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TMS-EEG signatures of glutamatergic neurotransmission in human cortex

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Für Mama – meinen Lebenskompass

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IV. Abbreviations

AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AMPAR	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
ANOVA	analysis of variance
rmANOVA	repeated measures ANOVA
APB	abductor pollicis brevis
CNS	central nervous system
DM	dextromethorphan
EEG	electroencephalography
EMG	electromyography
EPSC	excitatory postsynaptic current
EPSP	excitatory postsynaptic potential
fMRI	functional magnetic resonance imaging
FWER	familywise error rate
GABA	γ-aminobutyric acid
GABA-A-R	γ-aminobutyric acid type A receptor
GABA-B-R	γ-aminobutyric acid type B receptor
GABAR	γ-aminobutyric acid receptor
ICA	independent component analysis
IPSP	inhibitory postsynaptic potential
LTD	long term depression
LTP	long term potentiation
MCP	multiple comparison problem
MEP	motor evoked potential
M1	primary motor cortex
NMDA	N-methyl-D-aspartate
NMDAR	N-methyl-D-aspartate receptor
PET	positron emission tomography
RMT	resting motor threshold
RS-EEG	resting state electroencephalography
TEP	transcranial evoked potential
TMS	transcranial magnetic stimulation
TMS-EEG	transcranial magnetic stimulation with simultaneous electroencephalography measurement
TOI	time window of interest
VGCC	voltage gated calcium channel
VGSC	voltage gated sodium channel

1 Introduction

Recent advances in neurology have studied which mechanisms in the brain are driving neurological disorders. A key insight is that healthy brain function relies on a coordinated firing of neurons, while a break-down in synchronized neuron activity can explain many of the most serious neurological dysfunctions, such as schizophrenia, autism and epilepsy (Fritschy, 2008, Uhlhaas and Singer, 2012). Therefore, maintaining synchronized neuron firing is a key function of the healthy brain. An exciting new line of research describes the key bio-chemical mechanisms that control synchronized neuron firing. Two forces within the brain, the excitatory and inhibitory system, play an essential role for maintaining synchronized neuron firing (Isaacson and Scanziani, 2011). The excitatory system is responsible for mediating signals between cells that facilitate firing of the following cells. Whereas, the inhibitory system's signaling between cells makes them less likely to fire. The level of activity of the excitatory and inhibitory systems is controlled by neurotransmitters, which ensure that the two systems are in balance. The neurotransmitter responsible for the excitation in the human cortex and thus for an increase in activity is glutamate. The second neurotransmitter, that underpins the inhibitory system and reduces neural activity is γ-aminobutyric acid (GABA). However, their interrelations are not fully understood yet.

The aim of this study is to test whether glutamate receptors and voltage gated calcium channels affect that balance. Recent research utilizes drug applications whilst measuring the effect on the neural activity using a novel method: transcranial stimulation with simultaneously electroencephalography retrieving.

Different techniques have been developed in order to study whether brain activity is synchronized and what causes deviations. A unique technique is transcranial magnetic stimulation (TMS) combined with pharmaco-application (pharmaco-TMS) (Ziemann, 2004). This is a non-invasive and painless method which allows the investigation of different pharmacological targets in brain functioning (Hallett,

2007). To study the effects of central nervous system (CNS) active drugs we require a drug where the effects on the brain are well understood. Adding a method to evaluate cortex excitability and/or brain activity to the equation makes this method a door opener for studying drug effects on brain functioning. This goes beyond ordinary studies which focus exclusively on the pharmacology and are unable to provide direct evidence for the mechanism through which the drug takes effect.

Visualizing changes in brain functioning during laboratory testing remains challenging. One method that has developed is the parallel measurement of induced muscle activity. To do so the stimulation of the primary motor cortex (M1) has become an important stimulation site. Applying a stimulus at M1 provokes a visible and measurable muscle activity on the contralateral side. This muscle activity can be measured via electromyography, which shows a motor evoked potential (MEP) (Barker et al., 1985).

An even more detailed method to measure brain activity itself is the electroencephalography (EEG). Combining pharmaco TMS with simultaneous EEG recording (TMS-EEG) enables us to do so. Therefore, TMS-EEG has recently developed as a unique study and diagnostic tool (Ziemann, 2011, Ilmoniemi et al., 1997, Thut and Pascual-Leone, 2010).

In our context we want to study the underlying physiology of the evolution of potentials in the brain. These can be observed by looking at the response of the EEG to a single pulse TMS over the primary motor cortex. Such so called TMS-evoked potentials (TEPs) consist of specific positive and negative deflections (Premoli et al., 2014, Lioumis et al., 2009, Bonato et al., 2006). TEPs could serve as a novel biomarker for further characterization of inhibition/excitability of the brain. For now, the neurophysiology underlying TEPs still remains largely unclear.

Previous work has focused on the role of GABA receptors (GABARs) on TEP evolution and found that GABAR type A (GABA-A-R) have noticeable effects on brain activity that can be measured through TEPs. The distinctive pattern is as follows: About 45 ms after a stimulation of the receptor the TMS-EEG shows a negative deflection which is modulated by positive allosteric modulators and

antagonists of GABA-A-Rs (Premoli et al., 2014, Darmani et al., 2016). A second negative deflection can be detected around 100 ms post stimulation, which is modulated by GABARs type B (GABA-B-Rs) (Premoli et al., 2014, Premoli et al., 2018). Similarly, the application of a voltage-gated sodium blocker led to a reduction of the positive deflections around 25 ms (P25) (Darmani et al., 2018) and 180 ms post stimulation (Premoli et al., 2017, Darmani et al., 2018). Voltage-gated sodium channel blockers lower cortical excitability. Other studies showed a direct correlation between P25 and MEP amplitude (Maki and Ilmoniemi, 2010, Cash et al., 2017).

