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PHASE DEPENDENT MOTOR EXCITABILITY TMS APPLIED IN DIFFERENT tACS PHASES

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Phase Dependent Motor Excitability
TMS applied in different tACS phases

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Dedicated to my Parents and my Grandma Rosa.

Acknowledgements

"We've begun to blur the boundaries between humans and devices, and that will lead to a profound clinical effect for people with physical disabilities."

Hugh Herr

Abstract (EN)

Master of Science

Phase Dependent Motor Excitability:
TMS applied in different tACS phases

by Sandro Norim

Background: Non-invasive brain stimulation has proven to modulate brain activity as well as motor excitability. Experimental results depend on several parameters, such as time after a given event, stimulation intensities or frequency. Stimulation effects depend on the precise timing the stimulus are applied in a so called Brain State Dependent Stimulation (BSDS).

Objective: Observe the excitability changes in the stimulus response curves (SRCs) of a transcranial magnetic stimulation (TMS) intensities set, in which the pulse is triggered at determined wave phase. This study aims to contribute for a solution to neurological disorders, in this case, motor related disorders.

Methods: A 20 Hz transcranial alternate current stimulation (tACS), was applied over primary motor cortex (M1) to increase the phase stability of the sensorimotor β rhythms (13 to 30 Hz). TMS was applied at four different phases of a 20 Hz wave signal. The motor evoked potentials (MEPs) are recorded from the extensor digitorum communis (EDC) and the parameters of the SRC's were analysed for each phase and the state pre and post intervention.

Results: Although no significant difference between the phases parameters was observed, there was a significant difference between the pre and post SRCs.

Conclusions: The combination of tACS and TMS results in a significant increase of the corticomuscular excitability. Further work should be done to evaluate the contribution of each of the stimulations involved and verify if the potentiation observed in excitability is a long-term potentiation (LTP).

Abstract (PT)

Faculdade de Ciências e Tecnologia
Departamento de Física

Mestre em Ciências

**Excitabilidade Motora Dependente da Fase:
TMS aplicada em diferentes fases de tACS**

por Sandro Norim

Background: A estimulação cerebral não invasiva tem vindo a ser utilizada para modular a atividade cerebral assim como a excitabilidade motora. Os resultados experimentais dependem de vários parâmetros, como o tempo após um dado evento, a intensidade da estimulação ou a frequência. Os efeitos da estimulação dependem do intervalo de tempo em que esta é aplicada após um dado estado neuronal ser atingido numa então chamada, estimulação cerebral dependente do estado (BSDS).

Objetivo: Observar as diferenças na excitabilidade motora através das curvas de estimulo resposta (SRC) duma serie de diferentes intensidades de estimulação magnética transcraniana (TMS), na qual os pulsos foram aplicados em determinadas fases da onda produzida pela estimulação transcraniana de corrente alternada (tACS). Este estudo visa contribuir para solucionar problemas neuronais, neste caso, relacionados com a atividade motora.

Métodos: tACS a 20 Hz, foi aplicada sobre o córtex motor principal (M1) para estabilizar os ritmos sensorimotores β (13 a 30 Hz). TMS foi aplicada em quatro diferentes fases da onda de 20 Hz. Potenciais motores evocados (MEPs) do extensor digitorum communis (EDC) foram registados e curvas estimulo-resposta (SR) foram traçadas de forma a comparar os resultados de cada fase e do estado pré e pós intervenção.

Resultados: Pulsos de TMS aplicados em fases especificas de tACS resultam em diferenças nas SRC's dos diferentes sujeitos. Não foi observada uma differença significativa entre os resultados relativos às fases mas houve uma diferença significante entre as curvas traçadas para o pré e pós intervenção.

Conclusões: A combinação de tACS com TMS resulta num aumento significativo da excitabilidade corticomuscular. Trabalho futuro deverá ser realizado, para avaliar a contribuição de cada uma das estimulações envolvidas assim como verificar se a potenciação observada na excitabilidade é uma potenciação longo prazo (LTP).

Abbreviations

TMS	**T**ranscranial **M**agnetic **S**timulation
tACS	**t**ranscranial **A**lternate **C**urrent **S**timulation
FDI	**F**irst **D**orlsal **I**nterosseous
EDC	**E**xtensor **D**igitorum **C**ommunis
BCI	**B**rain **C**omputer **I**nterface
ISI	**I**nter **S**timuli **I**nterval
RMT	**R**esting **M**otor **T**hreshold
MEP	**M**otor **E**voked **P**otential
FES	**F**unctional **E**lectrical **S**timulation
MI	**M**otor **I**magery
SRC	**S**timulus **R**esponse **C**urve
MRI	**M**agnetic **R**esonance **I**maging

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Chapter 1

Introduction

1.1 Motivation

Non-invasive neurostimulation can modulate neuronal activity and disrupt the natural wave patterns of the brain. There are two main techniques used in this field, transcranial magnetic stimulation (TMS) and trancranial current stimulation (tCS), their capacity of changing brain plasticity[1] is well known and the research for their therapeutic usage has exponentially grown over the last two decades. This techniques are widely used as a research tool to study aspects of human brain physiology including motor function, vision, language and the pathophysiology of brain disorders. It's also used for therapy, for instance in motor related disorders, like the ones provoked by stroke, and in psychiatry, for problems as depression. Though, due to the complexity of the human brain, the existing methods, still require scientists to try novel approaches that adapt the stimulation parameters to individual subjects characteristics.

This introduction is focused in the motor effects of TMS and transcranial alternating current stimulation (tACS) to give the necessary information to the reader

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[1] The brain is able to reshape itself depending on the subject experiences, rebuilding it's connections according to the subject needs or the stimulus of the environment. With brain stimulation it's possible to force the brain to perform this changes.

to understand the protocols and the interpretation of the results. The brain stimulations used in this study are applied in other areas as it was referred in the first paragraph but previous work in this areas is not referred here.

Biomedical engineering is a field of studies that applies engineering concepts to solve health related problems. There is a big demand in the motor rehabilitation field, patients with motor disorders might benefit from neurostimulation studies as this one, where we combine TMS with tACS in a phase dependent approach.

1.2 State of Research

1.2.1 TMS

TMS was firstly brought to public by Anthony Barker (University of Sheffield, UK) in 1985, for the first time it was possible to stimulate neural tissue (cerebral cortex, spinal roots, cranial and peripheral nerves) in a safe, non-invasive and painless way. This technique is based in Faraday's law of induction, this law tells that a time-varying magnetic field, when in interaction with an electrical circuit originates electrical currents by forcing the charges to move inside this circuit, this force is known as, electromotive force. A TMS coil generates a time-varying magnetic field, perpendicular to the coil plane, over the scalp that induces electrical currents, in specific groups of neurons (circuit), which depolarizes them and originates action potentials (fig. 1.1) (Di Lazzaro, 2004). There are different shapes of coils, the round coils that generate a widely distributed field and figure-eight-shaped coils that produce more focal stimulus because they have maximum current at the intersection of the two round elements. Round coils allow bihemispheric stimulation, to do so with 8-figure coils it's necessary one for each hemisphere. TMS can be applied in single pulse, paired pulse or trains of pulses. The pulses can have different amplitudes, different widths and separated by different inter stimulus intervals (ISIs). Motor evoked potentials (MEPs) driven by TMS are
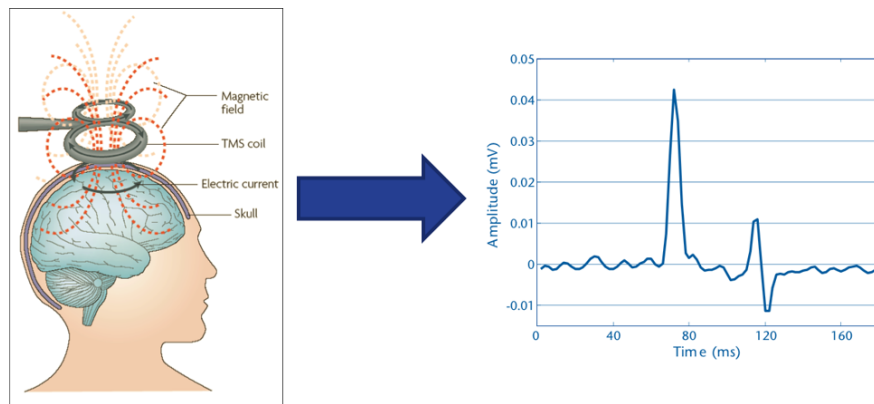
FIGURE 1.1: TMS produces a magnetic field (left image) that produces an action potential called motor evoked potential (MEP). The MEP is recorded by EMG on the target muscle (right image) the first peak in the signal is the TMS artefact and the next peaks describe the MEP.
Sources: Ridding and Rothwell, 2007,p.2 and Butler and Wolf, 2007,p.9

