Diplomarbeit

Effects of Transcranial Alternating Magnetic Stimulation on Motor Cortex Excitability

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Abbreviations

AC – alternating current
AMPA – α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA – analysis of variance
BDNF – brain derived neurotrophic facor
cAMP – cyclic adenosine monophospate
CREB – cAMP response binding protein
CSP – cortical silent period
DC – direct current
EEG – electroencephalography
EMG – electromyography
EPSP – excitatory postsynaptic potential
FDI – first dorsal interosseus
GABA – γ-amino butyric acid
GPT – Grooved Pegboard Test
ICF – intracortcal facilitation
ISI – interstimulus interval
LTD – long term depression
LTP – long term potentiation
MC – motor cortex
MEP – motor evoked potential
mGluR – metabotropic glutamate receptors
NGF – nerve growth factor
NIBS – non-invasive brain stimulation
NMDA – N-Methyl-D-aspartic acid
NO – nitric oxide
RC – recruitment curve
RM – rotating magnet
rMT – resting motor threshold
rTMS – repetitive transcranial magnetic stimulation
SD – standard deviation
SICI – short-interval cortical inhibition
tACS – transcranial alternating current stimulation
tAMS – transcranial alternating magnetic stimulation
tDCS – transcranial direct current stimulation
TES – transcranial electrical stimulation
TMS – transcranial magnetic stimulation

Zusammenfassung

Zur Beeinflussung der kortikalen Erregbarkeit eigenen sich verschiedene Methoden der nicht-invasiven Hirnstimulation, wie etwa die repetitive transkranielle Magnetstimulation (rTMS), die transkranielle Gleichstromstimulation (tDCS), oder die transkranielle Wechselstromstimulation (tACS). In der vorliegenden Studie wurde untersucht, inwieweit die transkranielle Wechselmagnetstimulation (tAMS) geeignet ist um diese Erregbarkeit zu beeinflussen. Dabei kam ein rotierender Permanentmagnet zum Einsatz der ein 20 Hz Wechselmagnetfeld mit einer Flussdichte von etwa 0,1 Tesla im Bereich des Motorkortex erzeugt. Die Hypothese war, dass die mit der tAMS einhergehende elektromagnetische Induktion stark genug sein sollte um die motorkortikale Erregbarkeit zu modulieren, vergleichbar wie nach einer 20 Hz tACS. Der Test erfolge mittels einer Blindstudie im Cross-over-Design. Es wurden 14 rechtshändige Probanden getestet, die sich drei Interventionen unterzogen (je 15 Minuten tACS, tAMS oder Schein-Stimulation). Vor und nach jeder Intervention wurde die kortikale Erregbarkeit mittels TMS bestimmt. Um dabei eventuelle motorische Lernleistungen festzuhalten, wurde vor und nach jeder Intervention ein Steckbretttest durchgeführt. Die TMS Pulse wurden am Motorkortex appliziert, so dass es zu einem motorisch evozierten Potential (MEP) im ersten Musculus interosseus der rechten Hand kam. Dabei wurden Einzelpulse bei den Intensitäten 110%, 130% und 150% basierend auf der motorischen Ruheschwelle appliziert um die kortikospinale Erregbarkeit und die kortikale Innervationsstille (CSP) zu testen. Zusätzlich wurden Doppelpulse appliziert, um die intrakortikale Hemmung bei kurzem Interstimulus-Intervall (SICI), und um die intrakortikale Bahnung (ICF) zu bestimmen (konditionierte MEP). Infolge der tACS erhöhten sich die Einzelpuls MEP-Amplitudenwerte bei allem Intensitäten (110% $p = 0.001$; 130% $p = 0.000$; 150% $p = 0.001$), es gab jedoch keine Effekte bei den konditionierten MEPs. Infolge der tAMS verringerten sich die MEP-Amplitudenwerte bei 110% und 150% (110% $p = 0.015$; 150% $p = 0.018$), und es kam zu einer Verringerung der konditionierten MEP Amplitudenwerte (SICI $p = 0.011$; ICF $p = 0.002$). Somit wirkt die tAMS inhibierend auf den Motorkortex. Keine der Interventionen zeigte Effekte bei der CSP oder dem Steckbretttest. Hinblicklich unserer Hypothese erzielte die tAMS zwar eine Veränderung in der kortikalen Erregbarkeit, die Richtung dieser Veränderung war jedoch entgegengesetzt zur tACS. Es sind daher weitere Untersuchungen notwendig um die zugrundeliegenden Mechanismen der tAMS aufzuklären.

Abstract

Non-invasive brain stimulation refers to well-known techniques that are able to modulate cortical excitability, such as repetitive transcranial magnetic stimulation (rTMS), transcranial direct current stimulation (tDCS) or transcranial alternating current

stimulation (tACS). For this study transcranial alternating magnetic stimulation (tAMS) was tested in order to evoke modulatory changes in motor cortex. The technique consists of a rotating permanent magnet, able to produce a 20 Hz magnetic field with a strength of 0.1 Tesla in motor cortex. Our hypothesis was that the electromagnetic induction as produced by this kind of tAMS, should be strong enough in order to modulate cortical excitability, similar as with tACS. For our study we applied a sham-controlled, double blind cross-over design. We tested 14 right-handed subjects, who underwent three interventions (15 minutes of tACS, tAMS or sham) and we assessed cortical excitability changes via single and paired pulse TMS in pre-post sessions. Furthermore, we assessed possible changes in motor performance by using the grooved pegboard test (GPT) before and after every treatment. The TMS pulses stimulated the motor cortex which lead to a motor evoked potential MEP in the first dorsal interosseus of the right hand. We recorded single pulse MEPs (at intensities 110%, 130% and 150% of resting motor threshold) to assess corticospinal excitability and cortical silent period (CSP), as well as paired pulse MEPs in order to assess short interval intracortical inhibition (SICI) and intracortical facilitation (ICF). The tACS increased the MEP amplitudes at every intensity level ($p = 0.001$ at 110%, $p = 0.000$ at 130%, $p = 0.001$ at 150%), but showed no effect on the other parameters. The tAMS decreased the MEP amplitudes at intensities of 110% ($p = 0.015$) and 150% ($p = 0.018$), and lead to a significant decrease of conditioned MEP amplitudes in SICI ($p = 0.011$) and ICF ($p = 0.002$). None of the interventions showed significant effects in CSP and GPT performance. Contrary to our hypothesis the tAMS showed a decrease of cortical excitability. Further research will be needed to elucidate the neuronal mechanisms underlying tAMS.

Preamble

Over the last thirty years non-invasive brain stimulation (NIBS) became a versatile tool in human brain research, for neuromodulation, and for therapeutic applications such as treatment of depression. Commonly NIBS employs local magnetic or electrical fields that enter the cranium in order to affect excitable structures within the brain. According to this two NIBS techniques are available, transcranial magnetic stimulation (TMS), and transcranial electrical stimulation (TES).

Commonly TMS utilizes impulse-like magnetic fields in order to induce small electrical currents in the brain tissue. For this purpose a flat coil, powered by short duration current pulses, is operated over the site of interest. Originally TMS was tested over the motor cortex (MC) in order to evoke electromyographic responses in associated hand muscles. Here it was shown that these motor evoked potentials (MEPs) not only reflect the strength of the pulse in the coil, but also the level of excitability in the motor cortex. This opened the door for the assessment of cortical excitability and related changes in cortical plasticity via TMS. Later on, repetitive TMS (rTMS) protocols came up that were also able to induce outlasting neuroplastic effects.

TES utilizes weak electrical currents applied to the head via a pair of electrodes. TES involves via a battery-driven current source, operated for some minutes either in the direct current (DC), or in the alternating current (AC) mode. Here, after application of transcranial direct current stimulation (tDCS), polarity dependent outlasting effects occurred: increased excitability in case the anode was operated over the MC, and decreased excitability in case the cathode was operated over the MC. Also after transcranial alternating current stimulation (tACS) outlasting effects on MC excitability were described. Here the induced effects showed dependent on stimulation amplitude and stimulation frequency.

For this diploma thesis transcranial alternating magnetic stimulation (tAMS) in contrast to tACS will be probed in order to examine whether outlasting changes in MC excitability can be induced. Therefore, a disk-shaped rear earth permanent magnet, constantly rotating at 1200rpm (accordingly to stimulation frequency of 20Hz), will be operated for a period of 15 minutes in close distance to the skull over the MC. To test for MC excitability changes MEPs will be compared pre and post to tAMS/tACS. Changes of MEP amplitudes

at this pre-post design can provide evidence whether tAMS is able to induce outlasting effects that are related to changes in cortical plasticity.

1. Neuronal plasticity

Neuronal plasticity describes the transformation of the human brain by experiences throughout live. It is the basic of learning. This means that, like the brain influences the environment, environment influences the brain. It works by strengthening and also weakening of existing synapses or a cortical "unmasking" of pre-existing synapses that are functional working but are inactive.[1] Because of this fact our brain can grow, develop and adapt to environmental factors. Another important ability is "forgetting". This describes a weakening of synaptic strength, which is necessary when new learning eliminates the need for previously established synaptic modifications.

Nowadays science has focused on motor learning[2,3], which gives important knowledge for therapeutical approaches in neurorehablitation. Neuronal transformation is quite difficult to understand, since many factors play their role in inducing changes in brain areas. The following chapters take a closer look at synaptic plasticity and structural modifications. The synaptic plasticity builds the basis for structural changes.

1.1. Synaptic plasticity

Synaptic plasticity describes the modification of synapses and post- and presynaptic processes. Synaptic plasticity itself can be divided into functional and structural changes.

1.1.1. Functional changes

The signal transmission between two cells runs in the space between the synapse and the postsynaptic cell, the so called synaptic cleft. When it comes to an action potential, Ca^{++} flows into the presynaptic cell and leads to a release of the vesicles, which are filled with transmitters. In an excitatory cell, the transmitter is glutamate. The postsynaptic cell has receptors, on which glutamate can dock. There are two glutamatergic receptors called α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-Methyl-D-aspartic acid (NMDA) receptor. Glutamate binds to both receptors. First the AMPA-receptor channel opens (fast response). Na^{+} and Ca^{++} flows from the synaptic cleft into the postsynaptic cell, which potential then becomes more positive. Only at more positive potential the NMDA-receptor channel can open, because it is coupled on a Mg^{+}-ion. This represents the late response. Through the depolarization the Mg^{+}-ion uncouples from the

channel and the channel opens. This leads to an additional Na^{+}- and Ca^{++}-influx and a second messenger systems is activated, which influences the cell activity.[4,5]

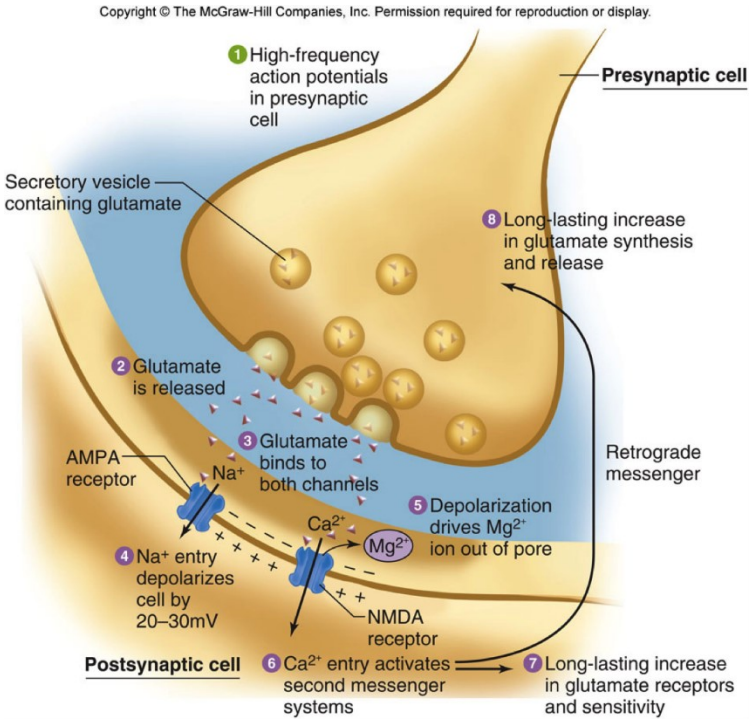

Figure 1 An action potential in a glutamatergic neuron and its long-lasting efects

If the brain learns something new, functional changes between the neurons, in synapses respectively, can be observed.

These include higher expression levels of receptors or transmitters, higher density and sensitivity of receptors, stronger Ca^{+}-release and lower transmitter reuptake and inactivation properties. For example, the second messenger systems, that the Ca^{++} influx activates, can lead to an increased expression of glutamate itself or an increased expression of glutamate receptors or an increase of their sensitivity. This works like a positive feedback.[6]

1.1.1.1. The Hebbian Theory

"cells that wire together, fire together"

The Hebbian Theory describes the development of a synaptic connection in relation to learning and neuronal plasticity. It concerns three elements: Two presynaptic axons (A, B)

and one postsynaptic neuron (C). If it comes to a simultaneous stimulation of B (through A) and C (trough B), it leads to an increase of the synaptic strength between them.[7]
Anyway synaptic connections can become stronger or weaker. In that content we talk about long-term potentiation and long-term depression.

