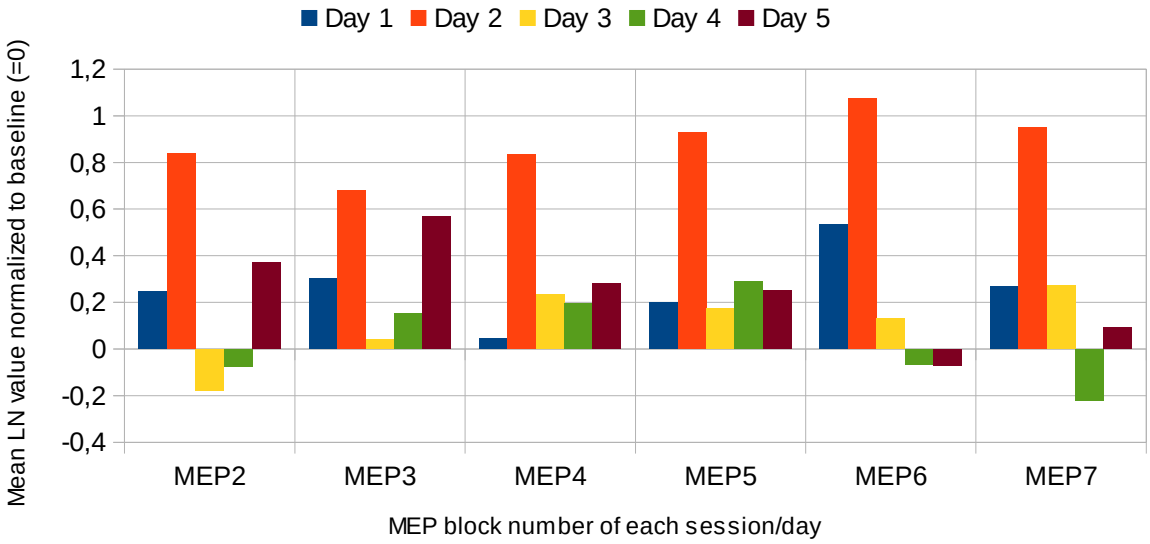
term data in section 3.1.1 (see graphs 1 and 2), we were not particularly concerned with the exploration of this aspect in this dataset. More interestingly, we focused mainly on the exploration of possible trends and differences among the same time-points within the sessions (comparison of the same MEP block numbers (e. g. MEP2 (5 minutes after cTBS)) in different following days/sessions). First we display table 3 with the individual RMT for the consecutive sessions.

Participant no.	Exp. Condition	Device type	Session number				
			1	2	3	4	5
1	30 Hz cTBS	Single pulse	47	43	44	47	47
		Repetitive	61	61	58	61	62
	50 Hz cTBS	Single pulse	47	46	49	45	47
		Repetitive	65	66	62	65	65
6	30 Hz cTBS	Single pulse	54	51	60	55	-
		Repetitive	61	68	65	68	-
	50 Hz cTBS	Single pulse	50	54	54	55	-
		Repetitive	61	68	65	66	-
10	30 Hz cTBS	Single pulse	43	42	46	42	46
		Repetitive	66	68	67	69	70
	50 Hz cTBS	Single pulse	50	51	53	40	48
		Repetitive	74	65	72	66	65