directly related to motor excitability of the target motor pathway. This potentials can be recorded through surface electromyography (EMG). Higher MEPs are recorded if the coil is placed tangentially to the skull with the handle oriented 45° medial to the anterior–posterior plane (fig. 1.2) (Mills, Boniface, and Schubert, 1992). From the EMG data two features are mostly analysed, latency, expressed in milliseconds (ms) and peak-to-peak amplitude, expressed in microvolts (μV). Latency is mostly related to the amount of synapses that are crossed by the signal from the pulse application site (cortex) till the recording site (muscle) and integrity of the white matter fibres, like the diameter and the thickness of myelin sheaths. The peak-to-peak amplitude is measured as the difference between the negative and positive peak, usually detected between 10 to 30 ms after the TMS pulse. Before starting a specific muscle stimulation the experimenter needs to find an area in the cortex defined has the "hotspot" of the muscle, that corresponds to the area that is more strongly connected to the muscle in study. Resting motor threshold (RMT) is defined as the TMS intensity where the reproducibility of 5 MEPs $> 50\mu V$ out of 10 (over the hotspot) by Rossini's method is verified (Rossini et al., 1994; Groppa et al., 2012), this is a reference value considering that after this intensity the chances of obtaining an MEP is higher than 50% and is also the beginning of the slope of the stimulus response curves (SRCs) described in the
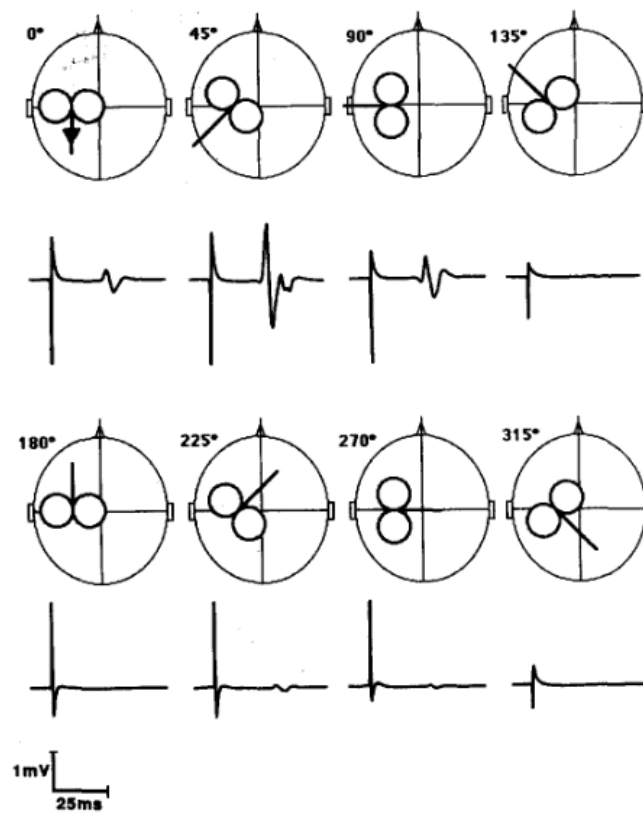
FIGURE 1.2: 45° produces much higher MEPs than the other orientations.
Sources: Mills, Boniface, and Schubert, 1992,p.3
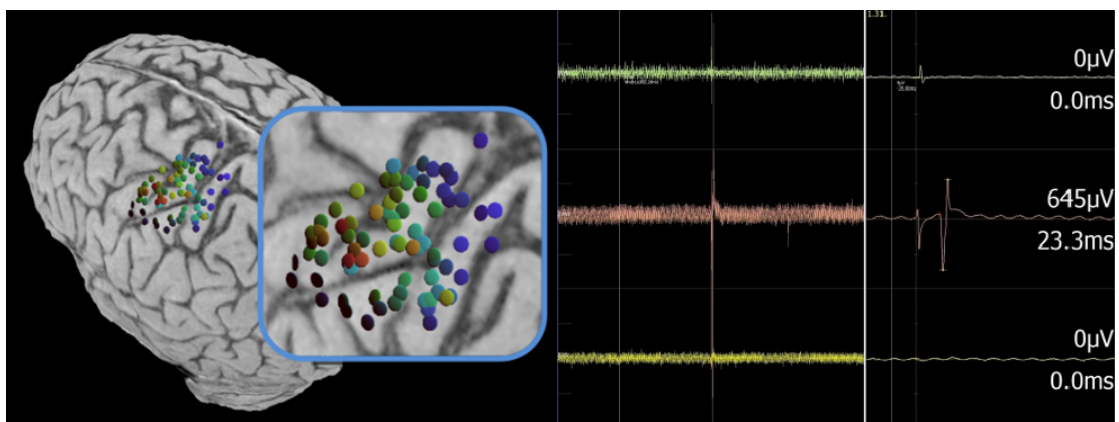
FIGURE 1.3: TMS hotspot finding. Red dots are the ones with better response from the muscle and blue/black dots the ones with worst response (left image). MEPs are analysed for each pulse on a second monitor (right image).
Sources: https://www.ant-neuro.com/show-case/motor-mapping-navigated-transcranial-magnetic-stimulation-0 and Alafaci, Conti, and Tomasello, 2013,p.9

next section. Active motor threshold (aMT) is basically the same as RMT but the subjects are asked to keep an isometric contraction[2] on the target muscle around 20% of their maximum force. A gauge is necessary to check first the maximum force and the for the subject to control the contraction and keep it around 20%. Another difference in relation to RMT is the minimum value of the MEP when applying Rossini's method, instead of $> 50\mu V$ the value has to be $> 200\mu V$ to distinguish the MEP from the EMG background activity due to the subject's contraction. aMT is used as reference to experiments where a isometric contraction is performed during the experiment. One pulse of TMS has an effect of some milliseconds on the target cells while multiple pulses might induce long term potentiation (LTP) represented in figure 1.4 or long term depression (LTD). Single
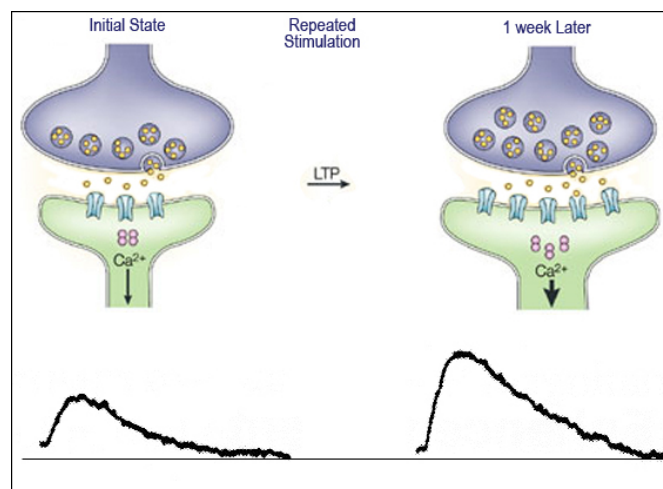
FIGURE 1.4: LTP after stimulation. The amount of neurotransmitters and neuroreceptors increases improving the communication between the two neurons after being stimulated.
Sources: http://www.sciengage.com.au/toying-memory-like-yo-yo/

pulse TMS is used as a probe to check the excitability or integrity of the neuronal tracts (Stinear et al., 2012) and repetitive TMS (rTMS) is used to enhance or inhibit the excitability of the target cells (Maeda et al., 2000). Significant phase resetting occurs at the TMS-targeted area and distant areas, showing that the disruption caused by TMS pulses resets the phase of brain ongoing signals (Kawasaki

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[2]When there is a balance in a muscular contraction between the agonist and antagonist muscles of a given movement (constant contraction). This activates the muscle fibres but keeps their length static.

et al., 2014). Age seems to influence interindividual variability in paired associative stimulation, LTD and LTP effects on plasticity were smaller in elder subjects than in young ones (Müller-Dahlhaus et al., 2008). Motor excitability depends on various factors like age, sex and handedness; this factors were not taken into account for this study due to the limited number of subjects recruited but their information was collected in case the sample of subjects is to be increased, so that group analysis might be done.

1.2.2 Stimulus Response Curves

Stimulus response curves (SRCs) also called input output curves or recruitment curves, describe the motor pathways (Devanne, Lavoie, and Capaday, 1997). To obtain this curves, several stimulus are applied from low to high output powers of the TMS device starting from the power where we can't record any MEP (none or few motor units recruited - minimum contraction) and increasing until we reach a saturation plateau (all the motor units recruited - maximum contraction). The MEPs obtained are averaged for each intensity and fitted to a Boltzmann sigmoid equation. There are three main parameters that we can extract from this curves, "range" that is the value of the upper plateau (or saturation plateau), "threshold" that is the function's inflexion point and "slope" is the function's steepness between the plateaus. "Range" is related with the maximum strength of the corticospinal projection, "threshold" reflects the bias level of the target motorneurons and "slope" reflects the gain. Steepness increases with tonic contraction and threshold decreases suggesting that this two parameters are related to the easiness of motor unit recruitment but they are determined by different neural mechanisms (Devanne, Lavoie, and Capaday, 1997). The turning point from the slope to the upper plateau with a preactivated muscle occurs around 140% of RMT, if the muscle is at rest this turning point occurs around 170% of RMT and these are also the intensities considered to be optimal to perform TMS diagnosis. The turning point intensity evokes MEP's at maximum strength at better comfort level for the subjects (Groppa et al., 2012). SRCs can be acquired using two different

methods ("ramped" and "random" mode), in "ramped" mode the intensities used are sequential (i.e. 50%, 60%, 70%, 80%) and in "random" mode they are shuffled (i.e. 70%, 50%, 80%, 60%) . Random mode was suggested by some authors to avoid increasing MEP's due to a serial order effect (Kazis et al., 2006; Kuijk et al., 2009), though it has been reported that there is no significant difference between them (Pearce, Clark, and Kidgell, 2013).

1.2.3 tACS

tACS is a very recent technique that belongs to the group of the tCSs, among transcranial direct current stimulation (tDCS) and transcranial random noise stimulation (tRNS). This technique can induce sinusoidal patterns in the brain driven by the alternating current between two electrodes (fig. 1.5) exerting behavioural changes in perceptual, motor and cognitive tasks (Feurra, Pasqualetti, et al., 2013). Through the induction of a low current electric field over a target area of the cor-
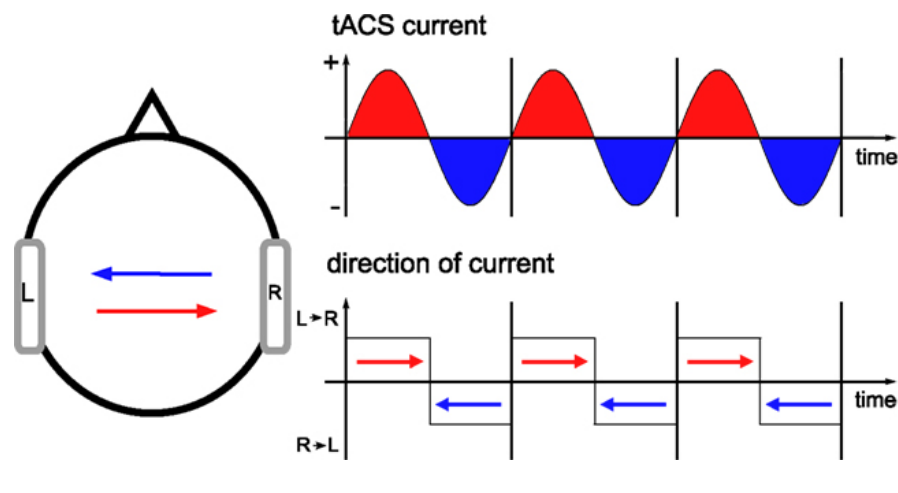
FIGURE 1.5: Current flow during tACS.
Sources: Neuling et al., 2012,p.8

tex, tACS drives the ongoing brain activity and forces the rhythmic brain patterns to synchronize on a given frequency (fig. 1.6), this is often referred as brainwave entrainment[3]. This entrainment can be registered even 10 min after stimulation