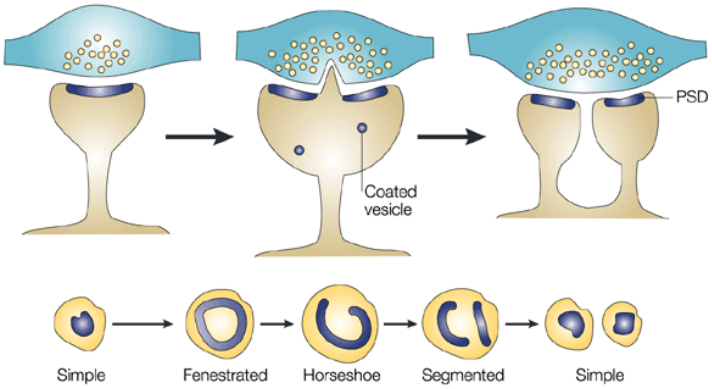

Figure 2 Division of dentritic spines due to LTP.

1.1.1.2. Long term potentiation (LTP)

The long-term potentiation describes the neuronal processes when long-term memory develops.
The mechanism of the long-term potentiation is important for the strengthening between neurons. This happens on the synaptic level and is mostly observed in pyramidal cells of the hippocampus. Most of the synapses are located on dendritic spines. If the AMPA-receptor concentration on one postsynaptic spine (and the glutamate release) increases due to LTP, this spine will divide itself (see figure 2).
This process leads over an addition of receptors to an addition of synapses.

Electrophysiology: A single pulse in an afferent neuron causes the release of neurotransmitters. In the postsynaptic neuron this leads to an excitatory postsynaptic potential (EPSP) with a certain amplitude. A low frequency of such pulses produces an amplitude, which always has the same height.
But if there are two or more impulses in a high frequency (100Hz), each of the EPSPs shows a higher amplitude. This was shown by Bliss and Lomo in 1973 on a hippocampal pyramidal cell.[8] A frequency of 100Hz that lasts for 1 seconds causes an LTP for some

minutes to hours (short-term effect). More trains of 100Hz can even lead to a LTP for hours to weeks (long-term effect).
A presynaptic neuron (neuron A) which is connected to another presynaptic neuron (neuron B) plays an important role in associative learning. If neuron A fires at the same time like neuron B the amplitude between those two increases.[9]

Molecular processes: The mechanism is the same like a single action potential. But when a volley starts, the glutamate concentration in the synaptic cleft increases even more and therefore more AMPA-receptors open. Thereby the postsynaptic neuron depolarizes stronger and this leads to a higher EPSP. Also second messenger systems are activated through the Ca^{++}-influx. There is a complex, called the Ca^{++}-Calmodulin kinase. This is part of a second messenger system and phosphorylates various other enzymes. One of them, for example, is the AMPA-receptor and the phosphorylation leads to an increased open probability.
It can be divided between a short-term and a long-term effect. The short-term effect depends for example on the increase of open probability of AMPA-receptors, the integration of glutamatergic receptors or an activation of the NO-synthase. The NO increases the presynaptic release of transmitters.[6]
The long-term effect is caused by modulation of gene transcription. A very important element is the transcription factor *cAMP responsive-element binding protein (CREB)* located in the nucleus. It is initiated by growth factors, the most important are *NGF* and *BDNF*, and the Ca^{++}-influx. Their activation and subsequently a changed gene transcription leads to cytoarchitectonic changes (s. 1.2.2. structural changes) and is called *sprouting*. [10]
But there is not only growth in the nervous system, also "forgetting" plays an important role in neuroplasticity.

1.1.1.3. Long-term depression

Long-term depression is the opposite of long-term potential. It becomes important when new things are learned and old synaptic connections are needed no more. The mechanism behind it is the removal of AMPA-receptors and this leads to a reduction of the amplitude of the EPSP and a weakening of the synaptic strength, respectively. It is best studied in hippocampal cells.
In contrast to LTP, here low-frequent impulses of the presynaptic cell are found. This activates a special receptor, the *metabotropic glutamate receptors (mGluR)*, which is G-

protein coupled and thereby activates phospholipase C and protein kinase C. This leads to a phosphorylation of AMPA-receptors and they dissolve from the membrane thereby.[9,11]

1.1.2. Structural changes

Structural changes in brain tissue describe bigger morphological variations then on the synaptic level. It is always caused by learning processes. It is based on modification on the synaptic level but this modification is widespread in a brain area.

Another importation process is the so called *sprouting*. This describes a growth of dendrites visible in a dendritic branching and an increase in the quantity of dendritic spines.

Johansson & Belichenko showed in 2002 that environmental enrichment leads to sprouting of neurons in the somatosensory layer II and III in rats that suffered from an infarct and a sprouting in the somatosensory layer II, III, V and VI in healthy rats (see figure 3).[12] They have shown in contrary to the belief that neuronal tissue can't be restored (e. g. after injury), that there are growth processes in the brain.

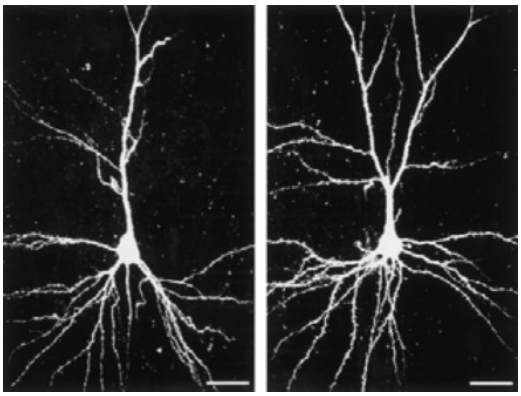

Figure 3 Dendritic morphology of pyramidal neurons in layer III of the somatosensory cortex in a rat housed in standard (left) and enriched (right) environments

The motor cortex lies in the gyrus precentralis. The muscles of the body are represented in this gyrus as a *homunculus* (see figure 4). It is not symmetric to the body relations. Frequently used areas like the hands with their fingers which we use every day are stronger represented than the shank for example.[13]

We can divide between sensory-, motory- and injury-induced plastic changes. In 1990 Jenkins et al. showed that cutaneous stimulation on digits of owl monkeys enlarges the representational region in the motor cortex.[14] But also the human brain reacts on stimulation. A short-term skill learning tasks over some days or weeks including the fingers show first an improvement in performance, second an enlargement of the corresponding area in the motor cortex and third a decrease in activation threshold.[15,16]

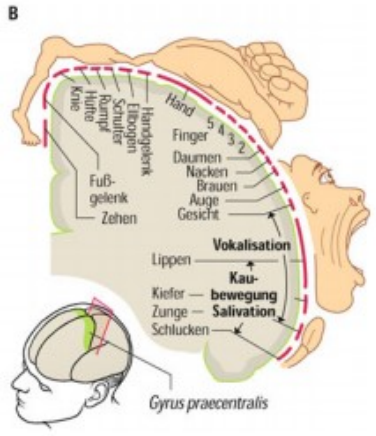

Figure 4 motoric homunculus, frequently used body parts are stronger represented.

Interestingly also mental practice only could show such an effect.[17]
Furthermore, cortical reorganisation can be observed in patients, who suffered from stroke or spinal cord injuries. After a thoracic spinal cord injury the somatosensory region liable to the fingers moved towards the region which normally represents the lower body due to loss of grey matter.[18]
This indicates that the picture of the homunculus isn't fixed, but is flexible and adapts to environmental circumstances, learning and experience.

Nowadays science puts effort in influencing and assessing changes of cortical excitability. Therefore, several ways have been found. One domain in the non-invasive brain stimulation (NIBS), which will be discussed in chapter 3 and 4.
The following chapter describes anatomical and histological conditions that are influenced by current or magnetic flux.

2. Functional organisation of the cerebral cortex

2.1. Anatomic way to the brain

The scalp consists of the Cutis, Subcutis and the Galea aponeurotica. Latter is an aponeurosis between the venter frontalis and venter occipitalis of the musculus occipitofrontalis and is connected to the skin by fibres. The thickness of the scalp is about 5mm.[19,20]

The scalp is connected to the periost of the skull. The skull consists of the Lamina externa, the diploe and the Lamina interna and is about 5mm thick.[21,22] The inner periost is connected to the dura mater which is the outer part of the meninges.

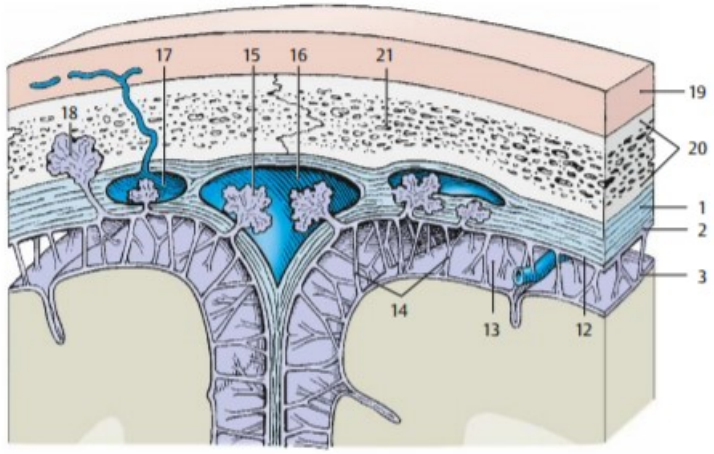

Figure 5 Scheme of the different layers.
19 – Scalp
20 – Scull
21 – Diploe
1 – Dura mater
2 – Arachnoidea
3 – Pia mater
12 – subdural space
13 – Cavum subarachnoidale
14 – trabeculae
15 – arachnoid villi
16/17 - Sinus

The meninges consist of the pachymeninx (dura mater) and the leptomeninx (arachnoidea + pia mater). The arachnoidea lies under the dura mater and between arachnoidea and pia mater there is the subarachnoid space, which contains vessels and liquor between its arachnoid trabeculae. The pia mater lies with a basal lamina directly on the brain and follows its surface. Between the basal lamina and the brain surface the glial limiting membrane is found, which is part of the blood brain barrier.[23]

2.2. Overview of the human brain[24]

The human brain consists broadly of white and grey matter. The grey matter forms the cortex, which is divided into six layers (see below) and is about 2mm to 4mm thick. This doesn't distinguish much from other species, the high performance of the human brain may be explained by the highly convoluted surface, so the number of neurons is much higher.

The brain is broadly divided into 4 parts: the frontal lobe, the parietal lobe, the temporal lobe and the occipital lobe. The grooves on the brain are called *Sulci* and they form the *Gyri*. There are prominent Sulci like the Sulcus lateralis, which divides the frontal from the temporal lobe or Sulcus centralis, which divides the frontal from the parietal lobe. They have quite the same position in every human brain, but there are also smaller Sulci, that differ from person to person.

The areas in the brain follow a specific task and are interconnected with each other to perform an information processing. The information "flow" in the cerebral cortex is hierarchically structured and thus the areas are called primary, secondary or tertiary. A primary area is nearest to the periphery.

The layers in the grey matter vary throughout the brain depending of their function. Typically, there are six layers. The layers of the motor cortex specifically are described below.

2.2.1. Cell Layers of the cerebral cortex

The neurons in the cerebral cortex can be classified regarding their function and/or length of axon. There are pyramidal cells and non-pyramidal cells.

The pyramidal cells have following in common: efferent neurons, triangular cell body with the apex pointing to brain surface, one apical dendrite reaching almost to the surface, basal dendrites, all dendrites with many spines, the axon emanates from the basis, gives away collaterals and reaches into the medullary layer. They mainly occur in layers III, V and VI and their neurotransmitter is the excitatory glutamate.

The non-pyramidal cells are interneurons and are very heterogeneous. They can more or less be found in every layer and their axons don't leave the region. Their function lies in the intracortical information processing and most of them are inhibitory by using the transmitter γ-amino butyric acid (GABA). Some examples are basket cells (axons terminate on cell bodies), chandelier (axons terminate on other axons) cells or martinotti cells.

In staining methods six different layers can be observed:

I. Molecular layer – hardly any cells and many fibres. There are dendrites from deeper cell bodies or axons that pass through or form connections.

II. External granule layer – compact small pyramidal cells and non-pyramidal cells.

III. External pyramidal cell layer – pyramidal cells and non-pyramidal cells. Cells are relatively small and the ones, that lie deeper are usually bigger than superficial ones.

IV. Internal granule layer – compact small pyramidal cells, similar to layer II

V. Internal pyramidal cell layer – pyramidal cells, which are larger than the ones in layer III, few non-pyramidal cells.