For a better understanding of brain activity many other mediators of the brain need to be considered. For instance, the role of the glutamatergic system is so far understudied, despite the fact that it plays a crucial role for the balance of neuronal excitability (Tatti et al., 2017). This study seeks to fill this gap and studies the glutamatergic system and the role of voltage-gated calcium channels and their effect on TEPs will be the subject of this work.

1.1 The major excitatory transmission: the glutamatergic system

Originally, it was speculated that glutamate has a metabolic function in the CNS (Krebs, 1935). It was not until the eighties that glutamate was acknowledged as a neurotransmitter (Fonnum, 1984). Today it is recognized as the major excitatory neurotransmitter in the mammalian brain. Due to the excitotoxic potential of glutamate, the glutamatergic system underlies multiple complex and energy-costly regulatory processes modulating glutamate metabolism.

Glutamatergic receptors are divided in two categories: ionotropic and metabotropic receptors. Ionotropic receptors consist of an ion channel which opens as a response to agonist binding and allows a cation influx. In contrast, metabotropic receptors interact with cognate G-proteins causing modulation of second messenger systems.

Subject to this study will be two of the ionotropic receptors involved in the excitatory neurotransmission of glutamate: the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) and the N-methyl-D-aspartate

receptor (NMDAR). These two receptors, each with distinct physiological properties, often coexist at the same synapse. In interaction with the third ionotropic receptor, the kainate-receptor, they mediate most of the rapid excitatory neurotransmission in the brain (Dingledine et al., 1999).

When an action potential reaches the presynaptic axon terminal it triggers a calcium influx through voltage gated calcium channels. The higher intracellular calcium concentration leads to the fusion of vesicular glutamatergic vesicles with the presynaptic membrane resulting in the release of glutamate into the synaptic gap. The neurotransmitter (glutamate) binds to a mixed population of NMDAR and non-NMDAR opening ion channels in the post synaptic membrane. This allows a rapid influx of positively charged ions resulting in an electrical event. Regularly, glutamate trans synaptic neurotransmission starts with a fast AMPAR response, leading to a sodium influx and depolarization of the membrane potential. The induced depolarization releases the magnesium blockade of the NMDAR allowing to open its channel and pass cations including calcium into the neuron (Figure 1.1.). Owed to this mechanism, action potentials generation is largely depended on AMPARs de/activation, whereas NMDARs enable summation of postsynaptic potentials (Niciu et al., 2012).

A dysfunction of the glutamatergic system plays a key role in various psychiatric and neurological disorders, e.g. schizophrenia (Hasan et al., 2014), epilepsy (Eid et al., 2008), or amyotrophic lateral sclerosis (Blasco et al., 2014).