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[3]When oscillators (neurons) are perturbed by an external force (tACS) and this force is periodic the oscillator might become synchronized in the same period and also in the same phase after some time of interaction.

has been ceased (Zaehle, Rach, and Herrmann, 2010), leading to the idea that tACS over M1 allows the increasing of the phase stability of the sensorimotor rhythms and because of this effect it's possible to target different phases of the induced tACS waveform with TMS pulses. The time needed for the tACS to entrain the ongoing activity of the brain can be achieved in few seconds but this time is dependent from the initial phase (onset phase) of the signal applied (Ali, Sellers, and Frohlich, 2013) having a faster entrainment when the initial phase is set to π rad. From 0 to π rad the entrainment time increases and for phases starting close to π till 2π rad the time of entrainment is shorter than in the previous interval increasing from π to 2π. Furthermore entrainment of tACS has been proved by invasive and non-invasive studies (Ali, Sellers, and Frohlich, 2013; Helfrich et al., 2014). Stimulation at $1mA$ intensity increases the MEP amplitude and intensities
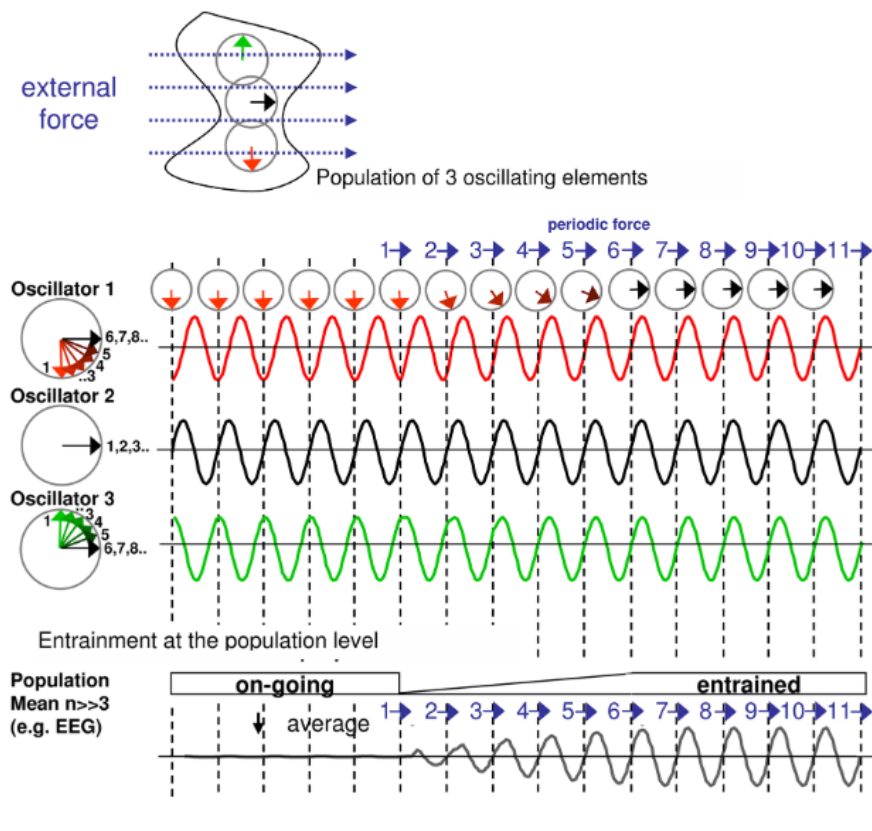
FIGURE 1.6: Groups of neurons (oscillators) are driven by an external force (tACS) to synchronize in the same period and phase after some time of interaction.
Sources: Thut, Schyns, and Gross, 2011,p.3

lower than $0.4mA$ provoke inhibition of the motor cortex. Intensities of 0.6 and

$0.8mA$ didn't have any effect (Moliadze et al., 2012). These data suggest that for intensities lower than $0.4mA$ the inhibitory neurons are more active and above $1mA$ excitatory neurons are favoured, between these two intensities the neuronal activity is equal so they cancel each others effects.

1.2.4 The Beta Band

The β band ($13 - 30Hz$ spiking at $20Hz$) of the brain waves frequencies is considered to transfer the information between the cortex and the muscle (Stuart N Baker, 2007). tACS at $20Hz$ (β frequency) has increased the MEP of TMS single pulses (Feurra, Bianco, et al., 2011) and slowed down voluntary movement (Pogosyan et al., 2009). A wide range of sensorimotor related studies have been performed showing it's relation with movement: the power of β increases while maintaining posture and decreases during motor initiation and performance (Conway et al., 1995; S. N. Baker, Olivier, and Lemon, 1997; K. J. Miller et al., 2007), motor imagery (MI) (Engel and Fries, 2010; Kilavik et al., 2013) and passive movement (Müller et al., 2003) also cause a decrease in β power (fig. 1.7) also called beta event related desynchronization (β ERD). The phase of oscillations in
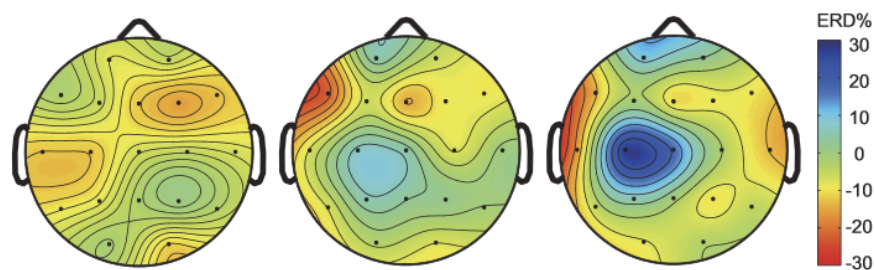
FIGURE 1.7: Progress of an EEG recording during the β ERD.
textitSources: Takemi et al., 2013,p.5

β band was recorded over M1 and has shown to be decisive for cortical computations (Kai J. Miller et al., 2012) and neuromuscular control (Lim et al., 2014), validating a relevance of pre-stimulus phase on TMS effects.

1.2.5 Stimulation effects depend on the brain state

Using tACS we can induce different brain states (Feurra, Pasqualetti, et al., 2013). Studies applying brain state-dependent stimulation (BSDS) have shown either increase or decrease excitability (Gharabaghi et al., 2014; Takemi et al., 2013). The difficulties of predicting the right timing for the stimulus application is within a very narrow time window, since the state of the brain changes very fast and a difference of few milliseconds, can provoke completely opposite effects (Gharabaghi, 2015; Devanne, Degardin, et al., 2009). Therefore is necessary to have an accurate control on the timings of stimulation.

1.3 Goals and Outline

This present work came out with the hypothesis that cortical excitability might be phase dependent. To test this two non-invasive stimulation methods were used: tACS was applied to entrain and stabilize the phase of the brain waves and TMS as a probe to check the neuromuscular excitability at four different phases of a $20Hz$ signal. Like this we can predict the brain state and stimulate in the pretended phase.

The ISI of the TMS pulse was set to 4.5 seconds to avoid frequency specific effects and once TMS leads to phase resetting it was necessary to have a large ISI to let tACS entrain the ongoing brain activity between TMS pulses.

Two experiments were done to complete this work the first one had too noisy data so changes were made in order to have better quality data. Both are reported so the reader can have a notion of the difficulties faced in this kind of protocols and how they were overcome. The main differences between them were:

- TMS system used (because of logistic reasons)

- muscle used to record the MEPs (to get more stable MEPs)

- placement of the EEG electrodes for the TMS artefact detection (due to noise problems)

- set of intensities (because of exponential SRCs problems - plateau was not reached in protocol I and neither the script for sigmoid fitting could predict it)

Due to safety measures, tDCS should not be applied to the subjects for more than 20 consecutive minutes (Bikson, Datta, and Elwassif, 2009). For this tACS protocol it was followed the same principle and also to avoid secondary neurosensory effects (Raco et al., 2014). Hence the reduced range of TMS intensities, which revealed to be problematic for the SRCs fitting because the saturation plateau wasn't always clear, this resulted in exponential fittings and measurements with exponential fittings were discarded.

Chapter 2

Protocol I: Materials and Methods

2.1 Participants

Nineteen subjects were recruited for this first study who had no history of psychiatric or neurological conditions, participated after giving a written informed consent (Appendix A), a form to check for contraindications to brain stimulation (Appendix B) and there was a monetary compensation according to the time of the procedure. The study was approved by the local ethics committee.

2.2 EMG recording

EMG data were recorded and digitized with a BrainAmpExG-Amplifier (Brainproducts GmbH, Germany) with 5 kHz sampling rate using a customized MATLAB (MathWorks, Natick, MA) code. EMG activity was recorded using surface Ag/AgCl adhesive electrodes (Neuroline 7200-S/25, Ambu/Medicotest, Denmark). The EMG electrodes were placed at the right first dorsal interosseous (FDI) with

the belly-tendon technique and the ground on the ulnar close to the humeroul-nar joint (Fig. 2.1). To lower the impedance between the skin where the electrodes were placed was used abrasive gel (Nuprep Skin Prep Gel) and ethanol, the impedance was checked using BrainVision software (Brainproducts GmbH, Germany) and kept $< 20k\Omega$.

FIGURE 2.1: EMG Setup for FDI MEP measurement

2.3 TMS

Participants were seated in a comfortable reclining chair for the duration of the mapping and the following intervention. During all TMS measurements, participants were requested to keep their muscles relaxed. A focal single-pulse TMS was applied over the FDI area of the left hemisphere M1 through a figure-of-eight coil ($17.3cm$) connected to an eXimia 3.2 magnetic stimulator (Nexstim, Helsinki, Finland). The coil was placed tangentially to the skull with the handle oriented 45° medial to the anterior–posterior plane. The FDI hotspot was defined as the point where the TMS pulse has maximal amplitude and minimal latency in the MEP. The points were acquired in a pseudo-random way. This point was marked on a standard template of a brain MRI and was used for pseudo-navigation using the eXimia NBS System 3.2 (Nexstim, Helsinki, Finland). RMT was calculated as the intensity that allowed the reproducibility of MEP >50 μV in 5 out of 10 times.