Table 3. % of MSO of TMS device at RMT in consecutive sessions

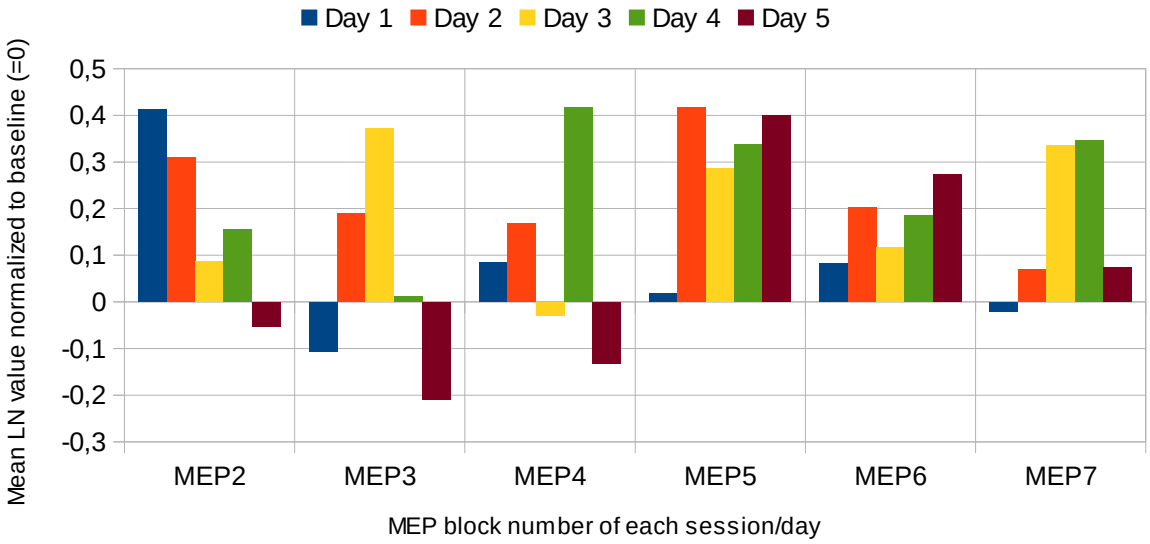
Without going into a deeper analysis of the individual characteristics and variability, we can observe oscillations and drifts of the RMT values in consecutive sessions, which could possibly indicate a long-term trend of changes in M1 excitability similar to what we saw in graphs 1 and 2 in a short-terms analysis. Graph 5 presents the mean values of MEP blocks grouped by their number for separate consecutive days of stimulation for the 30 Hz cTBS condition.

Graph 5. Mean values of MEP block normalized to baseline - 30 Hz cTBS



Again, drifts resembling a sinusoid are clearly visible here, as well as in the 50 Hz cTBS condition (see graph 6 below).

Graph 6. Mean values of MEP blocks normalized to baseline - 50 Hz cTBS



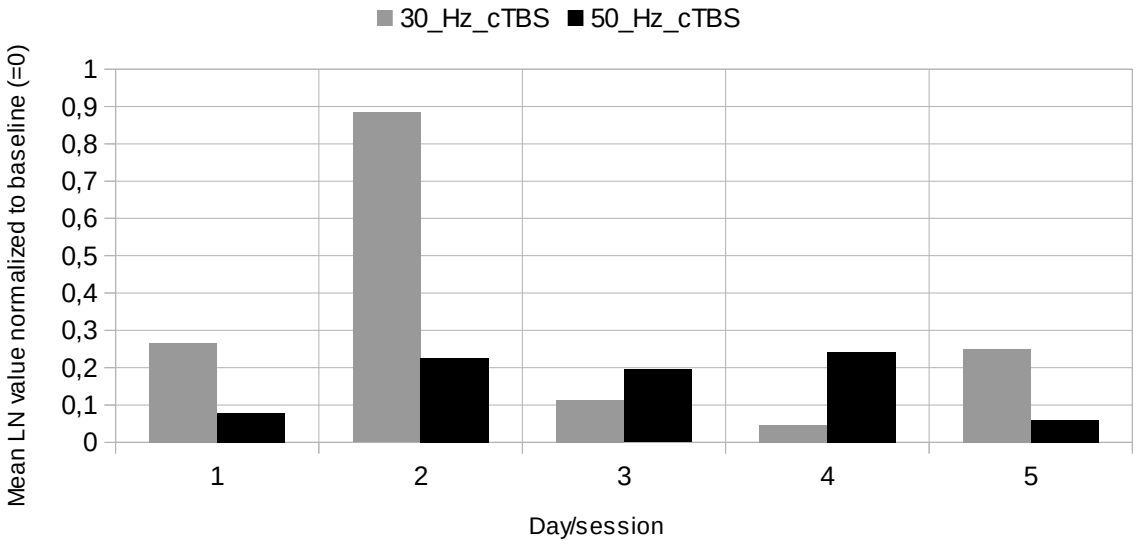
We summarized all the statistically significant differences between blocks at the same time-point for different days/sessions in table 4 below (note that due to the fact that one of the participants (number 6) did not participate in session number 5 in both conditions and since during the analysis with day 5 included, SPSS would exclude all cases with missing values (effectively excluding all data from participant number 6), we preferred to exclude the whole day/session number 5 from further analysis that is summarized in table 4, because we would lose less data by choosing this option).