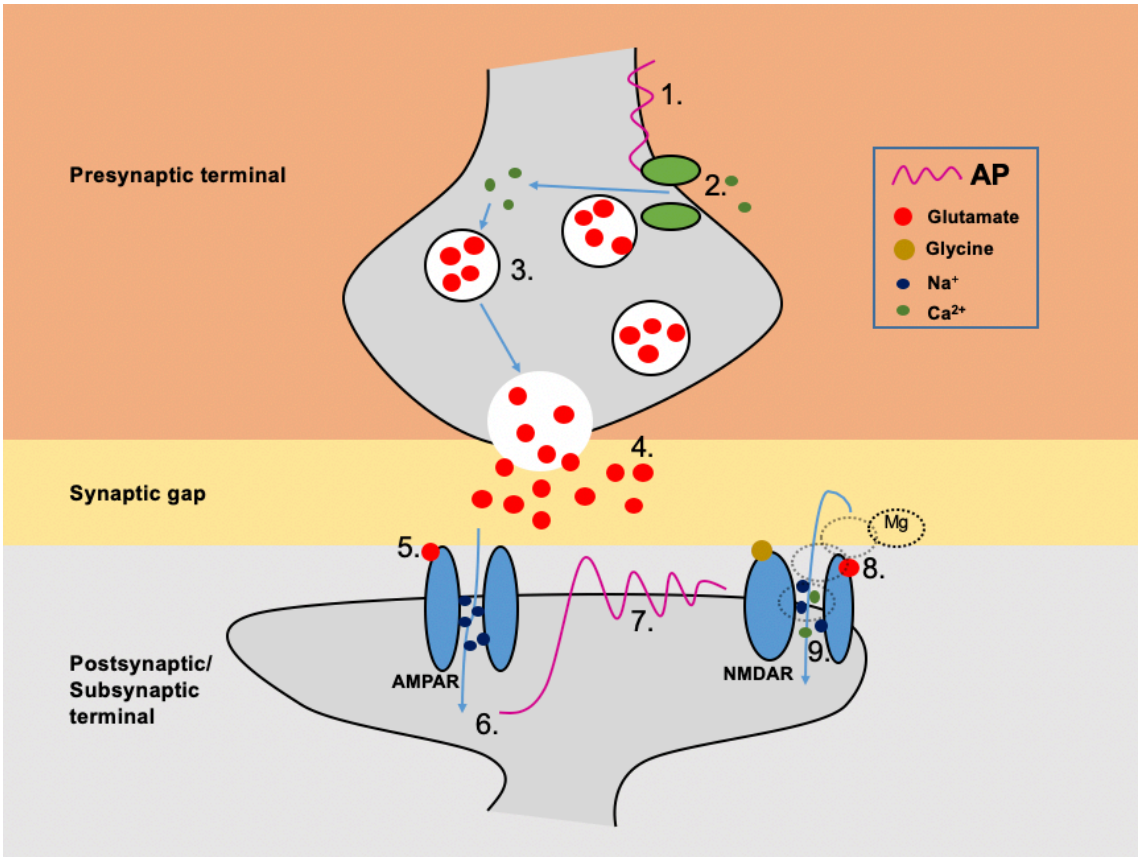

Figure 1.1 An excitatory glutamate synapse

When an action potential reaches the pre-synaptic terminal (1.), the depolarized membrane causes ion gated calcium channels to open up allowing a calcium influx (2.). The increased intracellular calcium leads to a fusion of glutamate vesicles with the membrane of the pre-synaptic terminal (3.) releasing glutamate into the synaptic gap (4.). Now the glutamate has various binding options. This illustration focuses on the two ionotropic glutamate channels AMPAR and NMDAR. When glutamate binds to the AMPAR receptor (5.) the ion gated channel within opens up allowing a cation influx, primarily sodium (6.). This leads to a depolarization of the postsynaptic membrane. When the NMDAR is reached by depolarization, the magnesium blockade is released (8.). This release plus the binding of two agonists (e.g. glutamate and glycine) activates NMDAR allowing a cation influx including a significant amount of calcium (9.). Intracellular calcium activates various second messenger pathways.

1.1.1 α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR)

Overall the majority of the excitatory synaptic transmission in the mammalian brain is conducted by AMPARs. AMPAR expression is spread throughout the CNS both in neurons and in glia (Belachew and Gallo, 2004, Wisden and Seeburg, 1993).

The AMPAR is a rather small glutamate-gated ion channel. It consists of four different subunits (GluR1-GluR4). These subunits are composed of 900 amino

acids. Together they form a tetramer located in the membrane with an extracellular N-termini and an intracellular C-termini. The agonist ligand binding domain (a lysine-arginine-othinine binding protein) is also located extracellular. The C-termini is the most variable and interaction-friendly part of the receptor (Soderling and Derkach, 2000, Malinow and Malenka, 2002, Collingridge et al., 2004) (Figure 1.2.). AMPARs have a small single channel conduction and fast kinetics, rapid inactivation and desensitization. Its conduction is proportional to the concentration of the agonist (Smith and Howe, 2000). Nevertheless, even small and brief glutamate contact leads to an excitatory potential (Niciu et al., 2012). Although AMPARs have a lower affinity to their substrate compared to the NMDARs. The activation of a native AMPAR induces a rapid opening of channels permeable mostly for sodium and potassium. AMPAR's ion specificity depends on the different mixture of subunit compounds. GluR2 for instance is not permeable for calcium ions. The cation influx causes a change in the membrane potential. This leads to an AMPAR-mediated excitatory postsynaptic current (EPSC) which initiates an AMPAR mediated fast excitatory postsynaptic potential (EPSP). Due to AMPARs' major role in EPSP mediation they are crucial factors in the evolution of epileptic activity (Rogawski, 2013).

AMPARs also play an essential role in long- and short-term synaptic plasticity of the brain (Bliss and Collingridge, 1993, Malenka and Nicoll, 1999, Malenka and Bear, 2004, Kullmann et al., 2000). Long term potentiation (LTP) involves an increasing AMPAR function, whereas long term depression (LTD) is expressed by decreasing AMPARs in the synapse. Short-term synaptic plasticity is mediated by an activity-dependent unblocking of AMPARs (Bowie et al., 1998, Rozov et al., 1998, Rozov and Burnashev, 1999)

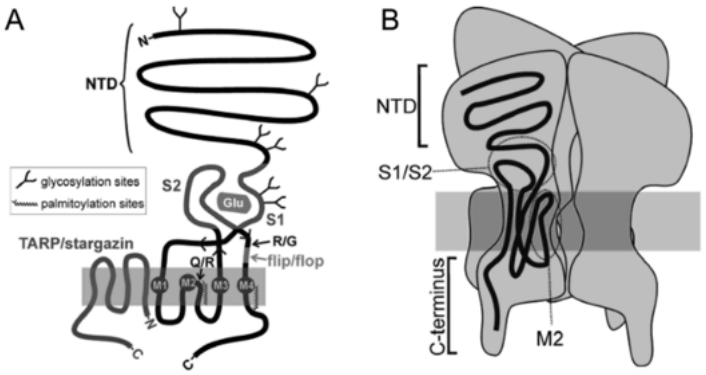

Figure 1.2 α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR)

(A) illustrates the schematic topology of the AMPAR transmembrane subunits (M1-4) and its extracellular N-terminal domain (NTD) and ligand binding domains (S1 and S2), as well as the intracellular C-terminal. Additionally, the receptor regulatory protein (TARP) is pictured. (B) shows a 3D representation of the subunit arrangement of an AMPAR. (Source: (Ashby M.C., 2008))

1.1.2 N-methyl-D-aspartate receptor (NMDAR)

Over 30 years ago the importance of MNDAR mediated synaptic activity for functional development of sensory brain circuits was discovered. Since then we have learned a great deal about this receptor, in particular about its shape and physiology.

NMDARs are found in neurons, glia and spinal cord (Hollmann and Heinemann, 1994, Cull-Candy et al., 2001, Wenthold et al., 2003, Salter and Fern, 2005) as well as in several organs (Skerry and Genever, 2001).

The NMDAR consists of different subunits from three subunit families (GluN1-GluN3). The heterogenic combination out of the subunits GluN1 with a subunits GluN2 and/or GluN3 (Khacho et al., 2015) form an NMDAR (Furukawa et al., 2005, Yao and Mayer, 2006). GluN1 and GluN3 contain a binding site for glycine while glutamate binds on subunit GluN2 (Figure 1.3.). NMDARs have the highest affinity for glutamate (EC50 1uM) (Niciu et al., 2012). The composition as well as the interaction with signaling proteins and the location of the NMDAR (postsynaptic, presynaptic and extrasynaptic) determine the specific function of the receptor (Niciu et al., 2012).