VI. Multiform layer – very heterogeneous layer, many modified pyramidal and non-pyramidal cells as well as axons, blurred transition into white matter

The apical dendrite of cells in layer V and VI reaches through layer I-III, the basal dendrites of cells in layer III and IV reach through layer V and VI.

Neurons are not only organized in horizontal layers but also form vertical columns. These form a functional unit and occur throughout the cerebral cortex, where they serve as crucial computing elements. The number of columns mainly differentiates the computing power of our brain from the ones of other animals.[23,24]

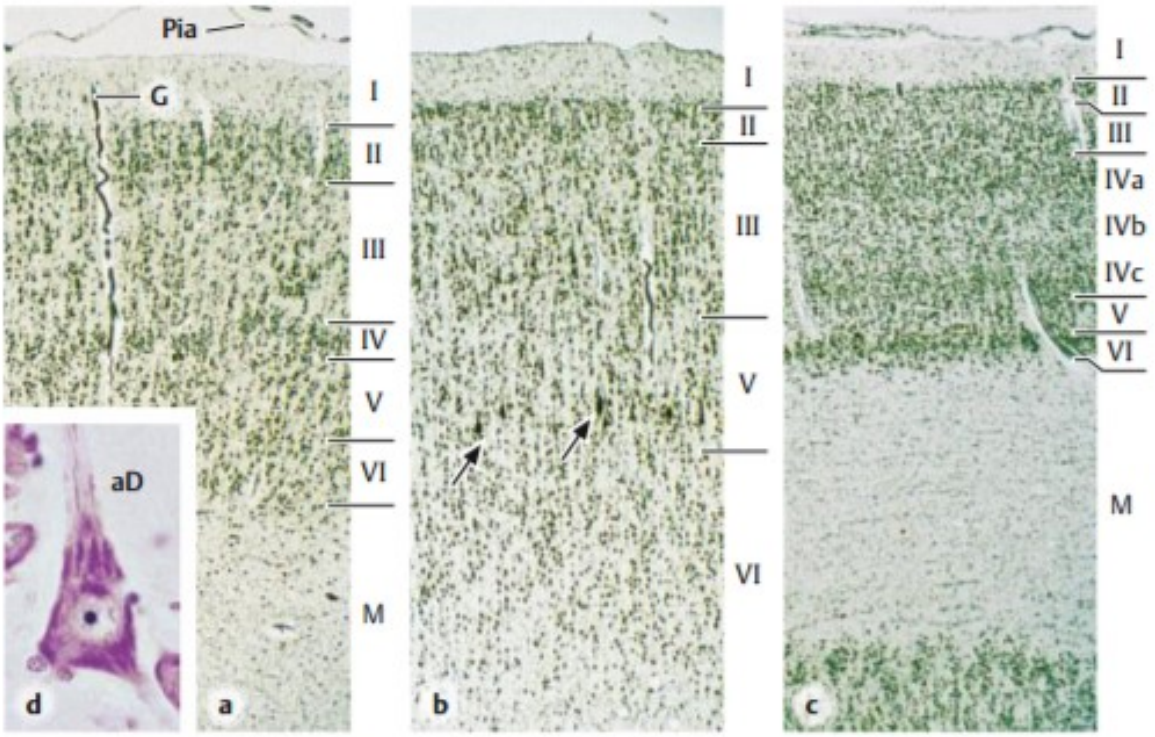

Figure 6 Nissl staining of cortex layers
a – homologous Isocortex
b – motor cortex, layer IV is missing, arrows point at betz cells
c – primary visual cortex
d – pyramidal cell (of a dog)

2.3. The motor cortex

The human motor cortex lies rostral the Sulcus centralis in the frontal lobe. It is the primary motor cortex and represents the initial site of motor activity information

processing. Secondary and tertiary areas would be more rostral and compute movement patterns which are conveyed to the motor cortex.

2.3.1. Specific elements of the layers of the motor cortex

When the six layers are clearly seen, the area is called homotypic cortex. A heterotypic area shows deviations like the motor cortex.

The pyramidal cells are prominently represented and layer V contains so called “Betz cells”. These are giant pyramidal cells building with their axons together with other axons an important part of the pyramidal tract. The motor cortex lacks the layer IV and therefore is called agranular layer. This fact can be explained by its function. Layer IV has usually many conection with the thalamus and serves as an “input” region. The primary visual cortex for example has a prominent layer IV because it gets much information from other brain regions like the thalamus whereas the motor cortex hardly receives any sensory information from outside and for his part works as an output system for motor control.[23,24]

2.4. Electrical conductivity

The current applied on the skin must enter the brain to have an effect. The way to the gray matter is the following: Skin/scalp – skull – cerebrospinal fluid – gray matter. They all have different conductivities.

The skull is separated into the lamina externa and interna and the diploe in between. The conductivity is about 5.4mS/m – 7.2mS/m in the lamina externa and about 2.8mS/m – 10.2mS/m in the lamina interna. The layer with the diploe is described with about 16.2mS/m – 41.1mS/m.[25] The conductivity of cerebrospinal fluid at body temperature is about 1.79 S/m.[26] The conductivity of the gray matter varies quite much between the studies. Some describe values of about 0.333S/m – 0.352S/m, but these derive from animal trials.[27] It is assumed that the values are similar to human gray matter, but trials with human tissue show different conductivities. Neuling et al computed a conductivity of about 0.24S/m for gray matter.[28]

2.5. Brain oscillations[29,30]

The brain oscillates in different frequencies depending on level of consciousness and mental/physical activity. This can be presented through an EEG. This chapter gives an overview of the different frequencies and what they mean.

2.5.1. EEG – Bands in cerebral cortex

The α-rhythm: Its frequency is about 8Hz – 12Hz and its amplitude about 15µV – 65µV. It's found mostly in posterior brain areas. The α-rhythm occurs mainly when we relax and especially close our eyes. In contrast it is blocked when we are mentally active with opened eyes. The frequency is dependent of the blood flow and varies with flow changes of about 1Hz – 2Hz. Another variation occurs in the follicular phase of women, when it's a little faster (0,3Hz). Very few people don't show any α-activity. There is a difference in the amplitude between left and right hemisphere. Usually it should not be more than 50% difference, otherwise it gives evidence to a lesion.

The β-rhythm: Its frequency is per definition anything above 13Hz, but it normally doesn't reach more than 35Hz. The most common frequency is between 18Hz – 30Hz and occurs in frontal and central areas of the brain. It the frontal regions it can reach higher frequencies, also while a subject is asleep it reaches up to 35Hz – 40Hz (see below "the γ-rhythm"). Posterior areas show a little slower β-rhythm and this one may be related to an α-rhythm, because it is reactive to eye closure. The amplitude is relatively low with <25µV. Benzodiazepines or barbiturates can increase the amplitude and medication that helps falling asleep can drive the frequency up.

The θ-rhythm: Its frequency lies between 4Hz – 7Hz and is shown in frontal and central areas. It can occur during disease or while falling asleep, but also while performance of difficult maths or spelling tests. Also it seems to be aging dependent, occurring more frequently above the age of 50.

The δ-rhythm: This is a very low frequency (0,5Hz – 3Hz), which only occurs during deep sleep stages.[31]

The γ-rhythm: The frequency of a γ-rhythm is high around 30Hz – 60Hz or even higher. It occurs when we perform mental tasks or when we learn something. Also while the REM phase this frequency can be found. It comes up because GABAergic neurons fire synchronously while mental tasks.[31,32]

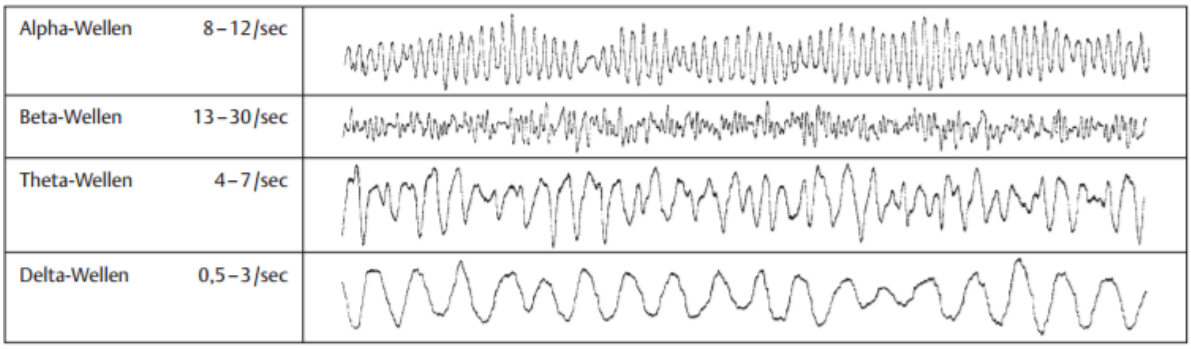

Figure 7 Diagram of the brain oscillation in different frequencies

2.5.1.1. EEG/Oscillations especially in MC

When looking at brain oscillation especially in the motor cortex a particular rhythm is described in literature, called the *rolandic μ-rhythm*. This frequency appears pre- and postcentral in the rolandic region. It can be divided between an alphoid (10Hz) and a fast (20Hz) component.[33,34] The alphoid frequency may arise from the sensory cortex and the fast occurs in the motor cortex. It is found in relaxation and is blocked by voluntary, but also passive and reflexive movement and even the thought of a movement. This effect can be shown bilateral, although its stronger on the contralateral site.

3. Non-invasive Brain Stimulation (NIBS) – technical issues

The currently available NIBS techniques for induction of cortical plasticity are transcranial electrical stimulation (TES) and repetitive transcranial magnetic stimulation (rTMS). For rTMS electrically powered coils producing trains of pulsed magnetic fields are state of the art. On contrary to this current approach, a rotating permanent magnet producing sinusoidal magnetic fields will be tested in this diploma thesis.

3.1. Transcranial electrical stimulation (TES)

Non-painful tES can be divided into transcranial direct current stimulation (tDCS) and transcranial alternating current stimulation (tACS). For both approaches electrical currents in a range between 1mA – 2mA are applied to scalp via skin electrodes with a typical size up to of 5cm x 7cm. The important factor is the scalp surface current density, which should be in range between 25µA/cm^2 – 35µA/cm^2. Basically currents in such a range slightly change the transmembrane potential of a cortical neuron, however such currents are too weak in order to activate action potentials directly.

3.1.1. Transcranial alternating current stimulation (tACS)

3.1.1.1. Basics of tACS

This method applies an alternating current on a specific target region in the brain in a particular frequency. The tACS works with two electrodes. In one half-cycle of the oscillation one electrode is the cathode and the other the anode and in the next half-cycle this traverses, which results in an alternating current. This procedure run with a particular frequency is thought to be able to manipulate the brain's own cortical oscillations.[35,36] Some studies in the last years showed that brain oscillations are associated with cognitive performance.[37,38,39] Brain oscillations are also thought to play a role in psychiatric disorders whereas this fact is still not clearly understood and needs further research.[40,41]

The physiological oscillation in the motor area is about 20Hz usually while tonic motor activation and abolishes while voluntarily movements.[29,42] Voluntary movements are shown to get slowed after application of tACS with 20Hz, which implicates that a β-rhythm refelcts the “resting mode” of the motor cortex.[43]

However, tACS can induce a change in excitability and this seems to be amplitude and frequency dependent. By measuring the motor evoked potentials (MEPs) after treatment

with tACS with a frequency of about 20Hz (β-frequency) it showed an increase of the MEPs. [44,45,46] This could not be demonstrated at other frequencies. Another finding showed an increase of the amplitude of α-waves on the EEG after stimulation with α-frequencies and this lasted for 30 minutes after stimulation.[47,48]

Moliadze[46] showed that a low amplitude of 0,2mA leads to an inhibition resulting in increased motor threshold whereas higher amplitudes like 1mA lead to a decrease of the motor threshold. They concluded that inhibitory interneurons are already affected at lower amplitudes, and only when the amplitude is high enough to affect the excitatory pyramidal cells, it results in an overall excitation, because the excitatory effect is higher that the inhibitory effect. An amplitude of 0,8mA showed no effect, probably because the inhibitory and excitatory effects cancelled each other.

In contrast to these findings there are some studies that couldn't show such an effect. Cappon et al. found a decrease in MEP amplitude after stimulation with β-frequencies, whereas other studies found no effect at all.[49,50] Observation of voluntarily movements could demonstrate that β-frequencies decline the speed of movements.[51,52]

So even after many years of investigation there are still conflicting findings in this topic. Therefore, in this thesis also the effect of application of 20Hz with 1,5mA by tACS on motor performance (see "6. Methods") will be investigated and may contribute important data to this problem.

3.1.1.2. Effect of applied stimulation current

When a particular current is applied on the skin, then only a small part reaches the target in the brain. The rest of the applied stimulation current is lost by resistances of the different tissues (skin, bone, cerebral fluid…) and goes directly from one electrode to the other.