MEP block	Exp. Condition	Statistically significant differences between	
		Conditions	Sessions (numbers) within MEP block and condition
2	30 Hz cTBS	No	1-3,4; 2-4
	50 Hz cTBS		None
3	30 Hz cTBS	Yes (part. η^2=0,119)	None
	50 Hz cTBS		3-1,2,4; 1-2
4	30 Hz cTBS	Yes (part. η^2=0,1)	None
	50 Hz cTBS		None
5	30 Hz cTBS	No	None
	50 Hz cTBS		1-2,3,4
6	30 Hz cTBS	Yes (part. η^2=0,275)	4-1,2,3
	50 Hz cTBS		None
7	30 Hz cTBS	Yes (part. η^2=0,103)	4-1,2,3
	50 Hz cTBS		1-3,4; 2-3

Table 4. Statistically significant differences found during overall analysis of cumulative effects

Furthermore, after excluding participant number 6 (in contrast to the analysis from table 4) to be able to compare all 5 days and MEP blocks within them, we found out that the sessions are significantly different from each other ($F(2,409; 94,802)=10,596$; $p<0,001$; partial $\eta^2=0,232$) in the 30 Hz cTBS condition. Post hoc tests with a Bonferroni correction revealed statistically significant differences among following days: 1-2 ($p=0,026$; mean difference=-0,165), 2-4 ($p<0,001$; mean difference=0,428), 2-5 ($p<0,001$; mean difference=0,311) and 3-4 ($p=0,011$; mean difference=-0,312). In the 50 Hz cTBS condition, days were statistically significantly different as well ($F(3,047;106,630)=15,999$; $p<0,001$; partial $\eta^2=0,314$). Post hoc tests indicated significant differences between days: 1-2 ($p<0,001$; mean difference=-0,438), 1-3 ($p<0,001$; mean difference=-0,568), 1-4 ($p<0,001$; mean difference=-0,447), 1-5 ($p=0,001$; mean difference=-0,302) and 3-5 ($p=0,001$; mean difference=0,266). According to a repeated measures ANOVA (factors: condition, day/session, MEP block number) Statistically significant differences have been found also between the 2 experimental conditions ($F(1;53)=11,961$; $p=0,001$; partial $\eta^2=0,184$) and interactions between: condition and day ($p<0,001$; partial $\eta^2=0,277$), condition and block ($p=0,024$; partial $\eta^2=0,044$), day and block ($p<0,001$; partial $\eta^2=0,066$); and condition and day and block ($p<0,001$; partial $\eta^2=0,059$).

Graph 7. Mean overall effects of sessions normalized to baseline MEP block



Additionally, we included a graph for illustrative purposes. Graph 7 shows predominant, but modest facilitatory effects in both conditions, which are not stable over time. This means that the cumulative effects of cTBS in relation to each session's baseline were not being gradually reinforced.

4. Discussion

4.1 Summary of relevant findings

In this section we would like to summarize the most important findings that are relevant with respect to the goals and aims of this master thesis and the overlaying project concerned with TMS and depression. As we were able to observe in the results section, the individual data of the short-term effects of cTBS generally suggested that these effects were very variable and inconsistent within as well as across almost all participants. There are relatively numerous participants showing both facilitatory and inhibitory effects within the same session depending on the time-point when the measure has been taken. Also, many participants exhibit insignificant effects in both conditions. What is more of a concern are extreme cases of both profound facilitation and inhibition in both conditions, indicating considerable interpersonal variability of the cTBS effects on M1. The pattern of drifts and oscillations of the effects are visible across all participants, frequently ranging from one polarity to the other (facilitation vs. inhibition or vice

versa).

On the overall level of the analysis of the effects in blocks rather than individuals, the MEP blocks were not significantly different from each other in the 30 Hz cTBS condition. In the 50 Hz cTBS, the baseline block (MEP1) was significantly different from the first post-TBS block measured at 5 minutes after cTBS (MEP2). By comparing the same time-points (corresponding blocks measured at the same time relative to the cTBS experimental condition), we found out that only the post-TBS MEP blocks measured at 45 minutes after stimulation (MEP6) were significantly different in each condition. The core finding that disproved our hypothesis was that the 2 experimental conditions were not statistically significantly different from each other. Based on the normalized mean values, we can conclude that the 50 Hz cTBS was slightly more facilitatory than the 30 Hz cTBS with both conditions having a negligible facilitatory effect on the M1.