2.4 tACS

Experiments were conducted using a multi-channel transcranial AC stimulator (NeuroConn, Ilmenau, Germany), a ring rubber electrode (internal diameter $2.5cm$ external diameter $5cm$) and a second rectangular electrode ($5x6cm$) (Fig. 2.2). The round ("active") electrode was positioned around the hotspot area and the rectangular ("passive") electrode over Pz according to the International 10-20 EEG System. The electrodes surfaces that were in contact with the scalp have been covered first, with Ten20 conductive paste and then with electrolyte gel. More gel was added in case the impedance was still high, impedance was kept below $5k\Omega$. During the whole duration of each run a tACS block at $20Hz$ ($1mA$) was delivered to the subject.
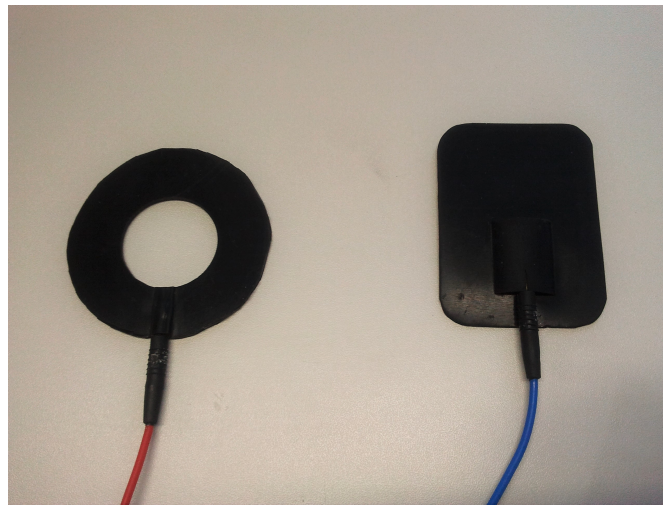
FIGURE 2.2: tACS electrodes: on the left the active electrode that was positioned above the hotspot and on the right the passive electrode placed over Pz.

2.5   Experimental Protocol

Six TMS intensities were set as the 90% to 140% (in 10% steps) of the RMT. During the experiment tACS was turned on and each of the six intensities represented a separate run whose order was randomized (SRC "random" mode acquisition). The duration of each run was around $3.5min$ and the total time of tACS applied was around $21min$. TMS pulses were triggered at one of 4 target tACS phases (maxima, falling flank, minima and rising flank), representing the four experimental conditions (Fig. 2.3). Each condition was repeated 10 times
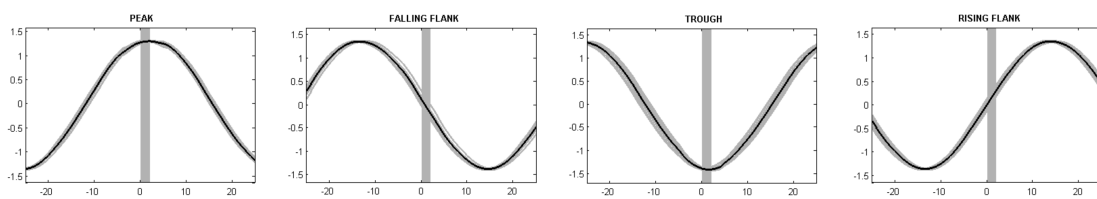
FIGURE 2.3: Recorded tACS waveform epoched and centred around the time of the TMS pulses (grey vertical patch). The gray sinuses represent the single waveforms and the thick black line is the mean across the whole run for each condition. The yy axis is in arbitrary units and xx axis is the time of one period $(50ms)$

and the full sequence randomized across the whole run (40 pulses per intensity). The interval between two consecutive TMS pulses is set as 4.5 seconds with a jitter of 0.05 seconds to limit frequency specific effects of the TMS stimulation. The TMS trigger is synchronized with the tACS signal with the help of a MATLAB script that calculates the time difference (Δt) between a test pulse and the tACS maximum just before this pulse (Fig. 2.4). Once the signal is periodic ($Period = 1/20Hz = 0.05s$) the script will always know when the next maximum will take place ($NextMax = 0.05s - \Delta t$) and also all the other phases (incrementing with $0.05s/4 = 0.0125s$).
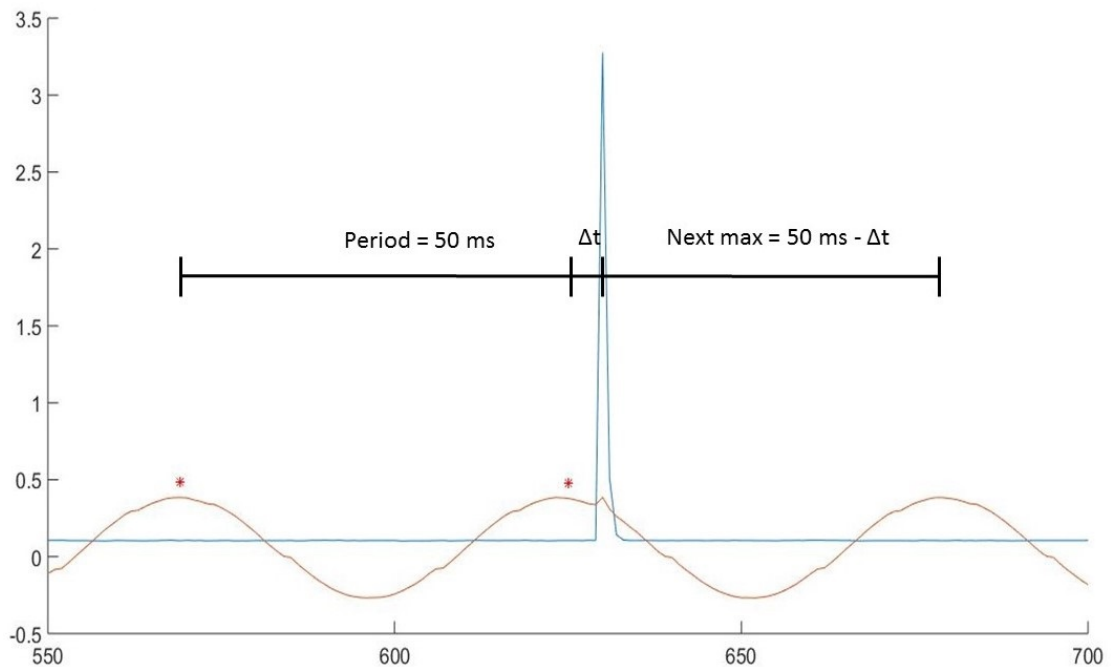
FIGURE 2.4: This figure shows how the time corrections for the TMS-tACS synchronization were calculated. The yy axis is expressed in millivolts and xx axis is in samples at a sampling frequency of $1100Hz$

2.6 Experimental Setup

The experimental setup is represented in figure 2.5. The tACS stimulator is connected to a splitter box, that splits the tACS signal in two pairs of cables, one pair to stimulate the subject's cortex and the other pair to the recording amplifier that sends the signals acquired to the computer. The EEG electrodes are placed over the subject's scalp to record the TMS artefact and also connected to the signal amplifier. The controlling computer acquires the tACS sinusoidal signal and the TMS artefact recorded by the EEG electrodes and triggers the TMS system according to the calculations described in the previous section. The EMG electrodes are also connected to the amplifier and the MEP information is stored in the computer.
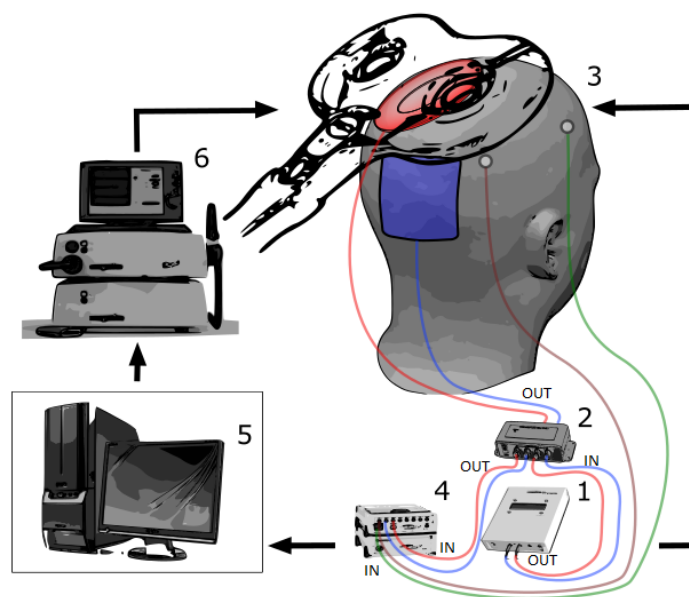
FIGURE 2.5: (1) tACS stimulator, (2) splitter box, (3) EEG (brown and green) and tACS electrodes (red and blue), (4) input-box and signal amplifier, (5) recording computer, (6) TMS system

Chapter 3

Protocol I: Problems and solutions

3.1 Online data quality check

During the experiment some data was plotted in a control screen (Fig. 3.1), this screen was used to check if the target phase was correctly targeted. The script defined which phase was going to be targeted and plotted the tACS signal when the TMS pulse was applied in the corresponding "box" according to the previously defined phase. For example, if there was a descending part of a sinusoidal signal in the maxima "box", the experimenter could observe that there was a mistake on the timing the TMS was triggered because the signal recorded at the same time as the TMS pulse didn't correspond to the targeted phase. For most of the subjects it was not possible to detect the TMS pulse (recorded by the EEG electrodes) and it was not possible to have an accurate phase targeting, so the measurements were discarded. When the EEG electrodes are placed over the scalp they capture the tACS signal and brain signals. The objective was to find the TMS artefact and most of the times it was mixed with other signals that were not of interest, the script used to make the calculations for the trigger timing couldn't trigger the TMS correctly due to this mixture of signals. The impedance registered (at