There are few studies that investigated the current density during stimulation with tACS, but if supposed that the mechanism is similar to tDCS there are some studies that could give evidence. Two studies examined the portion of current which reaches the brain by using head models. They found that an application of about 1mA or 2 mA respectively on the skin results in a current density of about 0,1mA/m2.[28,53] So estimated about 10% – 20% of the applied current reaches the brain.

On the cellular level the electric field has hardly an effect on the neuron soma, but on the axon, especially the hillhock and it is much more effective if it's orientated parallel to the axon fibres.[54,55]

3.1.2. Transcranial direct current stimulation (tDCS)

The tDCS device consists of a stimulator and two electrodes, like the tACS. The difference lies in the polarity of the electrodes. One is a cathode and the other an anode and this is fixed and won't change.

For anodal stimulation of the motor cortex the cathode is placed on the forehead and the anode about 3cm above the ear. This arrangement increases the excitability of the motor cortex whereas an arrangement vice versa decreases it.[17,56]

3.2. Transcranial magnetic stimulation (TMS)

A time-varying magnetic field induces an electrical field in electrically conductive brain tissue which in the following leads to eddy currents. If these eddy currents became strong enough they are able to influence membrane potentials and generate action potentials. This technique will be explained in this chapter.

3.2.1. Operating mode of magnetic stimulation

3.2.1.1. The magnetic field

The magnetic field is a vector field as generated by the current flow in a conductor, see picture at the left side. To achieve stronger magnetic fields, the conductors are often winded up to form a magnetic coil.

There are two variables describing the field. H stands for the field intensity and B stands for the flux density. The following relation exists for them:

$$B = \mu_0 * H \quad (1)$$

with μ_0 being the vacuum permeability (μ_0=1,257*10^{-6} Vs/Am).

The magnetic field is illustrated by magnetic flux lines. To know in which direction the field goes, there is the right hand rule (see figure 8). The thumb points in direction of the technical current flow and the other

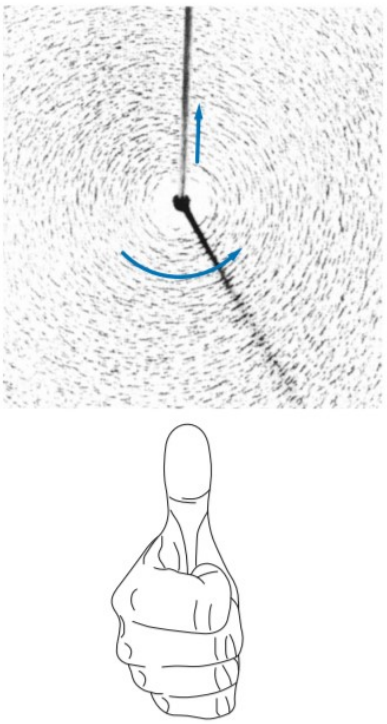

Figure 8 The upper picture shows a conductor with iron filings around it to represent the magnetic field.
The arrow, which points up shows the direction of the current flow, the other one shows the direction of the magnetic flux lines
The lower picture represents the right hand rule

fingers represent the flux lines.
The magnetic field theoretically goes unhindered through tissue. The field generating coil needs not to touch the tissue, but the strength of the field decreases with distance, so the coil should be positioned as close as possible to scalp. In this connection there is the law of Biot-Savart with H describing the field intensity:

$$H = \frac{I}{2\pi r} \quad (2)$$

For magnetic fields applies the principle of superposition. That means that the vectors can be added if the flux lines interfere with each other. Such an interference can be reached through an increase of the winding number of the magnetic coil.[57]

3.2.1.2. Faraday's law of induction[57]
The crucial element in the development of the magnetic stimulation is Faraday's law from 1831. This law states that the time-varying magnetic flux $\phi(t)$ induces the electric field E_{ind}. For a homogenous magnetic field the flux $\phi(t)$ is defined by the product flux density B(t) times area (A). The induced electric field E_{ind} is a vector field and stands perpendicular to the magnetic field. The way from one point to another along an electric field line represents the voltage and is given by U_{ind}.

$$U_{ind} = \frac{d\Phi(t)}{dt} = E_{ind} * 2\pi r \quad \text{with} \quad \phi(t) = B(t) \cdot A \quad (3)$$

3.2.2. Principles to induce transcranial electrical fields via electromagnetic induction
For generation of electrical fields via time-varying magnetic fields there are basically two possibilities. Either a time-varying current is supplied to a coil, or the poles of a permanent magnet are rotated. Coils, such as used for classical TMS, are commonly supplied with fast changing current pulses, and if placed on the head, impulse-like transcranial electrical fields are generated in brain tissue.[57] This was first shown in 1985 by Barker et al. by using a TMS device to produce impulse-like magnetic fields with a flux density of about 1 Tesla over the motor cortex.[58] According to Faraday's law the induced electrical fields are strong enough to evoke action potentials directly and transsynaptically, resulting into a motor evoked potentials (MEP).

On the other side, also rotating magnets can be used for electromagnetic induction. During rotation of a permanent magnet, north and south-pole alternate according to the rotation frequency and thus producing an alternating magnetic field around the magnet. If such a magnet is rotated at a close distance to the scalp the magnetic field enters the brain tissue and corresponding transcranial electrical fields are induced. These induced fields, however are too weak in order to evoke action potentials directly. Nevertheless, it appears feasible that this form of transcranial alternating magnetic stimulation (tAMS) should be strong enough in order to drive neuromodulatory effects comparable to tACS. This is what this study tries to find out.

3.2.3. Applications of TMS

For human studies magnetic stimulation can be used for different purposes. It can either be used for assessment motor cortical excitability (see "4.1. The transcranial magnetic stimulation (TMS)") or as a method to modulate brain excitability and to induce cortical plasticity. Examples of the latter would be repetitive TMS (rTMS) or tAMS, which is object of this thesis. More recently rTMS also was probed to substitute electroconvulsive therapy, in the treatment of patients suffering on major depression.[59]

4. Assessment of motor cortical excitability with TMS

There are different types of TMS devices on the market. These are monophasic, biphasic and the repetitive stimulators, which vary in the construction of their power circuit. For our study a monophasic device was used (Magstim 200) which is shortly explained here.

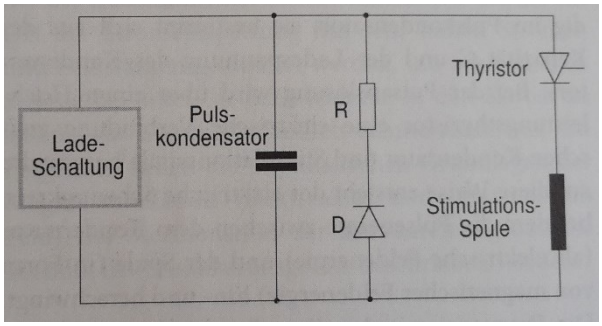

Figure 9 Schema of a monophasic TMS device

The power circuit of this device consists of the charging unit, the capacitor, the thyristor and the stimulation coil. For a stimulation first the charging unit loads the capacitor to a particular voltage until all the pulse energy is stored in the capacitor. When the thyristor connects the capacitor to the coil an electric current impulse is generated.[60]

4.1. TMS coils

An important element of a TMS device is a special air-core coil, suited to generate a short lasting (50µs – 100µs) but strong (about 1 Tesla) magnetic impulse. There are two types of stimulation coils in use. One is the round coil. Here the electric conductor is spirally winded up. The magnetic field produced by this coil reaches its maximum value annular around the centre while directly in the centre the electric field is not evident. That means that neurons under the centre of the coil are not stimulated and the focality of the magnetic impulse remains low (see figure 10). On the other hand, the magnetic field of a round coil has strong penetration, which is advantageous to stimulate deep laying peripheral nerves.
The second type is the double coil, further denoted as figure of eight coil because the shape of the coil reminds to a drawing an "8". Here the current first rotates through one coil, and then counter rotates through the second coil. Thereby the magnetic fields superpose at the point of intersection of the two coils (see figure 10). At this point, the field gradient becomes maximal which means that a higher focality is achieved with this coil. This is

necessary for stimulating specific cortical motor areas, in order to produce motor evoked potentials (MEPs) in their associated muscle groups. Further, also for cortical mapping such coils are suited.[61]

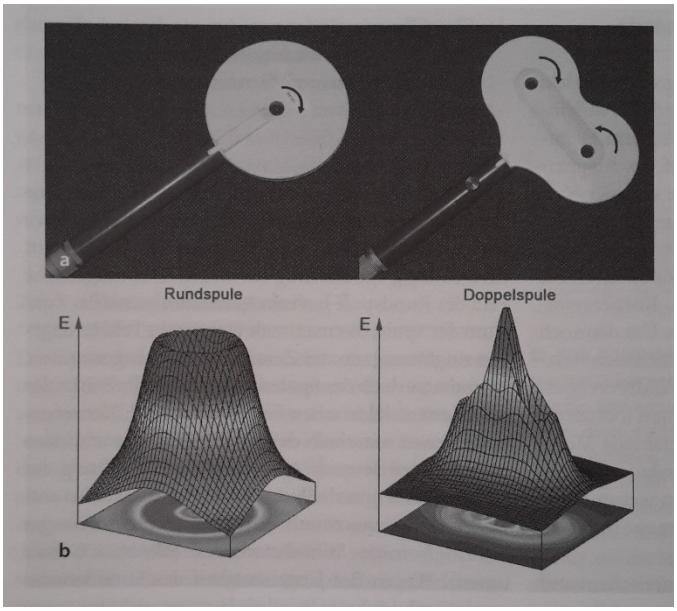

Figure 10 The produced magnetic fields of a circular and a double coil.

4.2. Motor evoked potential (MEP)

Here, for this study we used a figure of eight coil to assess motor cortical excitability at the hand area. Therefore, the intensity of the magnetic impulse has to exceed certain threshold in order to elicit MEPs. MEPs are the surface electromyographic signals over a target muscle in response to the stimulation of the motor cortex via TMS.[62] The stimulus excites pyramidal cells in the motor cortex and the corticospinal tract, that connects the motor cortex with the spinal cord, leads the excitation to the anterior horn. Here the stimulus passes on to the peripheral neuron, which action potential causes the movement in the muscle. So if the fast conducting neuronal tissue is stimulated, it builds up a connection to the target muscle and this potential at the muscle can be assessed as the MEP. Because the target muscle is not perfectly synchronous excited, and thus some electrical potentials also cancel each other, usually in an experimental setting 8 to 12 MEPs are stimulated consecutively and further the average is used. This provides information about motor cortical excitability.[63]

4.2.1. Resting motor threshold (rMT) and recruitment curve (RC)

RMT is defined as the minimum stimulation intensity which is necessary in order to induce a motor evoked potential (MEP) of 0.05mV in at least 5 out of 10 consecutive trials[64] over a relaxed muscle. This is the point when the stimulus from the TMS is strong enough to excite the neuron that innervates the muscle. The rMT value is defined by the percentage of the maximal output (100%) of the stimulator. They can differ between individuals but remains relatively constant within a single person and increases with age.[65,66]

Resting motor threshold (rMT) is a basic unit and necessary to dose TMS within an experimental setting. For example, to assess cortical excitability via single pulses, stimulus strength values relative to rMT are used. For example, a stimulus strength of 120% means that the stimulator output has to be adjusted 20% above the value needed for rMT.

It is distinguished between the activation of a single motor unit and the activation of the whole corticospinal pathway. Devanne et al. showed that the input-output relation of stimuli to the corticospinal pathway and to a single motor unit behave differently and thus, are independent from each other. Recruitment curves (RCs) record the relation between stimulus strength and MEP amplitude. The stronger the stimulus pulses become, the higher the MEP amplitudes become. After a certain strength a plateau is reached and the RC becomes sigmoidal.[67]

While rMT indicates the necessary stimulus intensity in order to activate an action potential, the RC represents the performance of the motor cortex and thus gives information about its excitability. It was suggested to be the most sensitive procedure to measure the cortical excitability.[68] For this study motor cortex excitability was assessed at stimulus intensities of 110%, 130% and 150% of the rTM.

4.2.2. Cortical silent period (CSP)

A silent period relates to partial or total reduction of EMG-activity of a contracted muscle triggered by a stimulation, and thus represents an inhibition. The length of a silent period may depend on any component of the motor system (from the motor cortex to the muscle) but also on inhibitory interneurons and afferent input from muscular spindles and Golgi tendon organs. The stimulation can be set at any point in the motor system (motor cortex or peripheral neuron) through magnetic or electrical stimulation, however only a silent period which a length of more than 100ms has its origin in the cortex.[69] This study uses TMS over the motor cortex to assess the cortical silent period (CSP) in addition to MEP amplitude.