From the analysis of cumulative effects of cTBS protocols only very limited conclusions can be drawn, especially considering the low number of participants (only 3 subjects participated, one only for 4 days/sessions per condition instead of 5). The percentages of the MSO at RMT indicated that there would be differences in the effects of cTBS over a longer time span that manifested also in the drifts and oscillations of the percentage at RMT. The values of MEPs from all stimulation sessions/days in pairs of corresponding MEP blocks compared by condition were significantly different in the case of MEP blocks number 3, 4, 6 and 7. Significant differences have been found also between mean values for whole days: between days 2 and 1, 4 and 5; and between 3 and 4 in the 30 Hz cTBS condition. For the 50 Hz cTBS, significantly different were day 1 from all following days and the 3rd day from the 5th. A gross analysis also showed that the 2 conditions were statistically significantly different and significant interactions have been found for all combinations of independent variables (condition-day, condition-block, day-block and condition-day-block).

4.2 Interpretation of results and findings

The assumption formulated in our hypothesis was heavily based on the findings and implications of a study carried out by Goldsworthy et al (2012). They found that when compared to the 50 Hz cTBS applied over the M1, whose effects

were interindividually variable, the novel 30 Hz cTBS produced universal and consistent inhibition in all 12 participants. These results constituted a very promising proposal and alternative to currently applied rTMS protocols as alternative treatment for depression. In clinical practice and therapeutic use, predictability, reliability and validity are very important, because their lack would be a potential health hazard for the patients. Therefore a universally inhibitory cTBS protocol, which is superior to conventional rTMS protocols (cTBS being generally delivered at lower intensities, more comfortable and shorter), seemed to be a very viable substitution for them.

That was also the justification and foundation for the decision to include 50 Hz cTBS and 30 Hz cTBS as potential candidates in a preliminary study testing the efficiency of both these protocols for their perspective application as alternative treatment for depression later during more advanced stages of the overlaying project at the Neurological clinic. Although this master thesis was never intended to be a strict replication of the study of Goldsworthy et al. (2012), a comparison of their results to ours is only a logical consequence.

In stark contrast to the findings of Goldsworthy et al. (2012), our results showed slight facilitatory effects of both 30 and 50 Hz cTBS protocols, which are not significantly different from each other. Furthermore, we found a high intraindividual and interindividual variability in both conditions, ranging from significant facilitation to significant inhibition, but in most cases with negligible effects on the excitability of the M1. Additionally (outside of the scope of Goldsworthy's study) we report notable variability and inconsistency in short-term effects of cTBS (within one hour after the stimulation at various time-points) as well as long-term effects (similar variability and inconsistency over consecutive days after daily stimulation).

The differences in the experimental design of both studies could not have accounted for the discrepancies of their results and findings. One possibility might have been the overextensive rejection/exclusion of the data in the study of Goldsworthy et al. (e. g. in case outliers have been excluded to make the data less variable, which then in turn affects the validity of the data). In our study, we did not exclude any data except for the 1st pulse per MEP block (taking into account the remaining 19/20 pulses) in the short-term effects analysis and in the

cumulative effects analysis we excluded the data for the 5th day/session or the whole participant number 6 respectively (which was a necessity considering the function of SPSS and the fact that subject number 6 did not participate in the 5th day/session in both condition and hence those data were missing).

To conclude, our data confirm the inherent nature of variability of TMS measures, ranging from the loose definition of the 1 mV intensity measure (which is only approximate) to short- and long-term effects of modulatory cTBS.

4.3 Implications of our study

The findings of our study have serious implications on both the overlaying TMS and depression project as well as the broad scientific community concerned with TMS research. In the next stages of the TMS and depression project, the usefulness and efficiency of cTBS as a candidate for alternative treatment of depression will have to be reevaluated. Based on our findings, both the 30 Hz and 50 cTBS protocols are not reliable and valid methods that could be applied to induce inhibitory effects in M1, which is an indication of their inhibitory inefficiency in the effects on the DLPFC as well. If the characteristics of cTBS in relation to its inhibitory properties are going to be studied for the purposes of this project also in the future, due to insignificant differences between the 2 compared cTBS protocols, the 50 Hz alternative is likely to be preferred because of its more widespread use and research of its mechanisms of action in comparison with the relatively infrequently used and non-standard 30 Hz cTBS (which is also practically relevant to the theoretical prospects of any of these protocols being potentially approved for the alternative treatment of depression in the future).