Each NMDAR contains a voltage gated Mg^{2+} blockage (Nowak et al., 1984), which is released through depolarization of the postsynaptic cell. Therefore,

NMDARs kinetics are slower in comparison to AMPARs. When the Mg^{2+} blockage is released and additionally two agonists (glutamate and a co-agonist e.g. glycine) attach to the binding site NMDARs are activated. NMDARs function as a non-selective channel for cations resulting in an influx of sodium, potassium and calcium. NMDAR activation leads to a prolonged EPSP, lasting up to several hundred milliseconds. Additionally, calcium acts as a second messenger activating different pathways including mediation of neuronal plasticity. The intracellular calcium activates the calmodulin-depended protein kinase leading to a phosphorylation of neighboring AMPARs at the postsynaptic membrane (Lisman et al., 1997, Barria et al., 1997a, Barria et al., 1997b). This leads to an insertion of new AMPARs into the postsynaptic membrane (Malinow and Malenka, 2002). Resulting in LTP, a long-lasting increase in neurotransmission (Bliss and Collingridge, 1993, Nicoll and Malenka, 1999). LTD, on the other hand, seems to be mostly modulated by non-synaptic NMDARs (Taylor et al., 2016).

EXCURSUS: Long-term potentiation (LTP) and long-term depression (LTD):
LTP describes the phenomenon of strengthened synaptic activity leading to a long-lasting increase in neurotransmitter signaling between two neurons. LTD, on the other hand, has the opposite effect – weakening of synaptic activity making two neurons less likely to communicate. Both, LTP and LTD, are properties of brain plasticity and therewith crucial for various forms of learning and memory formation (Lau and Zukin, 2007).
LTP and LTD are mainly modulated by the increase and decrease of AMPAR-mediated transmission. Many pathways have an influence on synaptic plasticity including also NMDAR-mediated neurotransmission (Malenka and Nicoll, 1993). Accordingly, it has been shown that the blockade of NMDARs with memantine and dextromethorphan inhibits LTP-like plasticity induced by non-invasive brain stimulation in humans (Schwenkreis et al., 2005, Stefan et al., 2002, Nitsche et al., 2003). In contrast, a study including a NMDAR agonist showed an enhancement in LTP-like plasticity (Nitsche et al 2003b).

Additionally, a role of voltage gated calcium channels (VGCCs) in LTP can be assumed. Studies showed that L-type and T-type VGCC blockers reduce LTP-like plasticity (Wankerl et al 2010, Wolters et al 2003; Nitsche et al 2003).

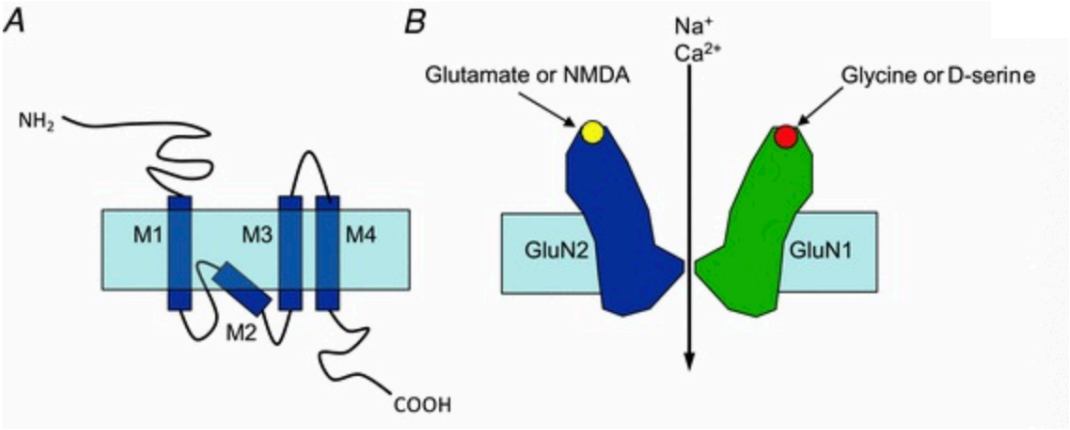

Figure 1.3 N-methyl-D-aspartate receptor (NMDAR)

(A) shows a basic sketch of the transmembrane topology of a NMDAR. Additionally, it illustrates the long extracellular N-terminal, which is involved in ligand binding, and the intracellular C-terminal. (B) presents a schematic NMDAR. It shows only one GluN2 and one GluN1 subunit, instead of the complete NMDAR consisting of four subunits. It is shown that two ligands (glutamate/N-methyl-D-aspartate and glycine/D-serine) have to bind in order for the non-selective ion channel to open up. Additionally, the magnesium (Mg) blockade has to be released to allow a cation influx (e.g. Na^+ and Ca^{2+}). (Source: (Stys et al., 2012))

1.2 Brain stimulation via transcranial magnetic stimulation (TMS)

Various neuro-imaging techniques and EEG give us the ability to map neuronal activity. TMS gives us the unique opportunity to study connectivity and functional organization of the brain. Furthermore, TMS allows stimulating and modulating neural activity (Wagner et al., 2007). TMS is a unique technique to noninvasively stimulate the human brain through a copper-wired coil placed on the scalp that induces a magnetic field. It allows to asses and study cortical network properties. TMS of the human cortex was first introduced in 1985 by Barker and colleagues (Barker et al., 1985). It has grown to great popularity as a noninvasive and painless tool to stimulate the cortex (Hallett, 2007) as well as a diagnostic technique without any long-term side effects (Chen et al., 2008).

An electric current flowing through a copper-wired coil creates a magnetic field in its surrounding (Figure 1.4.). A magnetic field can easily penetrate biological tissue. Therefore, a contactless and painless stimulation is possible. The time-changing magnetic field travels through the cranium and induces an electric current in the brain (Figure 1.4.) which causes a depolarization of neuronal membranes. The depolarization has the highest intensity on superficial layers (Heller and van Hulsteyn, 1992). If the depolarization overcomes the threshold potential, the membrane permeability increases inducing a sodium influx which results in an action potential (Ilmoniemi and Kicic, 2010).