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BrainVision software) in the EEG electrodes was also many times too high causing saturation on the amplifier and the signal couldn't be properly analysed. In this screen was also visible the EMG signal where the experimenter could check if the signal was correctly acquired and even the MEP was visible if the TMS intensity was enough to evoke it.
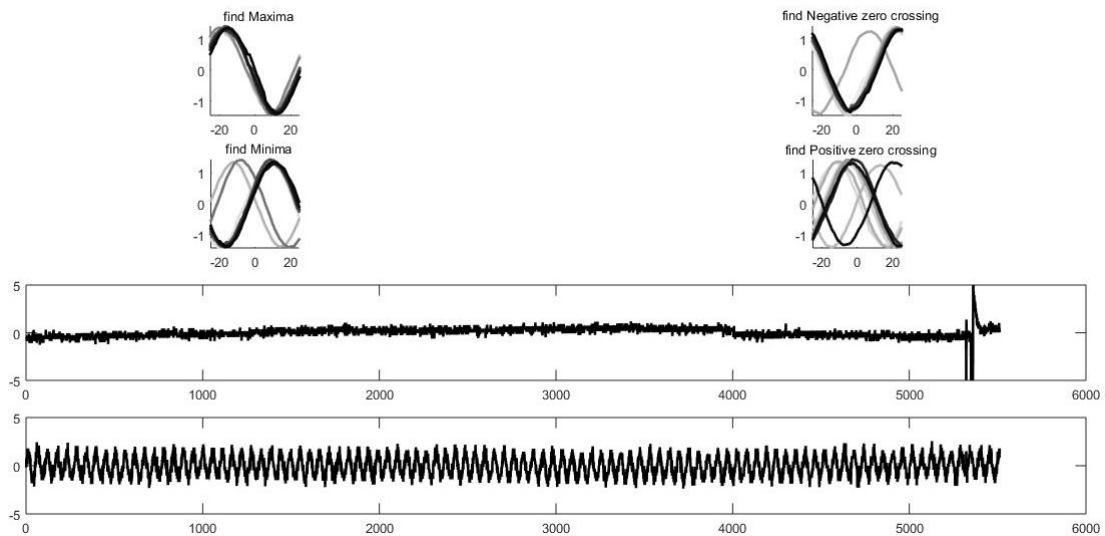
FIGURE 3.1: The four first plots correspond to the four phases that were targeted. Here it is visible that the TMS was not triggered in the right time showing an error at the time TMS was triggered. The first long plot was the EMG signal. The last plot was the signal recorded by the EEG electrodes, in this picture is visible that the tACS signal was strongly affecting the signal. The yy axis is in arbitrary units and the xx axis is a period time for the phases plots, for EMG and TMS xx axis is in samples at a sampling frequency of $1100Hz$.

Solution: EEG electrodes attached to the top of the TMS coil with the surfaces turned to each other. This reduced the impedance between them and there was no other signals registered besides environmental noise and the TMS artefact was precisely detected, as it is shown in chapter 5.

3.2 Exponential SRC's problem

The EMG data was cut into epochs of 1 second length centred on each TMS pulse. For the calculation of the MEP size it was considered the range of the signal from

10 to 30 ms after the TMS artefact in the the EMG signal. The MEP amplitudes were averaged and a Boltzmann sigmoidal function was fitted by a MATLAB script to the results of each condition using the equation:

$$MEPmax(S) = \frac{MEPmax}{1 + e^{\frac{S50-S}{k}}} \tag{3.1}$$

Where MEPmax (Range) is the estimated maximal MEP amplitude, S is the stimulus intensity, S50 (Threshold) is the stimulus intensity required to produce a response equal to half MEPmax and k is the slope parameter (inversely proportional to maximal function steepness). This script also made a prediction of values for the intensities not applied. Many SRC's had an exponential fitting due to the lack of values that could define or predict where the plateau would be (Fig. 3.2). The average for the turning point of the upper plateau is around 170% of RMT with the muscle relaxed. The amplitudes registered till 140% of RMT were only enough to define the lower plateau and the slope but not enough to define the upper plateau.
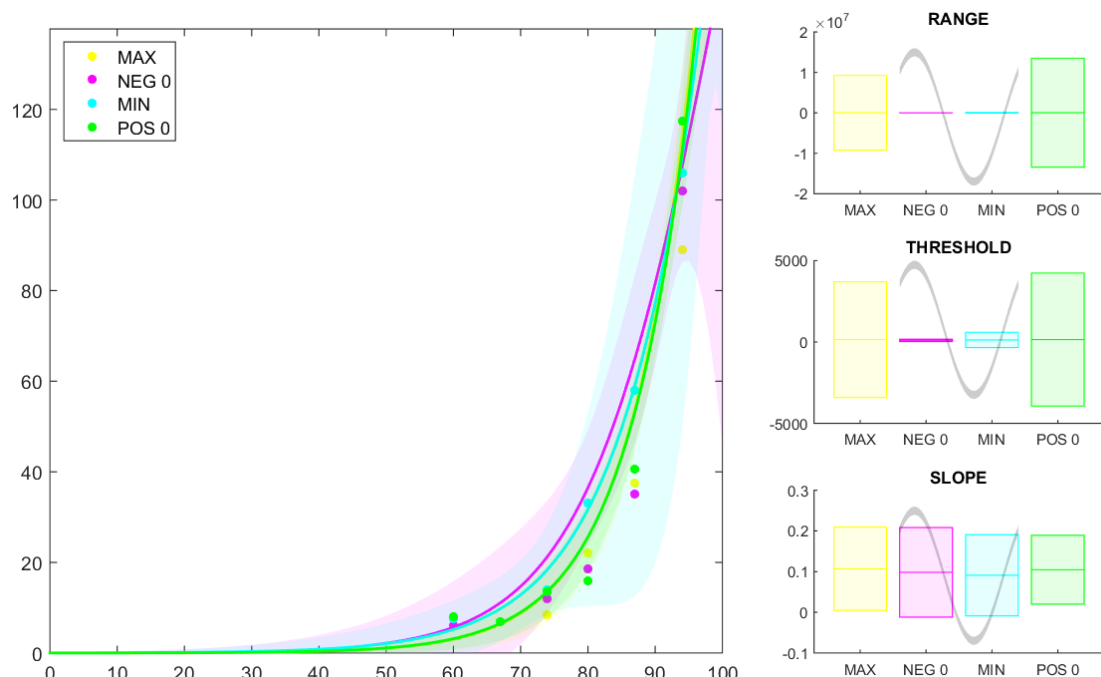
FIGURE 3.2: Exponential SRC's. The yy axis is in microvolts and the xx axis is the percentage of maximum TMS output.

Solution: TMS set of intensities changed to a wider range of intensities. Intensities set was defined to 80%, 100%, 110%, 120%, 140% and 150% so the plateau could be found by the script used to fit the sigmoid function. Although this intensities are not considered to define points after the turning point (170% of RMT for muscles at rest) from the slope to the upper plateau it was enough to do a prediction of this values.

3.3 Functional movement stability

In many subjects the stability of the functional movement in this case the finger pinch was not possible and for high intensities there was contraction of multiple muscles of the arm putting into question the reliability on the MEP's recorded. This multiple contraction might be caused by cross talking of the motor-neurons.

Solution: Target muscle was changed from FDI to EDC. EDC is bigger and a stable functional movement was observed even at high intensities.

Chapter 4

Protocol II: Materials and Methods

4.1 Participants

Twelve subjects were recruited for this second study (5 female and 7 male), 11 right-handed and 1 left-handed according to Edinburgh Handedness Inventory (Appendix C) (Oldfield, 1971), ages ranged from 21 to 30 years old (Avg: 25). Although the age, sex and handedness are reported, they were not taken in account to this protocol but it could be used to separate the subjects in groups for further statistical analysis. The subjects filled a form to check for contraindications to brain stimulation (Appendix B). They did not suffer from neurological or psychological disorders, didn't have metallic implants/implanted electric devices, didn't take any medication nor had a personal or family history of epilepsy. The experiment started after giving a written informed consent (Appendix A) and after the experiment there was a monetary compensation according to the time of the procedure. The study was approved by the local ethics committee.

4.2 EMG recording

EMG data were recorded with a BrainAmpExG-Amplifier (Brainproducts GmbH, Germany) with $5kHz$ sampling rate using a customized MATLAB (MathWorks, Natick, MA) code. EMG activity was recorded using surface Ag/AgCl adhesive electrodes (Neuroline 7200-S/25, Ambu/Medicotest, Denmark). The EMG electrodes were placed over the right extensor digitorum communis (EDC) and the ground on the ulnar close to the humeroulnar joint (Fig. 4.1). To lower the impedance between the skin where the electrodes were placed, abrasive gel (Nuprep Skin Prep Gel) and ethanol were used, the impedance was checked using BrainVision software (Brainproducts GmbH, Germany) and kept $< 20k\Omega$.
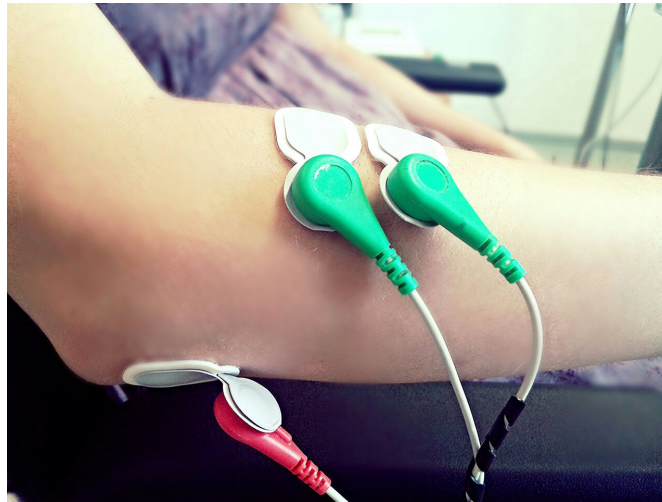

FIGURE 4.1: EMG Setup for EDC MEP measurement

4.3 TMS

Participants were seated in a comfortable reclining chair for the duration of the mapping and the following intervention. During all TMS measurements, participants were requested to keep their muscles relaxed. A focal single-pulse TMS was applied over the FDI area of the left hemisphere M1 using the MagVenture R30

stimulator (MagVenture A/S, Farum, Denmark) and a figure-of-eight coil (MCF-B70, inner diameter $23mm$, outer diameter $96mm$, $2.5kg$). The coil was held by the experimenter tangentially to the skull with the handle oriented $45°$ medial to the anterior–posterior plane. The EDC hotspot was defined as the point where the TMS pulse has maximal amplitude and minimal latency in the MEP. This point was marked on a brain model generated by the navigation software (Localite TMS Navigator, Localite GmbH, Sankt Augustin, Germany) and was used for pseudo-navigation. RMT was calculated as the intensity that allowed the reproducibility of MEP $> 50\mu V$ in 5 out of 10 times.