Another potentially applicable option would be to employ some kind of preconditioning prior to cTBS to moderate its effects in a desirable way (similarly as we described in section 1.3.5, but opted for the omission of its use due to practical reasons, while now in the light of our findings there may be stronger justification for its application). The solutions may include the application of tDCS or administration of a drug/substance (as has been mentioned in section 1.3.5), but an extensive review of literature focused on this aspect would have to be carried out first to select the most promising definitive alternatives. An additional approach might include the use of conventional rTMS (which are

already approved as an alternative treatment of depression) perhaps also combined with preconditioning.

Assuming the validity of our findings, their implications have considerable impact also on TMS research as a whole. Our results largely dispute the common notion of cTBS protocols having an overall inhibitory effect on M1, suggesting no significant effects or mild facilitatory effects at the most instead. The discrepancies between the findings of Goldsworthy et al. (2012) and ours raise doubts about the methodology and statistical analysis of one or both of these studies, because of the improbability of different findings that could be perhaps explained by the use of different TMS equipment or the different samples (although the physiological mechanisms of function of the brain are widely believed to be universal across all populations). We have to stress the importance of unbiased and ideally blinded data analysis where especially in the case of TMS measures (due to the large variability) outliers cannot be simply rejected (data rejection patterns and criteria should be explicitly stated and justified prior to the analysis, not applied ad hoc during the analysis while observing the data) just because they do not fit the prevalent or desirable data trends and distribution, since such an approach would be misleading and those outliers might be relevant in the applied fields (medicine, psychiatry, etc.).

4.4 Limitations of our study

We are aware of many limitations of our study that could have negatively influenced the progress and outcomes of this project. Among the conceptual limitations might have been the fact that we were studying the effects of cTBS on the plasticity of the M1 (because the response is directly measurable, which is not the case for the DLPFC), which is based on the assumption of possible generalization of the M1 response on other brain structures, while in practice it principally does not necessarily have to indicate the same effects of cTBS on the DLPFC (which is the brain region relevant in cases of depression). There is also the theoretical possibility of differences in effects between healthy subjects (which we used as participants) and depression patients (which would be the actual target population for alternative treatment of depression after the suitability of cTBS would have been established) due to differences in brain function, possible age mismatch or other factors.

The practical limitations and problems included our inexperience with TMS within the TMS laboratory of the Neurological clinic (compared to worldwide established TMS labs), which manifested in issues we continually had to address. Among them were the stability and general placement of the TMS coil, lack of exact determination of the hotspot and the imprecise approximation of the 1 mV stimulation intensity (due to the general variability of TMS). Furthermore, the realization of the experiment and data collection took a long time due to a generally slow recruitment of participants and with too few subjects volunteering for the full-scale cumulative effects version of the experiment, which might have been determined by the time-consuming experimental design in the cumulative effects version (it is only fair to add that this could not have been principally circumvented provided that we were really interested in the exploration of the cumulative effects). The final experimental design also might have been too complicated and ambitious, since we wanted to incorporate as many TMS measures as possible (MEPs, RC, CSP, SICI, ICF), which increased the duration of sessions and the total number of TMS pulses delivered to the participants.

Last but certainly not least (quite the opposite), the author of this master thesis was never involved in a complex study such as this before, providing him with a lack of experience, knowledge base and overview, which might have contributed to possible potential unintentional mistakes, omissions, misinterpretations and other types of fallacies during the data analysis as well as the composition of this thesis and he hopes that this particular research experience is just one more step in a long-term gradual process of accumulation of knowledge.

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Appendix A

General questionnaire for TMS safety and Informed consent

GENERAL QUESTIONNAIRE REGARDING ELIGIBILITY FOR PARTICIPATION IN A TMS STUDY

NAME OF THE PARTICIPANT:

DATE:

1. Are you being treated for epilepsy or have ever suffered an epileptic seizure or other kind of seizure? No ☐ Yes ☐

2. Have you ever lost consciousness or fainted? No ☐ Yes ☐

If so, please describe what happened:

3. Have you ever suffered a head injury resulting in unconsciousness? No ☐ Yes ☐

4. Do you have problems with hearing, or experience noise in your ears? No ☐ Yes ☐

5. Are you pregnant? Is it possible you're pregnant, but not aware of it yet? No ☐ Yes ☐

6. Do you have metal pieces in your brain or skull (except titanium pieces), for example shrapnel or clamps left after surgery? No ☐ Yes ☐

7. Do you have cochlear implants in your ears? No ☐ Yes ☐

8. Do you have a central nervous system stimulator inserted (for example Deep-Brain Stimulation, Vagus Nerve Stimulation)? No ☐ Yes ☐

9. Do you have a pacemaker, or other sorts of wires or metals in your heart or elsewhere in your body? No ☐ Yes ☐

10. Do you have a medicine infusion device inserted? No ☐ Yes ☐

11. Are you on any sort of medication? No ☐ Yes ☐

If so, please specify which:

12. Have you ever undergone a surgery on your spine? No ☐ Yes ☐

13. Do you have a spinal or ventricular fluid drain inserted? No ☐ Yes ☐

14. Did you have a TMS procedure in the past? No ☐ Yes ☐

DECLARATION OF INFORMED AND FREE CONSENT TO PARTICIPATION IN STUDY:

"Effects of Various Theta-Burst Transcranial Magnetic Stimulation Protocols on Plasticity of Primary Motor Cortex"

You're are invited to participate in a study examining the response of the brain and muscles to Transcranial Magnetic Stimulation (TMS) in healthy people, with the purpose of developing efficient protocols for treatment of people with depression. If you decide to participate in the study, you will be asked various questions and your answers will be recorded. Besides this, you will participate in recording of electromyographic and electroencephalographic responses to brain stimulation with TMS, all of which represent non-invasive methods for stimulation and recording of electric activity of brain and muscles.

You can revoke your consent to participate in the study without consequences at any time. Your data, collected during the study, will be kept private. It will be protected from unauthorized access by being stored under a personal code, and will not be available to third-parties without your consent. It will be stored only for the purposes of this study.

We expect that participation in the study should not cause you any significant discomfort. Individual experiments will last for approximately two hours, will not require any special mental efforts and should cause no side-effects apart from occasional unpleasant sensations on your skin or mild headaches. Rarely occurrences of epileptic seizures have been reported in TMS stimulation research and treatment, but only when using the more intensive TMS stimulation protocols, which is not the case in this study. You will be asked to participate in two experiments (in which we will evaluate effects of two different TMS protocols), separated by at least 2 weeks. In case you will be interested to take part in the longer version of the experiment, you will be asked to participate in daily repetitions of stimulation with one protocol (up to 7 days for each) to examine the cumulative effects of repeated stimulation, which is a conventional, medically approved and safe way of treating depressed patients with TMS.

Your eligibility for the study and your safety will be ensured with prior examination to see if you meet the safety requirements for TMS research. In case you find the clinical examination, TMS stimulation and recording of electrophysiological too strenuous at any time, please inform the researcher present, who will respond appropriately.

For all questions related to the study, please contact the project leader:

asist. mag. Jurij Bon, dr.med.spec.psihiater
Klinični oddelek za bolezni živčevja, Nevrološka klinika
Zaloška 2, 1000 Ljubljana

Email: jure.bon@kclj.si
Phone: (01) 5872 264

I consent to the participation in the study. I have read the explanation, or it has been given to me verbally, and I understand it.

Date and signature of the participant:

Date and signature of the person responsible:

Appendix B

Experimental protocol

1st and last sesion protocol (perhaps also 4th session):