An action potential develops after the all-or-none law after overcoming a threshold of -55 mV. Two channels are mainly responsible for the development of an action potential: voltage-gated sodium channels and potassium channels. In the beginning (-55 mV) the voltage-gated sodium channels open and a sodium influx starts. This leads to a lower concentration of positive ions on the outside of the cell/a higher concentration of positive ions on the insight off the cell, causing a depolarization of the cell membrane. At a potential of 30 mV the sodium channels start to close while potassium channels open, inducing a potassium efflux which leads to a repolarization and ultimately hyperpolarization (Figure 1.8.).

For the induction of an action potential the intensity of the stimulus, the conductivity properties of the head and the coil position/direction of the induced current are crucial factors (Ilmoniemi and Kicic, 2010, Janssen et al., 2015). The electric current flows rectangular to the magnetic field and, therefore, in parallel to the current in the coil, but in opposite direction (Figure 1.4.). The direction of the flowing current determines the activation of interneurons and pyramidal cells in the cortex (Hallett, 2007).

A coil used for a TMS application is always a compromise between handiness and overheating risk. Two types of coils are most widely used. A round coil which induces a broad stimulus underneath the ring with a relatively large stimulation depth, and an eight-shaped coil which allows a more focal stimulation of the area underneath the coil center (Cohen et al., 1990) but with less stimulation depth of approximately 2-4cm (Deng et al., 2014) and a higher heat evolution (Figure 1.4.).

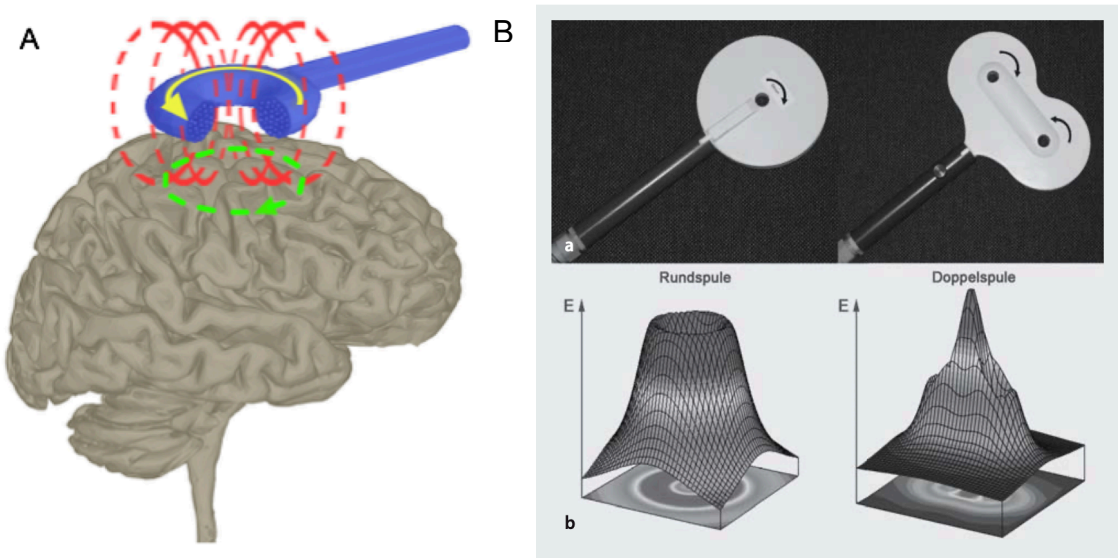

Figure 1.4 Coil functioning and form

(A) When a current flows through a coil (yellow line) it causes a magnetic field in its surrounding (red line) which again induces a current in the brain (green line). The induced current in the brain runs parallel but in the opposite direction of the current in the coil. (Own figure based on (Wassermann et al., 2008))
(B) Two different coil forms have been established. A round coil with a current running clock wise (left side). This coil induces a round shaped current in the cortex with a stimulation hole beneath the middle of the coil. It creates a deeper but less focal stimulus. On the other hand, there is a figure of eight coil with currents in each circle running in the opposite directions (right side). Therefore, this coil induces a very focal pyramidal shaped stimulation site with a less deep reach. (Source: (Siebner and Ziemann, 2007))

The transcranially induced activation is not just limited on the point of stimulation. Through transsynaptic transmission the activation is spread via cortico-cortico and cortico-subcortical pathways (Groppa et al., 2012, Voineskos et al., 2010). Stimulating the primary cortex, for instance, activates the corticospinal tract. Through glutamatergic transmission motor neurons are activated, resulting in a peripheral motor event, the so-called motor evoked potential (MEP) (Groppa et al., 2012, Barker et al., 1985) (Figure 1.8.). Although TMS is not limited to stimulation of the primary motor cortex, it has developed as a popular stimulation site because the stimulation effect can be measured with electromyography (EMG) as a MEP. The MEP latencies and amplitudes give indirect information about cortical excitability and conduction of the cortico-spinal tract (Hallett, 2000).

Various stimulation protocols with single or paired pulses exist to explore cortical excitability (Groppa et al., 2012). In order to elicit sufficient stimulation, usually the resting motor threshold (RMT) is being determined at the beginning of the measurements. The RMT is defined as the minimum single-pulse stimulus intensity that elicits regular but minimum muscle responses.