4.4 tACS

Experiments were conducted using a multi-channel transcranial AC stimulator (NeuroConn, Ilmenau, Germany), a ring rubber electrode (internal diameter $2.5cm$ external diameter $5cm$) and a second rectangular electrode ($5x6cm$) (Fig. 2.2). A pointer provided with the TMS system was used to help finding the right location for the round ("active") electrode, that was positioned around the hotspot area and the rectangular ("passive") electrode over the Pz area according to the International 10-20 EEG System. The electrodes surfaces that were in contact with the scalp have been covered first, with Ten20 conductive paste and then with electrolyte gel. More gel was added in case the impedance was still high, impedance was kept below $5k\Omega$. During the whole duration of each run a tACS block at 20 Hz (1 mA) was delivered to the subject.

4.5 Experimental Protocol

Six TMS intensities were set as the 90%, 100%, 110%, 120%, 140% and 150% of the RMT. The intensities set didn't have a constant increment between intensities due to the limited time for tACS stimulation and it was the strategy found in attempt to find the the SRC's upper plateau. SRC's were acquired without tACS in a

"ramped" mode to check the differences in the excitability before (Pre SRC) and after (Post SRC) the experiment. During the experiment tACS was turned on and each of the six intensities represented a separate run whose order was randomized (SRC "random" mode acquisition). The duration of each run was around $3.5min$ the total time of tACS applied was around $21min$. TMS pulses were triggered at one of 4 target tACS phases (maxima, negative zero crossing, minima and positive zero crossing), representing the four experimental conditions (Fig. 2.3). Each condition was repeated 10 times and the full sequence randomized across the whole run (40 pulses per intensity). The interval between two consecutive TMS pulses is set as 4.5 seconds with a jitter of 0.05 seconds to limit frequency specific effects of the TMS stimulation. The TMS trigger is synchronized with the tACS signal with the help of a MATLAB script that calculates the time difference (Δt) between a test pulse and the tACS maximum just before this pulse (Fig. 4.2). Since the signal is periodic ($Period = 1/20Hz = 0.05s$) the script will always know when the next maximum will take place ($NextMax = 0.05s - \Delta t$) and also all the other phases (incrementing with $0.05s/4 = 0.0125s$).
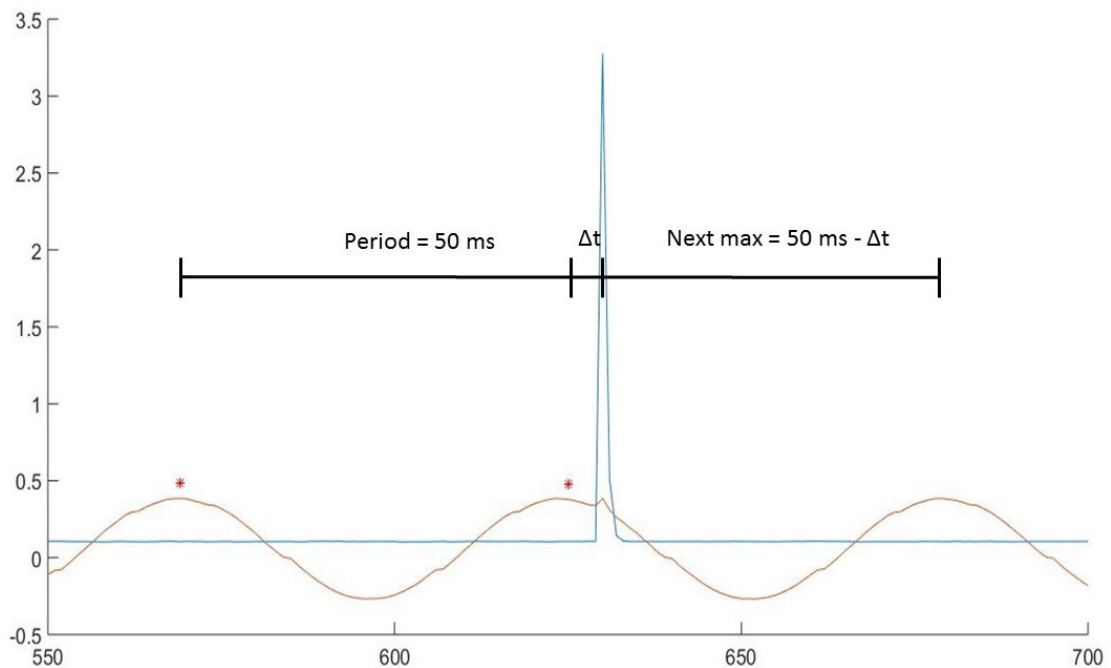

FIGURE 4.2: This figure shows how the time corrections for the TMS-tACS synchronization were calculated. The yy axis is expressed in millivolts and xx axis is in samples at a sampling frequency of $1100Hz$

4.6 Experimental Setup

The experimental setup is represented in figure 4.3. The tACS stimulator is connected to a splitter box, that splits the tACS signal in two pairs of cables, one pair to stimulate the subject's cortex and the other pair to the recording amplifier that sends the signals acquired to the computer. The EEG electrodes are also connected to the signal amplifier, the recording surface was touching each other and they were placed over the TMS coil to record the TMS artefact. The controlling computer acquires the tACS sinusoidal signal and the TMS artefact recorded by the EEG electrodes and triggers the TMS system according to the calculations described in the previous section. The EMG electrodes are also connected to the amplifier and the MEP information is stored in the computer.
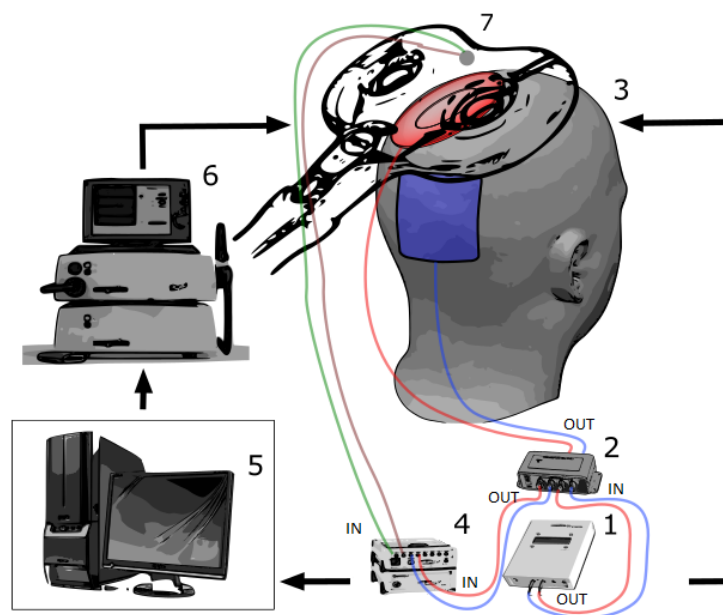

FIGURE 4.3: (1) tACS stimulator, (2) splitter box, (3) tACS electrodes, (4) input-box and signal amplifier, (5) recording computer, (6) TMS system, (7) EEG electrodes attached to the TMS coil

Chapter 5

Protocol II: Results

5.1 Online data analysis

During the experiment some data was plotted in a control screen (Fig. 5.1), this screen was used to check if the target phase was correctly targeted. The script defined which phase was going to be targeted and plotted the tACS signal when the TMS pulse was applied in the corresponding "box" according to the previously defined phase. For example, if there was a descending part of a sinusoidal signal in the maxima "box", the experimenter could observe that there was a mistake on the timing the TMS was triggered because the signal recorded at the same time as the TMS pulse didn't correspond to the targeted phase, like in protocol I. During the observation of the control screen during the experiments it was possible to observe much less noise on the signal of the EEG electrodes and the target phases were found correctly. The impedance registered (at BrainVision software) in the EEG electrodes was much lower than in protocol I and the amplifier channel was never saturated. In this screen was also visible the EMG signal where the experimenter could check if the signal was correctly acquired and even the MEP was visible if the TMS intensity was enough to evoke it.
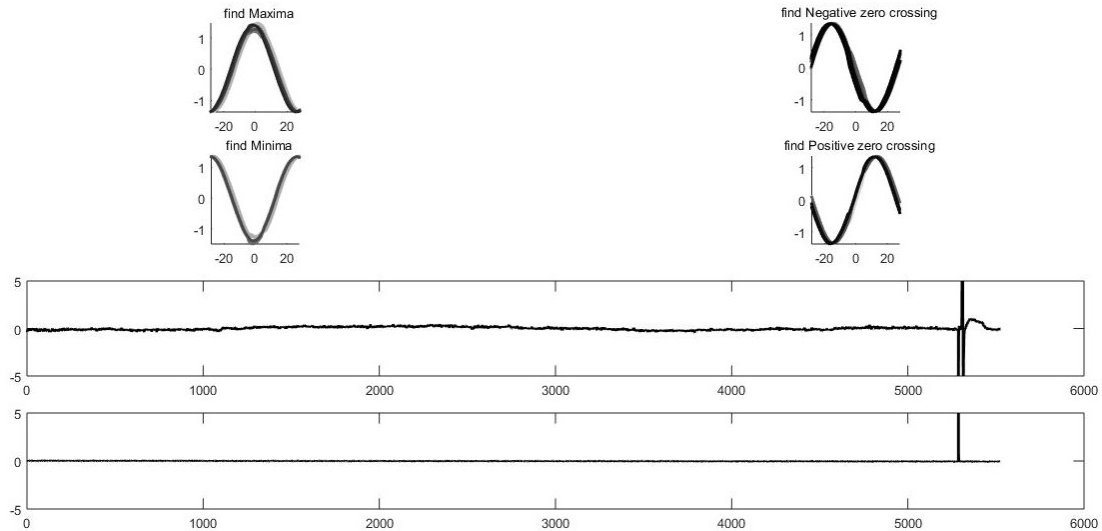
FIGURE 5.1: The control screen showing a good TMS pulse detection and the correct target phases. The yy axis is in arbitrary units and the xx axis is a period time for the phases plots, for EMG and TMS xx axis is in samples at a sampling frequency of $1100Hz$.