MEP amplitude correlates with the length of the CSP. The intra-individual variability of this quotient is quite low, whereas the inter-individual variability is high. This also holds true for the orientation of the TMS coil over the motor cortex. A stimulus in posterior-anterior direction induces a smaller MEP and a shorter CSP than a stimulus in anterior-posterior direction, but the quotient stays the same.[70] This is an evidence that the MEP and CSP depend on the same factors. There exist different explanations concerning the mechanism of CSP. One explanation considers collaterals from corticospinal neurons: TMS activates fast conducting neurons and their collaterals inhibit slow conducting neurons responsible for tonic activation.[71] However, other data show an association between CSP and the GABA-system. Modulation of GABA-receptors leads to a change of the CSP.[72,73] Wehrhan et al. compared MEP and CSP in recruitment curves. They have shown that the CSP recruitment curve begins and saturates earlier which leads to the conclusion that the mechanisms for MEP and CSP are separate from each other.[74]

4.2.3. Paired pulse paradigm[75]

The paired or double pulse paradigm describes applications of two TMS-stimuli set very shortly after another in order to assess excitatory and inhibitory modulations of motor cortex excitability. The first stimulus (conditioning stimulus) always is below rMT (typically 80% of rMT) while the second stimulus (test stimulus) always is above rMT (typically 120% of rMT). The length of the time interval between the two stimuli determines whether it comes to an increased or decreased MEP, compared to a MEP following a single pulse at 120% rMT.

A decrease of MEP amplitude occurs, if the interval between the two stimuli is a range between 2ms - 4ms and this effect is termed *short interval intracortical inhibition (SICI)*. On the other hand, if the interval is in a range between 10ms – 15ms, an increase of MEP amplitude occurs and this effect is termed *intracortical facilitation (ICF)*.[76] There is evidence that SICI and ICF represent cortico-cortical effects.

4.3.3.1. Short-interval cortical inhibition (SICI)

At the SICI the interval between the first and the second stimulus is something between 2ms – 4ms. This leads to a lower MEP amplitude in comparison to an unconditioned single test stimulus. The physiological basis of SICI is very complex. A TMS impulse leads at a supraspinal level to a series of spike volleys. These are so called direct waves (D-wave) and indirect waves (I-waves). There is one D-wave followed by several I-waves in a

periodicity of about 1,5ms. On the EMG they represent one tri-phasic MEP signal.[77] Pharmacological interventions, which influenced the GABAergic transmission, have shown that I-waves depend somehow on that transmitter.[78]

Stimulation of the motor cortex between 2ms – 4ms (SICI) resulted in a reduced MEP and also indicated reduced I-waves. So any changes in cortical GABAergic transmission are mirrored into the value of SICI. An increase of SICI indicates decreased intracortical inhibition, while a decrease is an indicator for increased intracortical inhibition. For example, within a pre-post experimental design, induced changes in SICI can give information about GABAergic circuits.

4.3.3.2. Intracortical facilitation (ICF)

If the time interval between the conditioning (S1) and the test stimulus (S2) is in the range between 10ms – 15ms this results in a higher MEP amplitude in relation to an unconditioned test stimulus. This facilitatory effect, in contrast to SICI, was explained by an increase in the amplitude of I-waves, and may be dependent on a glutamatergic pathway. Here an increase of ICF indicates increased intracortical facilitation, while a decrease is an indicator for decreased intracortical facilitation. Further, within a pre-post experimental design, induced changes in ICF can give information about changes in glutamatergic circuits.

5. Hypothesis

The hypothesis of this study is, that a tAMS intervention of 10 minutes applied over the motor cortex will we able to induce outlasting effects in cortical excitability, comparable to tACS. In order to verify our hypothesis that tAMS induces outlasting effects three experimental tests have to be conducted: TMS assessments pre and post to tAMS intervention, TMS assessments pre and post to tACS intervention, and TMS assessments pre and post to sham stimulation.

For the functioning of tAMS, the crucial element is the design of rotating permanent magnet. A permanent magnet primarily produces a static magnetic field. Because the magnet is rotated constantly at 1200rpm, a 20Hz alternating magnetic field is produced. This alternating magnetic field weakens with distance to the magnet. Based on the data of the permanent magnet an estimation of the field intensity was performed considering the distance between magnet and motor cortex.

For this study a disk-shaped magnet made of neodymium, iron and boron (NdFeB) with a diameter of 50mm was specially produced (www.neomagnete.de). This magnet was magnetized in diametral direction and had a remanence of 1.33T. If we assume that the

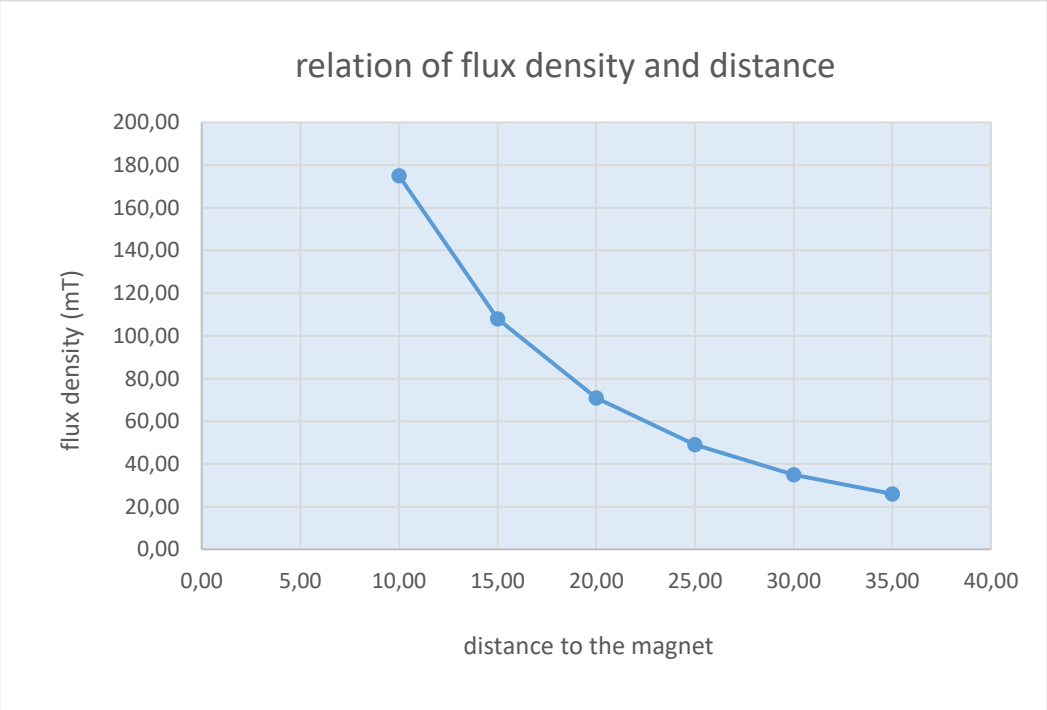

Figure 11 flux density is falling with the distance to the magnet

magnet is rotated closely at scalp, the distance between scalp and grey matter is about 15mm (5mm scalp, 5mm skull, 5mm other tissue). First we calculated flux density in radial direction of the magnet by using the field calculator of www.magnetfabrik.de. The flux density in a distance of 15mm (here begins the grey matter) is 108mT, in a distance of 20mm it is 71mT and so on (see figure 11).

The magnetic field induces an electrical field according to equation 3. If we assume a homogenous alternating magnetic field (71 mT flux density at the distance of 20mm) within an area A given by the radius of 10 mm, the intensity of the induced electrical field can be estimated according to equation 4. Calculations with a frequency of 20Hz results into a field intensity of 0.044 V/m in the motor cortex.

$$E_{ind} = \left(\frac{1}{2\pi r}\right) \cdot (r^2\pi) \cdot \frac{d(0{,}071\sin(2\pi f))}{dt} = 0{,}044 * \cos(2\pi f) \quad (4)$$

Some studies have estimated intracortical field intensities during tACS-stimulation. Neuling found that 1mA tACS corresponds to E = 0,42V/m and two other studies found synchronisational effects from 0,5V/m and 1V/m respectively.[28,35,79] Thus the field intensities as produced with tACS are expected one order higher as produced with 20 Hz tAMS.

6. Methods

6.1. The participants

We tested 14 healthy right-handed men (8) and women (6) with age 22 to 34 years (mean: 27,07 ± 3,07 SD). They were recruited at the Institute of Physiology at the Medical University of Graz using announcements. According to the informed consent excluding criteria were pregnancy, metal implants (cardiac pacemakers, …) and neurological diseases such as epilepsy. (see attachment "TMS-Abklärung")

The subjects were informed about the procedure and before participation they signed an informed consent (see attachment "Probandeninformation und Einwilligungserklärung zur Teilnahme an der wissenschaftlichen Studie").

6.2. Study design

The study consisted of 3 sessions with different interventions: tACS (on left motor cortex), tAMS (rotating magnet on left motor cortex), sham-condition (rotating magnet on the vertex).

The order of the sessions was randomized between the subjects and every subject took part in each session. There had to be 5 to 7 days between the sessions to avoid possible interfering post-stimulation effects.

Before every intervention the following parameters were assessed: rMT, different MEP-amplitudes (110%, 130%, 150%), the SICI, the ICF and the CSP and the motor performance in the Grooved Pegboard Test (GPT).

After the measurements in baseline, one of the three treatments were carried out and at the end we assessed all above mentioned parameters again (see scheme).

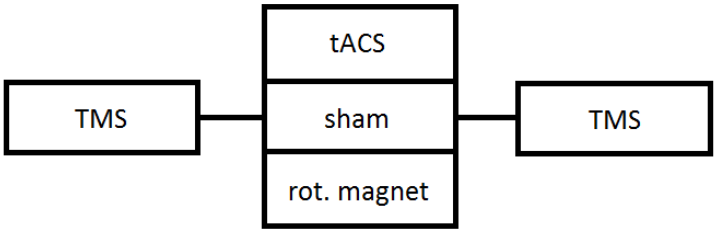

Figure 12 Scheme of the study set-up

6.3. The Grooved Pegboard Test (GPT)

To evaluate the possible effects of stimulation on motor skills of the hands (left and right) we used the Grooved Pegboard Test (Model 32025, Lafayette Instrument, USA). It has 25 holes (5x5) differently shaped and 25 pins. The task is to adjust and place the pins into them as fast as possible. The outcome parameter is the time (in seconds) it takes the subject to perform that.

All of the pins have the same form. To put them correctly into the holes, they must be adjusted into the shape of the particular hole. The subject was supposed to only use one hand and not take more than one stick in his/her hand and also fill it up from left to right and up to down.

The test was performed twice per session (once at the beginning and once at the end). The subject begins with the right hand to perform the test. The observer stops the time. Same procedure was repeated with the left hand.

Figure 13 The Grooved Pegboard Test

6.4. tACS intervention

A standard battery-driven device, called DC-stimulator PLUS from NeuroConn, was used. Before every treatment the stimulation parameters (alternating current), the frequency (20Hz), the amplitude (750µA peak to peak) and the duration of stimulation (15min) were adjusted manually by the investigator.

After the adjustment of the device the subject got explanation about experimental procedure and especially that visual effects like flashes might occur during the current application The active electrode (5cm x 5cm) was placed on the area of the left motor cortex and the other reference electrode (10cm x 10cm) was placed on the shoulder. To reduce the resistance Signa Gel was spread onto the electrodes before placement on the skin.

The resistance between the electrodes was controlled and had to be low enough (<10 kΩ), the intervention could be started and the subject got the stimulation for 15min. During the stimulation the resistance was controlled regularly.

Figure 14 The tACS - stimulator

6.5. tAMS intervention

For this study a special ring shaped rare earth magnet with an outer diameter of 50mm and a thickness of 8mm was produced (type R-040-D-15-008-N from Neotexx in Berlin, see figure 15). North and south poles are diametrically in opposition in order to achieve a sinusoidal waveform of the magnetic field during rotation. This magnet was connected to a precision motor, to be rotated at 1200 rounds per minute (corresponding to a frequency of 20 Hz). The motor was run using batteries.

The magnet was covered in a plastic sleeve before it was placed onto the area of the left motor cortex as defined by TMS and to start the rotation the magnet was linked to the batteries. The rotating magnet had a noise emission. The subject was exposed to the stimulation for 15min.

Figure 15 The rotating magnet

6.6. The sham condition

Here the rotating magnet was placed transversally onto the vertex, so that the magnetic stimulation cannot affect the motor cortex. The subject was exposed to the sham stimulation for 15min.

6.7. TMS-assessments

For the TMS assessment two Magstim 200 stimulators, that are coupled via a Bistim module, were used. The coil was connected to the Bistim module. The device produced a monophasic impulse (see. “4.1. The transcranial magnetic stimulation (TMS)”).

A figure eight-shaped coil (outer Ø = 9cm) delivered the magnetic stimuli. The coil was positioned on the left motor cortex in order to stimulate the contralateral first dorsal interosseous (FDI). The center of the coil was placed onto the scalp and was inclined by 45° to the median sagittal plane. The exact position of the coil was defined as the place with the largest MEP (hot spot). The subject was wearing a cap, where the hot spot was marked with a soft pen.