1.3 Recording brain activity via electroencephalography (EEG)

A key issue to make drug effects on brain activity visible is to measure regional neuronal activity. This is required to draw any conclusions regarding drug effect on brain activity. EEG is a tool to detect drug-induced changes in cortical activity. Contemporary EEG was first introduced in 1929 (Berger, 1938) and enables a noninvasive method to measure regional neuronal activity of the brain with an excellent resolution in the time domain. Therefore, the electric potential differences between an electrode pair is measured on the scalp. The potentials registered by EEG are the summation of postsynaptic potentials from thousands of neurons (Kirschstein and Kohling, 2009) (Figure 1.5.).

Signals of interest arise from synchronized activity of groups of neurons that are close to each other and exhibit a similar pattern of activity. Indeed, a single neuron alone will not be able to elicit a measurable signal on the scalp. But a whole population of several thousand neurons that are activated synchronously can. The measured currents by EEG reflect postsynaptic potentials and not action potentials. Postsynaptic potentials are not subject to the all-or-none-law and have a longer duration (10-100 ms) than action potentials (Kirschstein and Kohling, 2009) making them more accessible for EEG measurements.

During synaptic neurotransmission a chemical signal induces ion channels to open, resulting in electric potentials in the subsynaptic terminal. Postsynaptic potentials are defined as the charge difference between the subsynaptic membrane and the postsynaptic membrane (Figure 1.5.). Depending on their excitatory or inhibitory effect they are called excitatory postsynaptic potentials (EPSPs) or inhibitory postsynaptic potentials (IPSPs).

In order to understand the physiology behind EEG registration at an excitatory synapse one has to look at the outer cell membrane. The outer cell membrane

becomes more negative when sodium influxes, promoting an action potential. Due to the negative charge the outer cell membrane of the subsynaptic part becomes a negative pole, whereas the postsynaptic membrane becomes the positive pole. A structure including a negative pole and a positive pole is called a dipole (Figure 1.6.).

Looking at the physical convention of a dipole, an electric flow travels from the positive to the negative pole. Thus, the minus pole correlates with a current sink whereas the plus pole correlates with the current source (Figure 1.6.). Depending on their source and orientation the summation of these currents can form a negative extracellular polarity near the scalp surface. This displays as an upward deflection in EEG. An IPSP creates a similar dipole but with a reversed polarity.

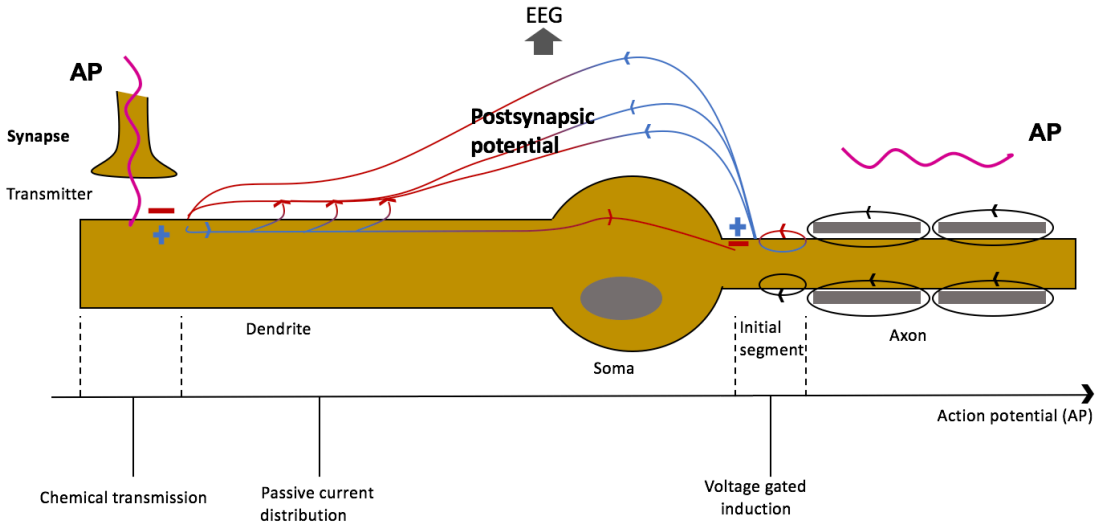

Figure 1.5 Physiology of electroencephalography derivation

When an action potential (AP) reaches a presynaptic terminal an activation is passed to the neighboring cell via a neurochemical synapsis. This triggers a passive current distribution along the postsynaptic membrane. At the axon this electron migration leads to a voltage gated induction of an action potential. Between the neuron terminal with a passive electro distribution, the subsynaptic terminal, and the axon itself exists a relative potential difference of the outer membrane creating a postsynaptic potential. These postsynaptic potentials of activated neighboring neurons sum up and can be recorded via EEG. (own figure based on (Zschocke and Hansen, 2012))

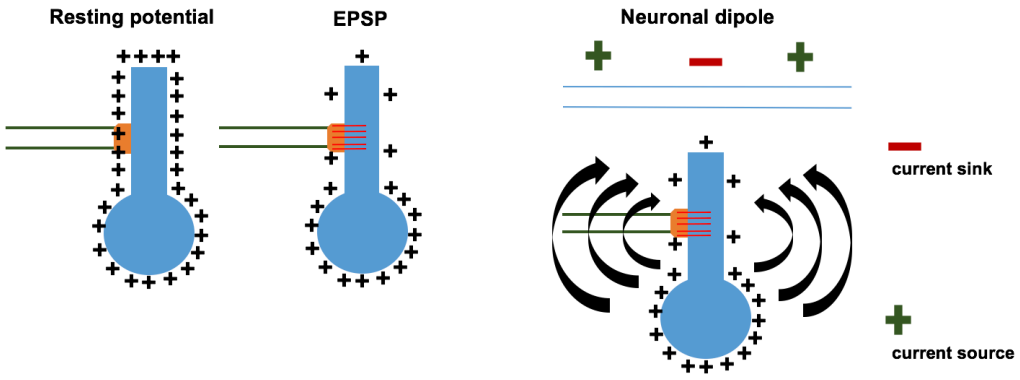

Figure 1.6 Neuronal dipole

When an excitatory synapse activates the following neurons, it induces a sodium influx into the subsynaptic terminal. Because positive charged ions migrate from the outside of the cell into the neuron, the outer membrane of the subsynaptic terminal becomes relatively more negative. Thus makes the subsynaptic terminal with its negative charge on the outer membrane the current sink. Whereas, the postsynaptic terminal becomes the current source because it remains more positive on the outer membrane in comparison to the subsynaptic terminal. Thus creates a dipole with a positive (current source) and a negative (current sink) terminal.