1. Put EEG cap on (32 channels out of 64).
   DC recording (DC MR+ AMP), 1000Hz low pass filter.
   Sampling rate 5000Hz
   Put EMG on FDI bely-tendon montage (disposable electrodes)
   20Hz low-cut / 2000 high-cut
   5000hz sampling rate
2. Find Hot-spot single pulse machine/alpha coil. **CONFIG2**
   Raw search 5 meps at 2cm apart locations, find response and place the 5x5 grid on that location.
   Find Hot-spot whithin grid in less than 50meps. Mark it.
   From here till the end, the subject will stare at a fixation cross (specially during 1mv blocks and brakes)
3. Find RMT. **CONFIG2**
4. Switch alpha coil to paired pulse Bitstim mode:
   Find intensity that elicits >=1mv in 5 out of 10 trials for TS intensity. **CONFIG2**
   Find RMT, use 70% for CS intensity. **CONFIG2**
5. Do SICI and ICF: **CONFIG4**
   3 ISIs + baseline and 10 trials each (40 trials total). Inter trial interval 5s no jitter.
   SICI ISIs: 2ms, 4ms, 15ms.
6. Determine maximum voluntary contraction and use 20% for CSP. **CONFIG3**
   Record CSP at 120% of RMT, 20 trials (7s ISI +-10% jitter). **CONFIG1**
7. Do Recruitment Curve at 90, 100, 140 and 150% of RMT, 5 trials per intensity = 20trials(7s ISI +-10% jitter). **CONFIG1**
8. Find 1mv intensity (5 trials per intensity until 1mv+-250microvolts in average found). **CONFIG2**
9. Record 1 baseline block of 20 trials (7s ISI +-10% jitter). **CONFIG1**
10. Switch to repetitive machine:
    Use Air cooled coil for finding RMT, use 80% for TBS. **CONFIG2**
    Deliver TBS (50Hz or 30Hz randomized and double blinded) with Air cooled coil (no vacuum on).
    50Hz: 600 pulses in bursts of 3 stimuli at 20 ms intervals (50 Hz), with bursts repeated at 200 ms intervals (5 Hz).
    30Hz: 600 pulses in bursts of 3 stimuli at 33.3 ms intervals (30 Hz), with bursts repeated at 167 ms intervals (6 Hz).
11. Immediately switch to single pulse machine: **Use CONFIG1 for all.**
    0min: Record RC (same as baseline).
    Brake 3,30 min.
    5min: Record one 1mv block.
    Brake 8,15 min.
    15min: Record one 1mv block.
    Brake 8,30 min.
    25min: Record one 1mv block.
    Brake 3,30min
    30min: Record RC (same as baseline).
    Brake 3,30min
    35min: Record one 1mv block.
    Brake 8,15 min.
    45min: Record one 1mv block.
    Brake 8,15 min.
    55min: Record one 1mv block
    Brake 8,15 min.
    60min: Record RC
    Brake 3,30 min.

Middle sessions:

1. Put EEG cap on (32 channels out of 64).
   DC recording (DC MR+ AMP), 1000Hz low pass filter.
   Sampling rate 5000Hz
   Put EMG on FDI bely-tendon montage (disposable electrodes)

20Hz low-cut / 2000 high-cut
5000hz sampling rate

2. Find Hot-spot single pulse machine/alpha coil. **CONFIG2**
   Raw search 5 meps at 2cm apart locations, find response and place the 5x5 grid on that location.
   Find Hot-spot whithin grid in less than 50meps. Mark it.
   From here till the end, the subject will stare at a fixation cross (specially during 1mv blocks and brakes)
3. Find RMT. **CONFIG2**
4. Do Recruitment Curve at 90, 100, 140 and 150% of RMT, 5 trials per intensity = 20trials(7s ISI +-10% jitter). **CONFIG1**
5. Find 1mv intensity (5 trials per intensity until 1mv+-250microvolts in average found). **CONFIG2**
6. Record 1 baseline block of 20 trials (7s ISI +-10% jitter). **CONFIG1**
7. Switch to repetitive machine:
   Use Air cooled coil for finding RMT, use 80% for TBS. **CONFIG2**
   Deliver TBS (50Hz or 30Hz randomized) with Air cooled coil (no vacuum on).
   50Hz: 600 pulses in bursts of 3 stimuli at 20 ms intervals (50 Hz), with bursts repeated at 200 ms intervals (5 Hz).
   30Hz: 600 pulses in bursts of 3 stimuli at 33.3 ms intervals (30 Hz), with bursts repeated at 167 ms intervals (6 Hz).
8. Immediately switch to single pulse machine: **Use CONFIG1 for all.**
   0min: Record RC (same as baseline).
   Brake 3,30 min.
   5min: Record one 1mv block.
   Brake 8,15 min.
   15min: Record one 1mv block.
   Brake 8,30 min.
   25min: Record one 1mv block.
   Brake 3,30min
   30min: Record RC (same as baseline).
   Brake 3,30min
   35min: Record one 1mv block.
   Brake 8,15 min.
   45min: Record one 1mv block.
   Brake 8,15 min.
   55min: Record one 1mv block
   Brake 8,15 min.
   60min: Record RC
   Brake 3,30 min.