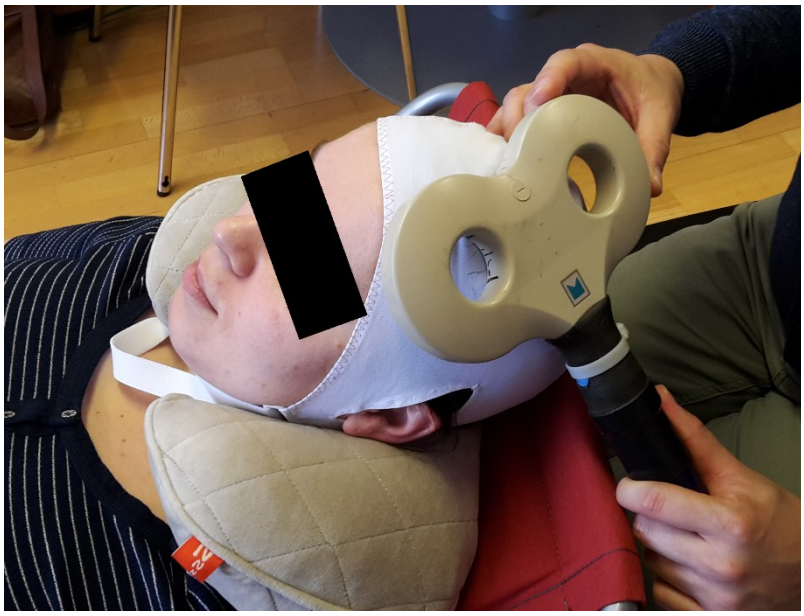

Figure 16 TMS-coil placed on subject’s motor cortex

To register the rTM the subject was sitting in relax in a chair and the observer searches with the coil for the hot spot. Once this is found, we seek for the minimal stimulus strength that can induce a MEP in at least 5 out of 10 trials. This then represents the rMT.[64]

To test higher MEP amplitudes 110%, 130% and 150% of the intensity of the rMT was delivered. 10 trials for each intensity 110%, 130% and 150% were performed (in total 30 trials).

For the SICI/ICF a pair of stimuli was applied. The first conditioning stimulus was 80% of the actual rMT and the second test stimulus represented the intensity that induces a MEP with amplitude at least 1mV. This is usually corresponding to 120% of the rMT.

For SICI those stimuli were set in an interstimulus-interval of 2ms and for ICF they are set in an interstimulus-interval of 13ms. Each of those stimuli were delivered 10 times in a randomized order.
The CSP was determined in the presence of weak voluntary contraction of the target muscle (about 20% of the maximal voluntarily contraction). Therefore, the observer asked the subject to press a ball between the index and the thumb. Then the TMS-stimulus with 130% of the rTM was delivered. This procedure was repeated 15 times.

6.7.1. Electromyographic recordings

MEPs were recorded from the first dorsal interosseous (FDI) via standard superficial Ag/AgCL electrodes (Ø = 9mm). The active electrode was placed on the FDI and the reference electrode on the distal phalanx of the index finger. A third electrode, the ground electrode, soaked in water, was attached on the wrist. The amplified signals were recorded on disc via MicroMed software (http://www.micromed.eu) and displayed on the computer screen in real-time for control.
After every tACS and tAMS interventions subjects were asked if they see flashes during the tACS.

6.8. Evaluation and statistics

Normal distribution was ascertained for all variables before performing parametric tests.
We tested within-subject effect by using repeated measures ANOVA (analysis of variance) with two factors: *time* (pre, post) and *STIM* (RM, tACS, sham). The parameters were recorded by a computer software called “SystemPLUSEvolution”. For evaluation the data were transferred in ASCII-files, from which we assessed the amplitude of the MEPs (MEP amplitude protocol, SICI, ICF) in mV and the latency (CSP) in ms. The latter was calculated from the end of the MEP until the recovery of the muscle activation.
The MEPs were presented in mV. First we performed a within-subject test of each intensity (110%, 130%, 150%) comparing time in connection with STIM and if that showed any significance, we performed a follow-up paired samples test, where we compared differences in time within one STIM. In this case we paired the pre and post levels of each STIM.
The MEP amplitudes of the SICI and ICF were presented in relation to a single pulse MEP amplitude. First we performed a repeated measures ANOVA with Bonferroni corrected

pairwise post-hoc comparisons to compare the conditioned MEP amplitudes with within-subject factors time and STIM and if that showed any significance, we performed a follow-up paired samples test, where we compared differences in time within one STIM. In this case we paired the pre and post levels of each STIM.

The SP was presented in ms and was defined as the latency between MEP offset and onset of muscle activity in the EMG recordings. First we performed a within-subject test comparing time in connection with STIM and if that showed any significance, we performed a follow-up paired samples test, where we compared differences in time within one STIM. In this case we paired the pre and post levels of each STIM.

Differences in the baseline values for all variables were compared using ANOVA with factor stimulation protocol.

Motor performance was quantified as the time (sec) to complete the GPT. Repeated measures ANOVA Bonferroni corrected pairwise post-hoc comparisons with within-subject factors time and STIM and hand (left, right) was used to evaluate the effect of the stimulation protocol on GPT performance. In all statistics a significance level of 0.05 was used.

7. Results

This chapter presents the results. It is separated in the two subchapters “MEP amplitude protocol” and “ISI”. The silent period and the GPT didn’t show any significant results, therefore they are only discussed shortly at the end of the chapter.

Our observations showed that the RM and the tACS was well tolerated by all our subjects. One subject reported slight tingling sensation in the right hand while RM stimulation which disappeared after the stimulation stopped. Three subjects reported visual flashes while tACS stimulation at 20Hz.

7.1. MEP amplitude protocol and rMT

The mean resting motor threshold (rMT) values at baseline were 45.7 ± 6.4 % for RM, 45.0 ± 5.6 % for tACS, and 46.5 ± 5.8 % for SHAM.

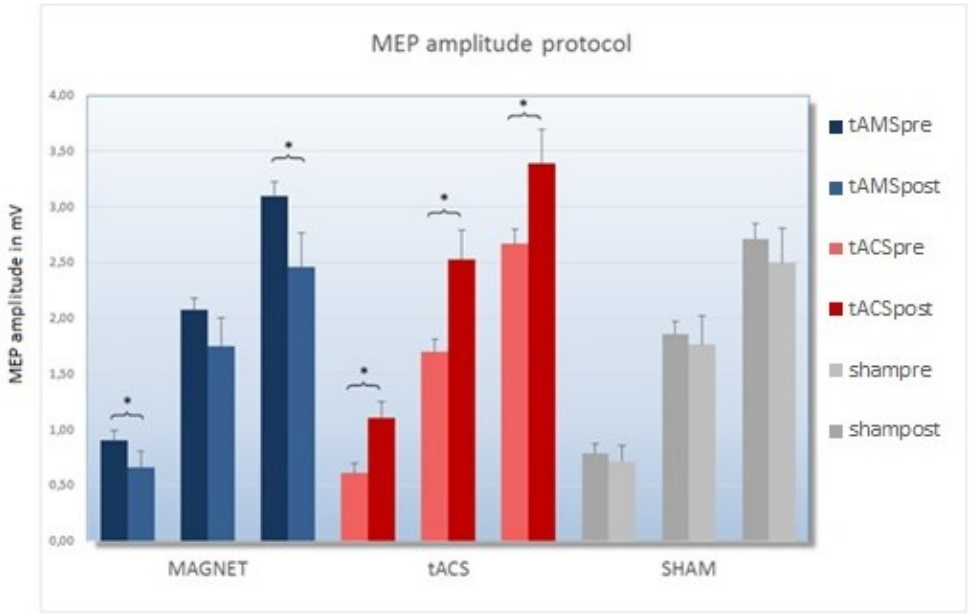

Figure 17 MEP amplitudes at intensity levels 110%, 130% and 150% in the three stimulation protocols. The Magnet lead to a significant TMSpost decrease at 110% and 150%. The tACS lead to a significant TMSpost increase at all intensity levels. The sham condition showed no effect.

Figure 1 shows the MEP amplitudes at stimulation intensities of 110%, 130% and 150% in each stimulation protocol.

To investigate whether the tAMS and/or the tACS influence the MEP amplitude protocol significantly a two-factorial ANOVA assessed a significance in the interaction effect of

time x *STIM* in every intensity (110% p = 0.000; 130% p = 0.007; 150% p = 0.001), whereas *time* and *STIM* at their own showed no significance (110%_time p = 0.294; 110%_STIM p = 0.255; 130%_time p = 0.42; 130%_STIM p = 0.629; 150%_time p = 0.719; 150%_STIM p = 0.49). Also significant differences between baseline MEPs were not found.

The transcranial alternating magnet stimulation (tAMS)

The histogram (see figure 17) showed that the intervention with the RM lead to a decrease of MEP amplitude at intensity of 110% and 150%. In the follow-up the paired samples test showed a significant decrease of MEP size from the pre-treatment to the post-treatment (p = 0.015) at intensity 110%. At an intensity of 130% there was no significant difference in the paired samples test (p = 0.233)

The paired samples test showed a significant decrease of MEP size from the pre-treatment to the post-treatment (p = 0.018) at intensity 150%.

The transcranial alternating current stimulation (tACS)

As the histogram shows (see figure 17) there was an increase of the MEP amplitudes in every intensity of the RC.

A paired samples test assessed a significance at intensity levels 110% (p = 0.001), 130% (p = 0.000) and 150% (p = 0.001).

The sham condition

There was no significance of MEP amplitude changes in any intensity in the sham condition at intensity levels 110% (p = 0.961), 130% (p = 0.59) and 150% (p = 0.398).

7.2. Paired pulse

The results of the ISI (SICI and ICF) are presented in figure 18 and 19. There were no significant differences between the three protocols at baseline.

A two-factorial ANOVA assessed a significance in the interaction effect of *time* x *STIM* in both, SICI and ICF (SICI p = 0.001; ICF p = 0.001), whereas *time* and *STIM* at their own showed no significance (SICI_time p = 0.306; SICI_STIM p = 0.073; ICF_time p = 0.215; ICF_STIM p = 0.095).

The transcranial alternating magnet stimulation (tAMS)

As figures 18 and 19 show, the tAMS as produced by the rotating magnet lead to a decrease of conditioned MEP amplitudes after treatment.

The following paired samples test showed a significant decrease from pre-treatment to post treatment in SICI to 34% ($F_{(2,24)} = 8.96$; $p = 0.011$) and ICF to 39% ($F_{(2,24)} = 9.19$; $p = 0.002$) respectively.

The transcranial alternating current stimulation (tACS)

There was no significant change in the paired samples test, neither for SICI ($p = 0.254$) nor for ICF ($p = 0.077$). There may be a tendency in the latter.

The sham condition

There was no significant increase or decrease in the paired samples test, neither for SICI ($p = 0.066$) nor for ICF ($p = 0.572$).

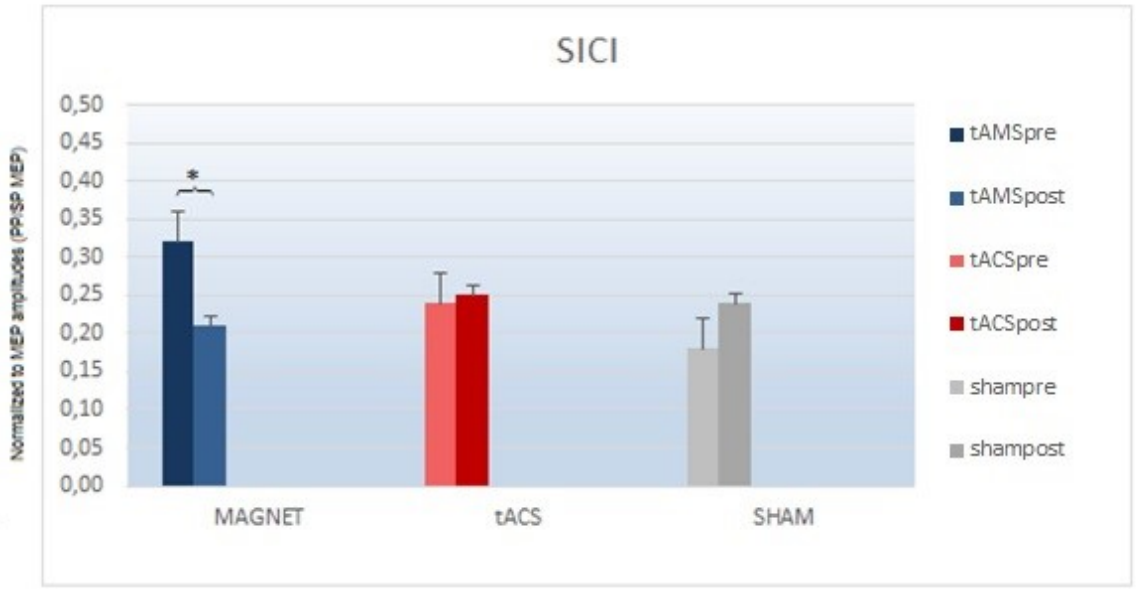

Figure 18 Results from SICI. Normalized to MEP amplitudes (PP/SP MEP). Only the Magnet lead to a decrease of the TMSpost stimulus.