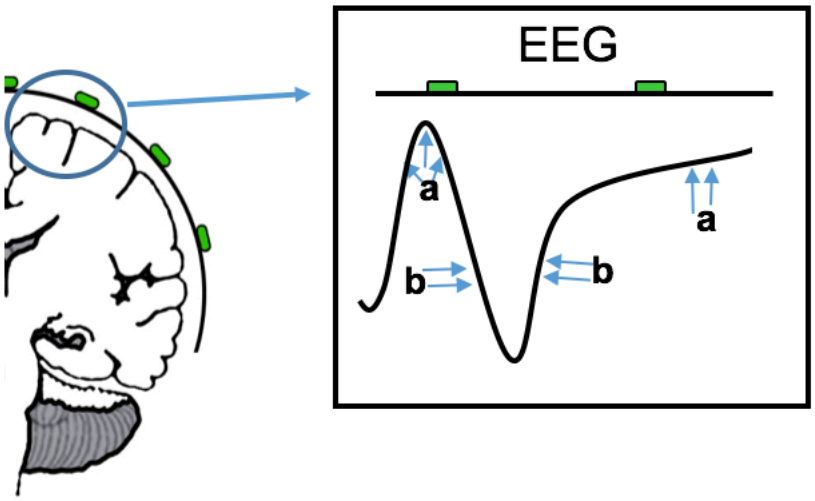

Figure 1.7 Dipole orientation

The orientation of the neuronal dipole decides over the availability for EEG registration. Is the dipole perpendicular oriented to the scalp surface (a) a clear surface negativity can be recorded. Whereas, when the dipole is oriented parallel to the surface (b) , e.g. in the gyri, a lot of the signal does not reach the scalp surface and can therefore not be recorded by EEG with scalp electrodes,

The representation of cortical activity via EEG depends on various factors: orientation of the electric dipole, extent of synchronized neural activity, conductance of the tissue, distance between the evolved potential source and the EEG-electrode and which electrode derivation program is used.
Among cortical neurons, pyramidal cells are believed to be the main source of EEG signals. This is because they are roughly parallel to each other and oriented perpendicular to the cortex surface. Only dipoles that are perpendicular to the scalp surface can fully be registered by EEG (Figure 1.7.). Therefore, when they are activated simultaneously their contributions sum up into a macroscopic current. Pyramidal neuron somata are mainly located in the cortical layer V. They have long apical dendrites extending towards the cortex superficial layers creating a perpendicular oriented dipole when activated.
Neurons have numerous synapses, for instance a single pyramidal cell can have up to 10.000 synapses. Each synapse develops a dipole when activated. During simultaneous synapse activation, newly formed dipoles add up to a summation dipole. Only a summation dipole has a sufficient potential to be registered by EEG.

EEG is recorded by a differential amplifier. An amplifier takes two different inputs and defines the output as the difference of those two (input 1 minus input 2). This technique is useful for recording very small electrical differences and discarding common noise waves. The ability to extract the common waves while differences remain is called the common mode rejection ratio. Due to the measurements of differences, EEG retrievals are always relative measurements rather than absolute ones.
Even though EEG has an excellent time resolution, its regional resolution is not as accurate. The electrodes are 1-2 cm apart which equals the regional resolution of the EEG data. One single electrode receives data from 100 million up to 1 billion neurons (Olejniczak, 2006). Another known limitation of EEG is its 2 dimensionality. The problem of drawing conclusions from 2D data for mapping of a 3D structure is called inverse problem and has no unique solution yet (Olejniczak, 2006).

EEG investigations may add valuable information to the clinical differential diagnosis in neuropsychiatric disorders (e.g. schizophrenia or Alzheimer's disease) (Sponheim et al., 2000, Babiloni et al., 2011), epilepsy (Renzel et al., 2016), stroke or tumor (Selvam and Shenbagadevi, 2011) and is frequently used for perioperative monitoring. The simultaneous application of an event that creates a potential and EEG recording enhances this procedure as a diagnostic tool to explore central nervous system diseases (e.g. multiple sclerosis) and neurophysiological mechanisms (Daskalakis et al., 2012).

However, EEG is often only used as an additional diagnostic tool due to its limitations to draw specific conclusions of the location of brain abnormalities (Niedermeyer, 1996) as well as a frequently late EEG involvement in diseases. Combining EEG with other neuronavigation, neuroimaging or neurostimulation techniques can drastically improve EEG's diagnostic capability.

1.4 The evolution of pharmaco TMS-EEG

TMS gives us the opportunity to study connectivity and pathways of evoked potentials but leaves a blind spot regarding the direct visualization of brain activity.

In order to retrieve an interpretable readout whilst applying TMS, a combination of TMS with EMG was developed. Therefore, the hand area of the primary motor cortex (M1) became a popular stimulation site. Stimulation of the primary motor cortex activates the corticospinal/corticomotoneuronal pathways (Hallett, 2007, Barker et al., 1985) eliciting a direct motor response. The muscle activity can be measured via EMG and interpreted as an indirect marker for cortical excitability (Daskalakis et al., 2002). But TMS-EMG is only significant when a motor area is stimulated and studied (Ilmoniemi et al., 1997).