5.2 SRC's

The EMG data was cut into epochs of 1 second length centred on each TMS pulse. For the calculation of the MEP size it was considered the range of the signal from 10 to 30 ms after the TMS artefact in the the EMG signal. The MEP amplitudes were averaged and a Boltzmann sigmoidal function was fitted to the results of each condition using the equation:

$$MEPmax(S) = \frac{MEPmax}{1 + e^{\frac{S50-S}{k}}} \tag{5.1}$$

Where MEPmax (Range) is the estimated maximal MEP amplitude, S is the stimulus intensity, S50 (Threshold) is the stimulus intensity required to produce a response equal to half MEPmax and k is the slope parameter (inversely proportional to maximal function steepness). This script also made a prediction of values for the intensities not applied (Fig. 5.2). Subjects with exponential SRC's were rejected from further analysis (Fig. 5.3).
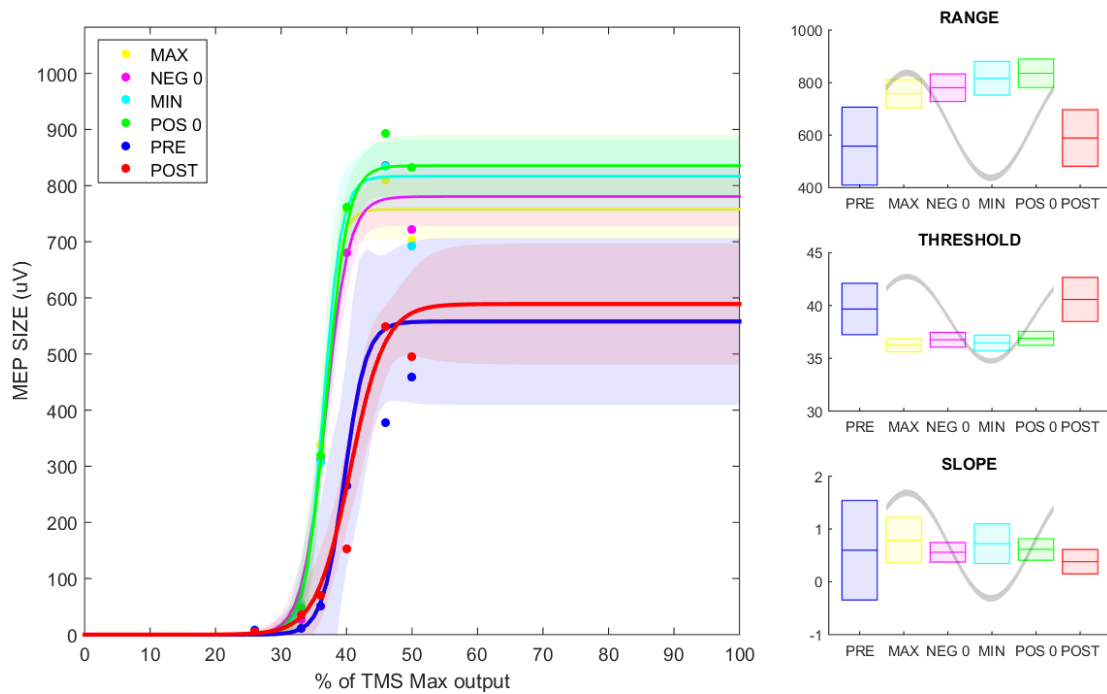
FIGURE 5.2: Single subject SRC's analysis. The yy axis is in microvolts and the xx axis is the percentage of maximum TMS output.
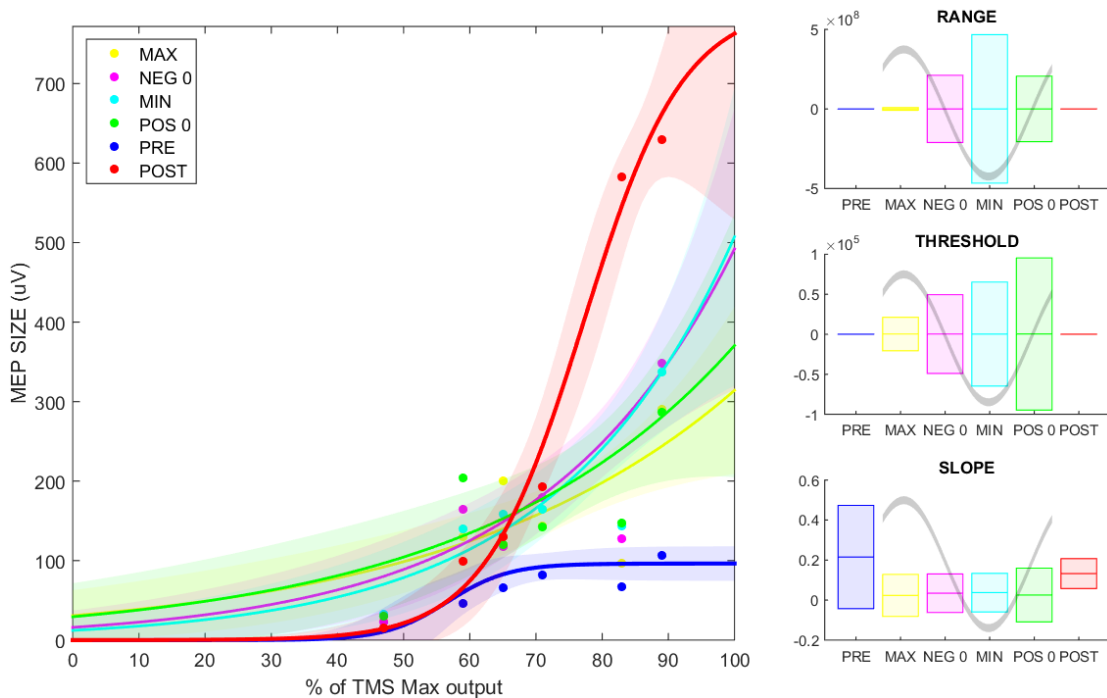
FIGURE 5.3: Exponential single subject SRC's analysis. The yy axis is in microvolts and the xx axis is the percentage of maximum TMS output.

5.3 ANOVA test

An ANOVA test was made to check the variance of each condition (pre,maxima,negative zero cross, minima, positive zero cross and post) within the three SRC's parameters (Figs. 5.4, 5.5, 5.6). The ANOVA test of the range parameter shows significant

FIGURE 5.4: Range ANOVA

FIGURE 5.5: Threshold ANOVA

differences between the pre and post SRC's ($P < 0.0322$). In the ANOVA test of the threshold parameter there is significant differences between the tACS phases

Click on the group you want to test

No groups have population marginal means significantly different from X1=6

FIGURE 5.6: Slope ANOVA

and the post SRC's post-maxima: 0.0066, post-negative zero: $P < 0.0003$, post-minima: $P < 0.0004$, post-positive zero: $P < 0.0047$. For the slope parameter it was not found any significant difference between the conditions.

5.4 Discussion

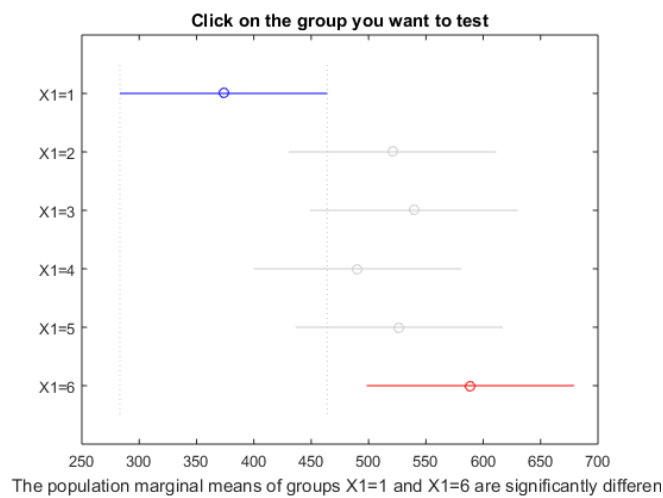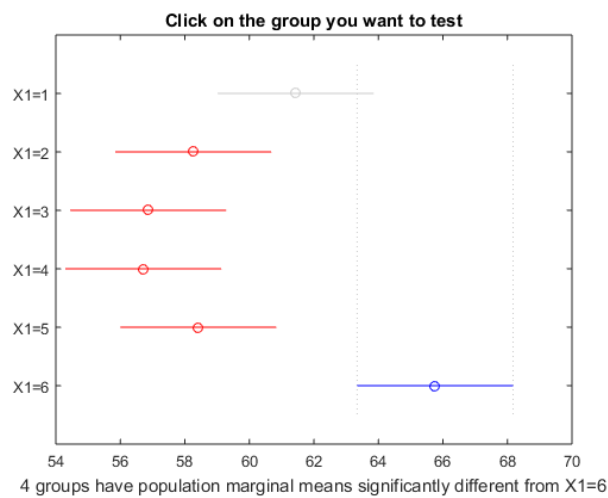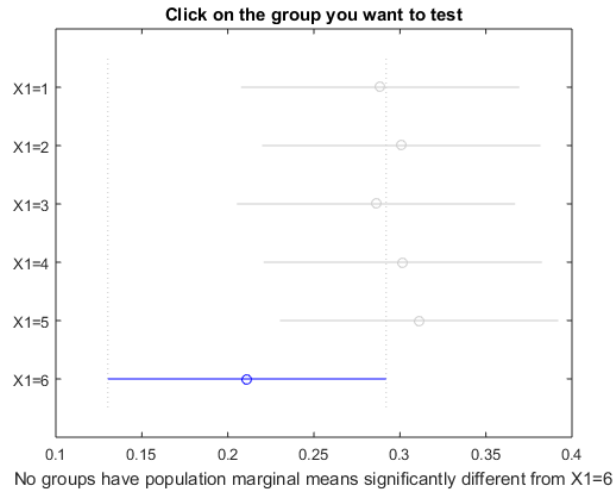The significance observed in the range parameter between pre and post SRC's and the less significant but still noticeable increase of the the maximum MEP's values during tACS confirms that $20Hz$ tACS at $1mA$ increases the power of the MEP's during tACS (Feurra, Bianco, et al., 2011;Feurra, Pasqualetti, et al., 2013) and had an even more noticeable increase after the intervention, revealing that the stimulation applied strengthened the corticomuscular communication. This would be interesting for a clinical application if the increase after the experiment is kept during time enough to be considered a LTP of the corticomuscular pathways. In this protocol it was not recorded more SRC's after the one that was taken immediately after the end of the tACS. There was an immediate effect in the strengthening of the communication between the cortex and the target muscle but it's not possible to state that this is a long term effect also because the effects of tACS are kept for several minutes after the intervention (Zaehle, Rach, and

Herrmann, 2010).

The significance observed in the threshold parameter shows that during tACS ($20Hz$ @ $1mA$) is facilitated the excitability of the corticospinal components revealing that more motor units are recruited at lower intensities.

In the slope parameter there was no significant results and once slope parameter is related to the gain of the MEP's in this experiment there wasn't a factor that could add this gain like in experiments with active contraction while TMS is applied where somehow there is an offset to obtain the MEP's.