Figure 19 Results from ICF. Normalized to MEP amplitudes (PP/SP MEP). Only the Magnet lead to a decrease of the TMSpost stimulus.

7.3. The silent period (SP) and grooved pegboard test (GPT)

The ANOVA didn't reveal any significant differences on CSP for any of the stimulation protocols. The mean values for RM were 119,38ms TMSpre and 124,23ms TMSpost; for tACS 101,58ms TMSpre and 103,3ms TMSpost; for sham 109,13ms TMSpre and 123,43ms for TMSpost.

The mean execution times at GPT at baseline in the right hand were: 56.84 ± 9.31 sec for RM, 57.89 ± 8.46 sec for tACS and 54.29 ± 6.92 sec for SHAM. The mean execution times at baseline in the left hand were: 59.94 ± 7.03 sec for RM, 62.43 ± 10.12 sec for tACS and 59.52 ± 6.62 sec for SHAM. There were no significant changes between these baseline values and post-stimulation values in both hands. Only a significant main effect of *hand* ($F_{(1,13)}$= 14.47, p< 0.002) was shown, what means that with the right hand a shorter performance time was achieved.

8. Discussion

The purpose of this thesis was to find out whether a 15-minute intervention with 20Hz tAMS is able cause a significant change in motor cortex excitability. The tAMS has been compared to a sham condition and to the well-known 20 Hz tACS intervention, which has already shown that it can increase motor cortex excitability. Cortical excitability was assessed with single and paired pulse TMS within a pre-post experimental design.

8.1. Intervention with 15min tACS

This study indicated an enhancing effect of the tACS intervention on motor cortex excitability shown by the results of the MEP amplitudes (Fig. 17). This enhancing effect fits to most of the other investigations that show similar results. The tACS is thought to entrain the brains own oscillations.[47,48] If the tACS runs with a frequency of 20Hz it appears conceivable that this frequency increases cortical excitability, because it "boosts" the β-frequency. There are several studies that give evidence to this hypothesis.[80,81,82] But also higher frequencies showed enhancing effects.[46,83] But further the amplitude seems to be important as well (see below).

However, there are also some other studies, that didn't find any effects or even inhibiting effects on cortical excitability.[43,49,84] Cappon et al was the one who found inhibition.[45] This result seems to be conflicting, as it doesn't fit to our findings. An explanation could be that they used lower current densities or too short time of application. For example, Antal et al. used for the tACS 0.25A/m^2 and applied the current for period between 2min – 10min. Rjosk et al. used a current of 1mA and stimulated for a period of 10min, which might have been to too weak or too short. In comparison Moliadze found an increase of MEP potentials at 1mA applied for 10min, but they used a stimulation frequency of 140Hz.[46] Thus these results are conflicting and therefore it is difficult to compare these studies.

But also the current density seems to be an important factor. Moliadze showed in 2012 that a tACS run with 140Hz and 0.4mA lead to decreased MEP amplitudes, 0.6mA and 0.8mA showed no effect and 1.0mA lead to an increase of the MEP. Although the frequency was not 20Hz but 140Hz, this shows that the modulation of cortical excitability seems to be

dependent on the current density and here our results match with these findings. Considering that 20Hz with 1mA showed no effect, we should further discuss whether both stimulation frequency and current strength are important in their combination.[49] Like, if the frequency is very high, the current can be a little lower and still induce a significant MEP increase. On the other hand, the 20Hz tACS is thought to be crucial because of the brains resting oscillations, and most of the studies on this topic nowadays use 20Hz because of that background. This thesis also used 20Hz and a quite high current strength with 1,5mA for 15min, so we can expect that 1,5mA are strong enough to transport the electric impulse into the motor cortex and the impulse can do its work also at a relative low frequency of 20Hz and this for 15min which is strong and long enough to induce measurable post-effects.

Another aspect to mention is the direction of the electrical field. First we know that the direction of the current is important. With regard to the tDCS we find increased excitability in case the anode was over the MC and decreased excitability in case the cathode was over the MC.[85,86] And already in 1975 we knew that current that flows parallel to an axon fibre, has the most effect.[55] This leads us to another consideration. The pyramidal cells rather lie perpendicular to the surface, reach until he medullary layer and their transmitter is Glutamate.[24] In contrast, the interneurons lie parallel to the surface, they don't leave their layer and their neurotransmitter is GABA. We could hypothesize that if the electric field is weak, it only reaches the top of the gyri. If the field extends and only affects the axon fibre, that lie parallel to it, we could suppose that it only affects the interneurons. But if it gets stronger, the sulcal fibres also are affected. In the sulcus the same electrical field affects the pyramidal cells, but not the interneurons. Now the pyramidal cells lie parallel to the field, because the orientation of the cortex surface is rotated about 90°. This consideration could explain why low current strengths cause an inhibition and higher current strengths cause an excitation.

Considering the paired pulse stimulation tACS had no effect. There are hardly studies about tACS in combination with the paired pulse stimulation, so it was not possible to compare something. SICI and ICF are both transmitter associated. At SICI, two stimuli are set 2ms after another and this activates inhibitory $GABA_a$ergic pathways. That's why the following MEP is much lower or even not existent. At ICF, the two stimuli are set 13ms after another and this, in contrast, activates the excitatory glutamatergic pathway.

There are studies that describe a modulation of tDCS effects after medication[87] and there is a new study that showed modulation of the $GABA_a$ergic pathway via tACS.[88] In the latter this effect was shown with a γ-frequency of 75Hz of the tACS but had no effect with a β-frequency. Considering that γ-frequencies occur because of synchronous firing of GABAergic neurons, maybe induced γ-frequencies entrain such GABAergic transmission.[31] Although such high frequencies are able to modulate the $GABA_a$ergic pathway lower frequencies seem to have no effect.

With the thoughts in mind, that weak currents lead to inhibition, a littler stronger currents show no effect and even stronger currents lead to an excitation, we could assume, that maybe for an effect on SICI and ICF we need even stronger currents. With the decrease of excitability with tAMS in mind that would explain, that the tACS showed no effect (because the excitatory and inhibitory neurons cancelled each other). However, it must be mentioned here that this is just a speculation and further work on this topic will be necessary.

8.2. Intervention with 15min tAMS

This is the first study that investigated whether 20Hz tAMS as produced by a rotating magnet is able to induce electric fields that are able to affect motor cortex excitability in comparison to the well-known tACS. The hypothesis was that tAMS generates a time-varying magnetic field, which on its part induces an intracranial electrical field, and which after an intervention of 15min leads to an increase of motor cortex excitability.

According to biological effects the crucial thing is the rate of change of the magnetic field. We know from Barker et al. that magnetic fields are able to induce a MEP, when the field changes are fast enough.[58] Barker worked with very fast changing magnetic fields (rise time 100μs) which were able to depolarize cortical neurons in the vicinity of the stimulating coil. That's the mechanism of the TMS. A permanent magnet that rotates primarily produces an alternating magnetic fields, here the field changes are much slower than with TMS but its strength is much weaker. But nevertheless, as seen here by this study, it could modulate brain's excitability.
Alternating magnetic fields are also able to affect other deep tissue layers. In this context pulsed magnetic stimulation was used for many treatment methods like bone grafting[89] or

pain therapy with arthrosis patients.[90] In these trials the magnetic field had a range of about µT to low mT. To generate the efficient strength of the electric field, the current to the stimulation coil is pulsed at frequencies between 50Hz – 200Hz.

In this study we compared tAMS with the tACS and therefore we chose 1200rpm which corresponded to the 20Hz tACS. Also the stimulation time was the same. What is not comparable is the current strength to the strength of the magnetic field. Contrary to our expectations tAMS showed a decrease in MEP amplitudes. Until today there are no other studies that investigated the effects of tAMS, so we were not able compare our results with previous findings.

As we found a decrease in MEP amplitudes we can assume, according to the results of Moliadze, that the field strength was not strong enough (see discussion above). Indeed, the estimated field intensity in the motor cortex as produced by tAMS was one order lower (0.044V/m) as produced with 1mA tACS (see chapter "5. Hypothesis"). If that assumption holds true further investigations with bigger magnets are needed. They may show that at a little stronger electrical fields lead to no effect and even stronger fields lead to an increase of MEP amplitudes, but this still remains to be elusive.

Also concerning the ISI we can explain the decrease of the MEPs by comparing it to literature of tACS. We suppose that for an increase of MEP amplitudes at SICI or ICF even stronger impulses are needed. So the tACS showed no effect because excitatory and inhibitory effects were the same, and tAMS was still strong enough to reach the surface of the gyri and to activate inhibitory pathways there. Also this assumption needs to be confirmed through further studies.

Another thought about our results give studies that used a static magnet. It has been proved that static magnetic fields play a role in biological systems and ion channels.[79,91] Na^{+} and Ca^{++} channels are shown to be inactivated by a static magnetic field, because the phospholipids change their position and thereby the kinetics of the channel are changed.[91] Oliviero et al. found a decrease of motor cortex excitability after application of a bigger static magnet (360 weight of grams) for 10 min over the cortex.[92] We could suppose that a static magnetic field modulates ion channel properties in direction of a decrease. Although these studies are about a static magnetic field and there is no induced electrical field, like

our study has, they show similar effects and may give evidence about the function of our rotating permanent magnet. It would also explain why tAMS showed effect on the ISI, which is GABA- and glutamate-dependent, respectively. There are no studies that show whether a static or a changing magnetic field also have influence on GABA- or glutamate receptors, but if they had comparable to the effect on ion channels, this could be a different explanation why the RM decreased the MEP at SICI and ICF. But if that holds true, a stronger magnet should show stronger decrease in excitability but not an increase as mentioned above.

The main limitation of this study is that there are no comparable studies. We only investigated whether there is an effect, but we can just suppose the mechanism behind it. Another thing to mention is, that the calculated field intensities from the magnet are only theoretical estimations and we don't really know how strong the field in the motor cortex really was and therefore it is difficult to compare with tACS. We only had one magnet and so we couldn't assess whether there are differences between stronger and weaker magnets.

Still little knowledge exists about the effects of alternating magnetic stimulation on the entrainment of the rhythms of the brain. Therefore, further studies including EEG would be necessary to elucidate whether 20Hz tAMS is able to synchronize the β-rhythm of the motor cortex. At least the hypothesis, that tAMS leads to comparable changes in cortical excitability than tACS, must be rejected. We now know that a rotating magnet applied onto the region of the motor cortex causes a decrease of motor cortex excitability in a setting like this study, but we actually don't know much about the underlying neurophysiological mechanisms. This study shall represent a basis for further research on this topic.

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Probandeninformation[1] und Einwilligungserklärung zur Teilnahme an der wissenschaftlichen Studie:

Wirkungen lokaler Wechselmagnetfelder auf die Erregbarbeit des Motorkortex

Sehr geehrte Teilnehmerin, sehr geehrter Teilnehmer!

Wir möchten Sie einladen an einer Studie teilzunehmen, die den Einfluss eines Wechselmagnetfelds auf die Erregbarkeit der primären motorischen Hirnrinde zum Thema hat. Das am Kopf einwirkende Wechselmagnetfeld wird mittels eines rotierenden Dauermagneten erzeugt, und als Methode zur Bestimmung der Erregbarkeit der motorischen Hirnrinde kommt die transkranielle Magnetstimulation (TMS) zur Anwendung. Die TMS ist eine nicht-invasive, schmerzlose Untersuchungstechnik. Sie gilt als sicher, schmerzlos und neben-wirkungsfrei. Aus Sicherheitsgründen gelten dabei folgende Ausschlusskriterien:

- neurologische und psychische/psychiatrische Erkrankungen (insb. Epilepsie)
- Metallimplantate im Kopf (außer Zahnersatz)
- elektronische Implantate (Herzschrittmacher, Kochlearimplantat)
- Schwangerschaft

Die Teilnahme an dieser Studie ist freiwillig und kann jederzeit ohne Angabe von Gründen durch Sie beendet werden, ohne dass Ihnen hierdurch Nachteile jeglicher Art entstehen.

Voraussetzung für die Durchführung dieser Studie ist jedoch, dass Sie Ihr Einverständnis zur Teilnahme schriftlich erklären. Bitte lesen Sie den folgenden Text als Ergänzung zum Informationsgespräch sorgfältig durch und zögern Sie nicht Fragen zu stellen.