Chapter 6

Conclusion and future directions

The increase in post intervention maximum excitability revealed to be significant and it might be of clinical interest. In this study it was combined two kinds of stimulation and might be interesting to make further experiments that could distinguish which stimulation is the one that contributed more to this result or it might be that only their combination reproduces such results. The reduced threshold parameter during tACS suggests that stimulation with 20Hz at 1 mA positively influences motor excitability facilitating the recruitment motor units for muscular contraction. The results show once more the relation of β band in corticomuscular communication. There was not foreseen in this protocol a period after the intervention in which SCR's were recorded periodically to check if LTP was achieved or if it was just a short lasting effect.

The lack of significant results in the phase parameters, doesn't mean that further experiments shouldn't be executed, there are several parameters that can be adjusted, like changing the time between the TMS ISI's applied, different set of TMS intensities, other tACS frequencies, intensity, montage, add control conditions (Herrmann et al., 2013) or reduction of the relative movement between the head of the subject and the coil during the runs, with a chin rest or other kind of apparatus, in a way that MEPs amplitudes get more stable. An important change to this protocol could also be to ask the subjects to keep an active contraction so that the turning point for the upper plateau could be achieved at 140% of RMT

and the SRC's would be more reliable. The force would have to be the same during the whole intervention and a gauge to measure the force would be required; in this case there could be also a limitation due to possible muscular fatigue of the subject.

Brain stimulation experiments are very susceptible to human errors, parameters as the angle and perfect position of the coil is not easy to achieve even with navigated stimulation. Involuntary movements provoked by the subject (i.e. respiration or reaction to muscle twitches caused by stimulation) and small movements from the experimenter while holding the coil changes the angle or the distance of stimulation and might stimulate neurons in the neighbourhood of the marked "hotspot" resulting in high variability of the MEP's. Even with navigated TMS, MEP variability doesn't have a significant decrease and it might be influenced by neurophysiologic factors (Jung et al., 2010). A rigid support as a mechanical arm can be used to perform TMS experiments but if the subject makes a slight movement with the head the problem is not solved once the arm will stay static, while if you have an experimenter holding the coil, the experimenter can always adjust as good as possible the position with the help from the navigation system. Robotic TMS could be a solution to reduce the margin of human error (fig. 6.1) in this protocols and achieve more reliable results (Richter et al., 2013) but is always conditioned by the budget of each research institution.
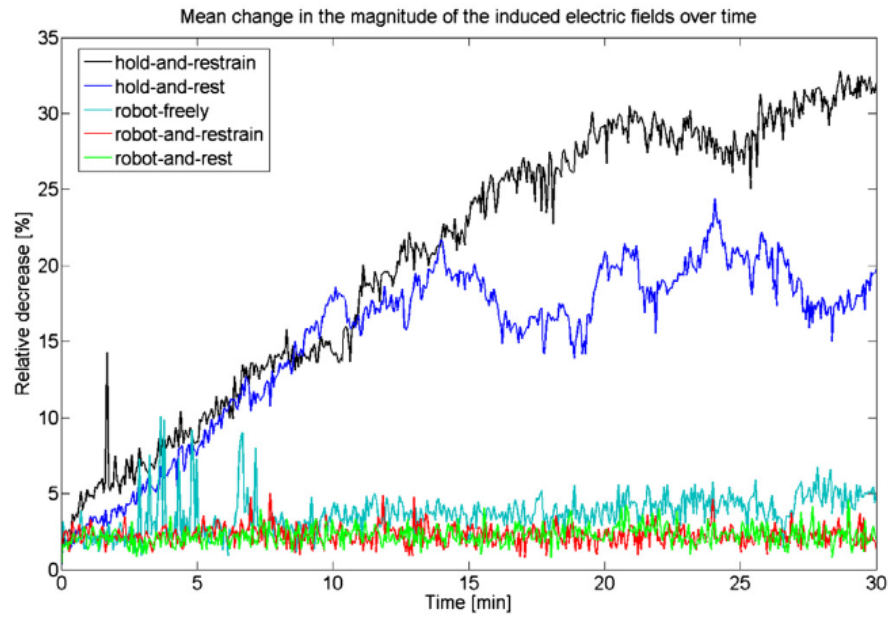
FIGURE 6.1: Human error induces high variability in the Magnetic fields produced by TMS. Here is possible to see that robotic TMS in much more stable. *Source:* Richter et al., 2013,p.5

Appendix A

Written Consent

UNIVERSITÄTS KLINIKUM TÜBINGEN

Klinik für Neurochirurgie

Clinics for Neurochirurgie • Hoppe-Seyler-Str. 3 • 72076 Tübingen

Neurochirurgie
Uniklinik Tübingen

**Motor Effects of Transcranial Alternating Current
Stimulation**

Medical Director:
Prof. Dr. med. Marcos Tatagiba

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special consultations:
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Private consultations:
            07071/ 29-8 03 25
Occupancy management:
            07071/ 29-8 36 23
Normal station:
Station 24   07071/ 29-8 20 55
Station 27   07071/ 29-8 66 54
Station 42   07071/ 29-8 55 53

Intensive care unit:
Station 23   07071/ 29-8 58 48

Consent form

Sir/Madam Dr. _____ explained the possibile
benefits and the existing risks of the above mentioned study. I do not have any
additional questions and hereby accept the presented treatment.   This is to
cerfify that I have given all the information about my medical history truthfully.

Sir/Madam Dr. _____ explained the handling of the
collected data. I do not have any additional questions and hereby accept the
terms. I understand that the data does not contain any personal information
and thereby cannot be traced back to me.

I know that I may decide to stop being part of the study at any time without any
repercussions.

Tübingen, at _____

_____

Name of the expimenter and signature

Tübingen, at _____

_____

Name of particepant and signature

Seite 1 von 1

Appendix B

TMS Assessment

Assessment for TMS_tACS experiment

Before you are able to take part in our TMS experiment, we need to make sure that it is safe for you to do so. For this purpose, we need information about the possible factors that would enhance the risk for you to experience unintended side effects. Please fill out the questionnaire carefully and honestly.

Personal Information

Surname,Name:	
Date of birth:	
Gender:	☐ male ☐ female
Adress:	
Emailadress:	
Phone number:	
Handedness:	☐ left ☐ right

Short Anamnesis

If you answer 'Yes', please provide an explanation
(What, when, under which circumstances, and if applicable where).

1. Are you currently suffering from any medical conditions?
No ☐ | Yes:

2. Do you have any diseases? (High blood pressure, diabetes, etc.)
No ☐ | Yes:

3. Do you suffer from epilepsy, have you ever experienced seizures or does someone in your family suffer from epilepsy?
No ☐ | Yes:

4. Have you ever had a severe head trauma?
No ☐ | Yes:

5. Have you ever lost consciousness without any known reason?
No ☐ | Yes:

6. Have you ever undergone surgery to your spinal column or head
No ☐ | Yes:

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72076 Tübingen

Paraph: _____

1/4

Assessment for TMS_tACS experiment

	7. Do you have any of the following implants in your body?
Nein ☐	☐ Yes (metal) plates and /or screws
	☐ Yes Vascular clips
	☐ Yes Artificial heart valve
	☐ Yes Metallic splinters/shrapnel/etc.
	☐ Yes Pacemaker
	☐ Yes Insulin pump
	☐ Yes Internal hearing aid (cochlear implant)
	☐ Yes Any other implant not mentioned above
	If you answered 'yes' to any of the questions above, Please specify:

	8. Do you have any deviations of the spinal cord, bone marrow, or the ventricular system?
No ☐	Yes:

	9. Have you ever (at present or in the past) had a brain-related, neurological illness?
No ☐	Yes:

	10. Do you have frequent severe headaches? If yes, please describe how often, and on which occasions
No ☐	Yes:

	11. Are you currently under any form of medical treatment?
No ☐	Yes:

	12. Are you currently taking antibiotics (a medication that helps alleviate bacterial infections)?
No ☐	Yes:

	13. Do you ever take antihistamines (anti-allergy medication)? If yes, how often and when was the last time you took them?
No ☐	Yes:

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2/4

Assessment for TMS_tACS experiment

14. Have you used any recreational drugs during the past year (such as marijuna, ecstays, cocaine, etc.)? If yes, how often, which drugs did you use and when was the last time that you used them	
No ☐	Yes:
15. Have you ever suffered from substance dependence or abuse?	
No ☐	Yes:
16. Do you drink alcohol, coffee or do you smoke? If yes, how often?	
No ☐	Yes:
17. Do you have sleeping problems?	
No ☐	Yes:
18. Have you ever undergone a MRI for clinical purpose?	
No ☐	Yes:
19. Have you ever undergone TMS?	
No ☐	Yes:
20. Are you pregnant, or is there a chance that you might be?	
No ☐	Yes ☐

Paraph: _____

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3/4

Assessment for TMS_tACS experiment

Information and explanation

I am aware of the fact that a false statement can influence the quality of the experiment and declare hereby that I have answered all questions to the best of my knowledge and belief.

I was pointed out that all information will be treated confidentially according to the Verschwiegenheitsgebotes (Secrecy commandment) in § 203 StGB.

I am sufficiently informed, have no further questions and agree to conduct the experiment.

Place, Date:	Signature participant:
	Signature experimenter:

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Translationale Neurochirurgie
Otfried-Müller-Str. 45
72076 Tübingen

Paraph: _____

4/4

Appendix C

Edinburgh Handedness Inventory

Edinburgh Handedness Inventory

Surname_____ Given Name_____

Date of Birth_____ Sex_____

       Please indicate your preferences in the use of hands in the following activities by *putting + in the appropriate column*. Where the preference is so strong that you would never try to use the other hand unless absolutely forces to, *put ++*. If any case you are really indifferent put + in both columns.

       Some of the activities require both hands. In these cases the part of the task, or object, for which hand preference is wanted is indicated in brackets.

       Please try to answer all the questions, and only leave a blank if you have no experience at all of the object or task.

	Left	Right
1. Writing		
2. Drawing		
3. Throwing		
4. Scissors		
5. Toothbrush		
6. Knife (without fork)		
7. Spoon		
8. Broom (upper hand)		
9. Striking Match (match)		
10. Opening box (lid)		
i. Which foot do you prefer to kick with?		
ii. Which eye do you use when using only one?		

L.Q.	Leave these spaces blank	DECILE

45

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