Bitte unterschreiben Sie die Einwilligungserklärung nur

- wenn Sie Art und Ablauf dieser Studie vollständig verstanden haben
- wenn Sie bereit sind der Teilnahme zuzustimmen

[1] gilt für beide Geschlechter, auch wenn es im Text nicht explizit angeführt ist!

- und wenn Sie sich über Ihre Rechte als Teilnehmer/ Teilnehmerin an dieser Studie im Klaren sind.

Zu dieser Studie, sowie zur Probandeninformation und Einwilligungserklärung wurde von der zuständigen Ethikkommission eine befürwortende Stellungnahme abgegeben.

1. Was ist der Zweck dieser wissenschaftlichen Studie?

Es ist schon seit einiger Zeit bekannt, dass die menschliche Gehirnaktivität mittels schwacher Gleich- und Wechselströme sanft beeinflusst werden kann. Diese Ströme werden dabei transkraniell, mittels feuchter Schwamm-elektroden, am Schädel appliziert. In der Fachsprache spricht man dann von der transkraniellen Gleichstromstimulation (engl. tDCS) oder von der transkraniellen Wechselstromstimulation (engl. tACS). Im Rahmen dieser Studie soll nun überprüft werden ob auch ein lokales Wechselmagnetfeld geeignet sein könnte um die Gehirnaktivität messbar zu beeinflussen. Es ist ein Grundgesetz der Physik dass ein Wechselmagnetfeld ein elektrisches Wechselfeld hervorruft (Induktionsgesetz), wobei in leitenden Stoffen dann auch Wechselströme entstehen (Trafoprinzip). Die dabei lokal im Gehirngewebe induzieren Wechselströme sind ähnlicher Natur wie die durch Schwamm-elektroden direkt applizierten Wechselströme.

Die für diese Studie applizierten Wechselmagnetfelder werden durch konstante Rotation eines kleinen Dauermagneten erzeugt. Der rotierende Magnet ist dabei durch einen Plastiktubus mechanisch geschützt, so dass nur der Tubus an der Schädeldecke aufliegt. Durch die Rotation des Dauermagneten innerhalb des Tubus (Drehzahl ca. 900 Umdrehungen pro Minute, entsprechend einer Frequenz von 15 Hz) wird dann das gewünschte Wechselmagnetfeld generiert. Dieses Wechselmagnetfeld selbst wird nicht wahrgenommen, es ist jedoch ein leises Laufgeräusch (Antriebsmotor) hörbar.

Um hier messbare Wirkungen zu erhalten wird dieses Wechselmagnetfeld für eine durchgehende Dauer von 20 Minuten Dauer appliziert. Der Plastiktubus (mit dem rotieren Magneten) wird dabei am Kopf, über der motorischen Hinrinde in konstanter Position gehalten. Einmal vor und dreimal nach Applikation dieses Wechselmagnetfelds kommt die transkranielle Magnetstimulation (TMS) zur Anwendung. Zweck der TMS ist es eventuelle Veränderungen in der Erregbarkeit der motorischen Hirnrinde infolge der

Wechselfeldeinwirkung festzustellen. Um sicherzustellen dass die Veränderungen in der Erregbarkeit durch lokale Einwirkung an der motorischen Hirnrinde entstehen wird das Wechselmagnetfeld dann noch an einer anderen Stelle am Kopf appliziert und dabei die TMS Messungen wiederholt. Schließlich, um die vergleichsweise Wirkung der transkraniellen Wechselstromstimulation zu studieren, wird das Prozedere noch ein drittes Mal mittels wiederholt.

Die Untersuchungen dazu erfolgen an einer Studiengruppe bestehend aus insgesamt sechzehn rechtshändigen Frauen und Männern. Dabei sind drei Termine geplant: einmal zur Applikation des Wechselmagnetfelds über der motorischen Hirnrinde (TEST1), einmal zur Applikation des Wechselmagnetfelds über einen Hirnareal abseits der motorischen Hirnrinde (TEST2) und einmal zur Applikation der transkraniellen Wechselstrom-stimulation an der motorischen Hirnrinde (TEST3).

2. Wie ist der Ablauf bei dieser Studie?

Die Studie besteht aus drei unabhängigen Tests (TEST1, TEST2, TEST3) an drei verschiedenen Untersuchungstagen. Aus statistischen Gründen erfolgt die Zuteilung randomisiert, d.h. Eine Person beginn mit TEST1, die andere mit TEST3 usw. Jeder der drei Tests sollte dabei möglichst zur gleichen Tageszeit stattfinden.

Der zeitliche Ablauf ist für alle Tests gleich. Zuerst erfolgt die Bestimmung der kortikalen Erregbarkeit mittels TMS. Gleich danach erfolgt die 20-minütige Einwirkung des Wechselmagnetfeldes bzw., der transkraniellen Wechselstrom-stimulation. Gleich nach der Einwirkung erfolgt dann nochmals die Bestimmung der kortikalen Erregbarkeit mittels TMS. Schließlich nach 10 Minuten, und dann noch einmal 30 Minuten nach der Einwirkung des Wechselmagnetfeldes wird die kortikale Erregbarkeit mittels TMS noch zweimal bestimmt. Insgesamt dauert jeder Test etwa 90 Minuten. Alle Tests werden sitzend, in entspannter Ruheposition, durchgeführt.

3. Worin liegt der Nutzen einer Teilnahme an der Studie?

Die Ergebnisse dieser Studie sollen beitragen zu klären, ob und inwieweit sich durch Einwirkung solcher Wechselmagnetfelder Veränderungen in der kortikalen Erregbarkeit hervorrufen lassen. Nutzanwendungen sind in der Neurorehabilita-

tion (Stimulation motorischer Areale bei Hirnschlagpatienten) zu sehen. Durch Ihre Teilnahme an dieser Studie werden Sie keinen persönlichen Nutzen haben.

4. Gibt es Risiken, Beschwerden und Begleiterscheinungen?

In dieser Studie kommt die transkranielle Magnetstimulation (TMS) zur Anwendung. Durch Applikation einzelner Stromimpulse über dem motorischen Hirnareal kommt es dabei zu kurzzeitigen Nervenerregungen, die zu messbaren Muskelantworten führen (sogenannte motorisch evozierte Potentiale). Bis auf eventuelle leichte, aber vorübergehende Kopfschmerzen, sind für die Teilnehmer dabei aber keine gesundheitlichen Risiken, Beschwerden und Begleiterscheinungen zu erwarten. Epilepsieerkrankung, Metallteile im Bereich des Kopfes, und das Tragen von Implantaten sind jedoch Ausschlussgründe (siehe Seite 1).

In dieser Studie kommt auch die transkranielle Wechselstromstimulation (tACS) zur Anwendung. Die Methode gilt als sicher und bis auf ein leichtes, vorübergehendes Kribbeln kurz nach dem Einschalten dieses Stimulators sind für die Studienteilnehmer keine Begleiterscheinungen zu erwarten.

Schließlich sind auch infolge Einwirkung des 15 Hz Wechselmagnetfelds (in Form des rotierenden Dauermagneten) keine Begleiterscheinungen zu erwarten.

5. Zusätzliche Einnahme von Arzneimitteln?

Keine.

6. Hat die Teilnahme an dieser Studie sonstige Auswirkungen auf die Lebensführung und welche Verpflichtungen ergeben sich daraus?

Keine.

7. Wann wird die Studie vorzeitig beendet?

Sie können jederzeit, ohne Angabe von Gründen, Ihre Teilnahmebereitschaft widerrufen und aus der Studie ausscheiden ohne dass Ihnen dadurch Nachteile entstehen. Es ist aber auch möglich, dass der Versuchsleiter entscheidet Ihre Teilnahme an der Studie vorzeitig zu beenden, ohne vorher Ihr Einverständnis einzuholen. Gründe hierfür können sein, dass Sie den Erfordernissen der Studie nicht entsprechen.

8. In welcher Weise werden die im Rahmen dieser Studie gesammelten Daten verwendet?

Sofern gesetzlich nicht etwas anderes vorgesehen ist, haben nur die am Projekt Beteiligten und deren MitarbeiterInnen Zugang zu den vertraulichen Daten, in denen Sie namentlich genannt werden. Diese Personen unterliegen der Schweigepflicht. Die Weitergabe der Daten erfolgt ausschließlich zu statistischen Zwecken und Sie werden ausnahmslos darin nicht namentlich genannt. Auch in Veröffentlichungen von Daten dieser Studie werden Sie nicht namentlich genannt.

9. Entstehen für die Teilnehmer Kosten? Gibt es einen Kostenersatz oder eine Vergütung?

Durch Ihre Teilnahme an dieser Studie entstehen für Sie keine Kosten. Nach Beendigung der Teilnahme an der Studie (3 x 90 Minuten) erhalten sie als Vergütung den Betrag von *€ 50.-* in bar ausbezahlt.

10. Möglichkeit zur Diskussion weiterer Fragen

Für weitere Fragen im Zusammenhang mit dieser Studie steht Ihnen Frau Mag. Dr. Monica Christova, sowie der Laborleiter Herr ao. Univ.-Prof. DI. Dr. Eugen Gallasch gerne zur Verfügung. Auch Fragen, die Ihre Rechte als Teilnehmer an dieser Studie betreffen, werden Ihnen gerne beantwortet.

Name der Kontaktperson: **Mag. Dr. Monica Christova**

Während der Untersuchungszeiten erreichbar unter: **0316 380 4261**

Name der Kontaktperson: **ao.Univ.-Prof. DI. Dr. Eugen Gallasch**

Ständig erreichbar unter: **0316 380 4265**

11. Einwilligungserklärung

Name des Probanden[2] in Druckbuchstaben: .....

Geb.Datum: ..... Kennzahl: .....

Ich erkläre mich bereit, an der Studie „Wirkungen lokaler Wechselmagnetfelder auf die Erregbarbeit des Motorkortex“ teilzunehmen.

Ich bin von Frau *Dr. Mag. Monica Christova* oder von Herrn *DI. Dr. Eugen Gallasch* ausführlich und verständlich über mögliche Belastungen und Risiken, sowie über Wesen, Bedeutung und Tragweite der Studie, sowie der sich für mich daraus ergebenden Anforderungen aufgeklärt worden. Ich habe darüber hinaus den Text dieser Probandenaufklärung und Einwilligungserklärung, die insgesamt

[2] Gilt für beide Geschlechter

5 Seiten umfasst gelesen. Aufgetretene Fragen wurden mir verständlich und genügend beantwortet. Ich hatte ausreichend Zeit mich zu entscheiden. Ich habe aktuell keine weiteren Fragen mehr.

Ich werde den Anordnungen die für die Durchführung dieser wissenschaftlichen Studie erforderlich sind Folge leisten, behalte mir jedoch das Recht vor meine freiwillige Mitwirkung jederzeit zu beenden, ohne dass mir daraus Nachteile entstehen.

Ich bin zugleich damit einverstanden, dass meine im Rahmen dieser Studie ermittelten Daten aufgezeichnet werden. Beim Umgang mit den Daten werden die Bestimmungen des Datenschutzgesetzes beachtet. Eine Kopie dieser Probandeninformation und Einwilligungserklärung habe ich erhalten. Das Original verbleibt am Institut für Physiologie.

.....

(Datum und Unterschrift des Probanden)

.....

(Datum, Name und Unterschrift des verantwortlichen Untersuchungsleiters)

(Der Proband/ die Probandin erhält eine unterschriebene Kopie der Probandeninformation und der Einwilligungserklärung. Das Original verbleibt beim Untersuchungsleiter)

TMS-Abklärung

Nur von VL auszufüllen!

Kennzahl: …… Datum: …….. VL: ……

Bitte ausfüllen!

Name in Druckbuchstaben ……

Geburtsdatum: ……. männlich □ weiblich □

Ich hatte:

Schädelverletzung	ja □	nein □
Gehirnerschütterung	ja □	nein □
Augenverletzung an der Metallsplitter beteiligt waren	ja □	nein □
Sonstige Verletzung an der Metallsplitter beteiligt waren	ja □	nein □
Rückenmark Operation	ja □	nein □
Synkope (kurzzeitige Ohnmacht)	ja □	nein □

Ich habe:

Epilepsieerkrankung	ja □	nein □
Epilepsieerkrankung in der Familie	ja □	nein □
Aneurysma Clip	ja □	nein □
Herzschrittmacher	ja □	nein □
Neurostimulator	ja □	nein □
Cochlear Implantat	ja □	nein □
Metallische Implantate im Körper	ja □	nein □
Hörgerät	ja □	nein □
Zahnspange, Retainer	ja □	nein □
Tätowierung, Permanentes Make-up	ja □	nein □
Metallische Platte, Nägel etc. im Körper	ja □	nein □

Ich bin:

schwanger	ja □	nein □

Sonstiges: Medikamente: ja □…… nein □

Ich habe bereits an einer: TMS Studie teilgenommen	ja □	nein □
MRI Studie teilgenommen	ja □	nein □