Combining TMS with EEG overcomes this limitation and can be used to stimulate both motor and non-motor regions and study sensory pathways and subcortical structures (Siebner et al., 2009). EEG alone draws as a great picture of ongoing brain activity but leaves out giving information about connectivity of brain pathways and brain plasticity. With TMS-EEG it is possible to evaluate if a brain area is hyper- or hypoexcitable, e.g. due to drug application or disease. Studies

have shown that alcohol reduces prefrontal excitability (Kahkonen et al., 2003). In schizophrenia the spreading of a TMS stimulus is modulated (Ferrarelli et al., 2008). And during deep sleep the general cortical excitability is reduced (Massimini et al., 2005a). EEG can show location and distribution of postsynaptic currents which allows to draw conclusions of local excitability and area-to-area functional connectivity (Komssi et al., 2004, Massimini et al., 2005b). It has a high time resolution allowing the direct measurement of the TMS-evoked EEG potentials (Ilmoniemi et al., 1997). This is a big advantage in comparison to the combination of TMS with other neuroimaging techniques such as functional magnetic resonance imaging (fMRI) (Bohning et al., 1998, Bohning et al., 2000), near-infrared spectroscopy (Furubayashi et al., 2013) or positron emission tomography (PET) (Fox et al., 1997).

However, combining TMS and EEG has shown a lot of technical challenges. But recent technical developments allow a combination of TMS and simultaneous EEG measurements providing a new possibility to study the brain's excitability, time-resolved connectivity and plasticity (Kimiskidis et al., 2014, Rogasch and Fitzgerald, 2013). Although TMS-EEG measurements and analysis are rapidly evolving (Rogasch et al., 2017) this procedure still presents a technical and methodical challenge. Due to the induced electrical artifact at the time of stimulation it is challenging to recover a clear EEG-signal with conventional amplifiers. It would take them several hundreds of milliseconds to recover from the TMS induced electrical signal (Fuggetta et al., 2005, Izumi et al., 1997, Virtanen et al., 1999, Veniero et al., 2009). The recorded data must have a low noise level and at the same time be insensitive or recover quickly from the stimulation impulse. Therefore, specific TMS-compatible amplifiers need to be used for TMS-EEG measurements. There are two kinds of amplifiers with two different techniques to solve this problem.
In 1999 Virtanen et al. developed a TMS-compatible EEG system to deal with the electromagnetic artifact. This EEG system can gain control by sample-and-hold circuits preventing the TMS artifact from being passed along to the amplifier (Ilmoniemi et al., 1997). Afterwards a high-pass filter (f>0.1 Hz) and a low pass

filter (f<500 Hz) are applied. Then the data is recorded via A/D conversion with a sampling rate of 1450 Hz (Virtanen et al., 1999).
Another option was introduced by Ives and coworkers using an amplifier with a limited slew-rate that allows a continuous measurement (Ives et al., 2006). However, this technique requires a baseline measurement and has an increased noise level due to combining the two signals (Ilmoniemi and Kicic, 2010).
The use of an BrainAmp (BrainProducts GmbH, Munich, Germany) amplifier allows to adjust the operation range and the sensitivity to the applied TMS strength. This permits a continuous measurement without the use of sample-and-hold circuits with a recovery rate of approximately 5 ms after the TMS pulse (Ilmoniemi and Kicic, 2010).
To summarize, EEG recording systems must be insensitive to TMS stimuli or need to recover quickly from the TMS pulse. In addition, it is necessary to repeat stimulations dozens of times and average the trials in order to receive a sufficiently good signal-to-noise ratio (Ilmoniemi and Kicic, 2010).

One needs to be aware of various other artifacts during TMS-EEG data cleaning. Not just the impulse itself but also the recharging of the coil can interfere with EEG recordings (Veniero et al., 2009). This needs to be detected and removed offline during data cleaning.
Direct contact of EEG electrodes with the coil and vibrations can lead to movements of the electrodes which disturb the distribution of charge resolution in a potential change (Ilmoniemi and Kicic, 2010). A direct or indirect connection between two electrodes leads to electrode polarization caused by electric currents between electrodes (Ilmoniemi and Kicic, 2010). During TMS measurements heat is produced. In order to avoid overheating of the electrodes a small diameter and a special coating with Ag-AgCl can reduce overheating while maintaining high-quality recordings (Ilmoniemi and Kicic, 2010). Nevertheless, low impedances (<5 kΩ) are the foundation of keeping thermal voltage noise and artifacts from electrode movements or polarization to a minimum (Ilmoniemi and Kicic, 2010).

Eye movements and blinking trigger an EEG-potential (Regan and Regan, 1989) which should be additionally monitored with periorbital electrodes. Depolarization of scalp muscle fibers due to TMS leads to muscle activity. Artifacts induced by evoked scalp muscle activity may be reduced by coil reorientation or reduction of the stimulus intensity (Komssi et al., 2004, Komssi et al., 2007, Kahkonen, 2005). Charging of the TMS coil leads to a brief deformation of the coil which makes a loud clicking noise when released. This activates the auditory system eliciting an auditory evoked potential (Nikouline et al., 1999). TMS triggers scalp sensations which lead to somatosensory evoked potentials. These potentials have in general a large amplitude over the contralateral hemisphere (Bennett and Jannetta, 1980, Hashimoto, 1988).
During TMS-EEG measurement and analysis the awareness of the described artifacts is important in order to avoid misinterpretation as TMS evoked potentials.

TMS of the motor cortex with an intensity above the threshold induces a specific EEG response characterized by positive and negative deflections at highly reproducible latencies (Bergmann et al., 2012, Bonato et al., 2006, Ferreri et al., 2011, Ilmoniemi et al., 1997). TMS-evoked EEG potentials (TEPs) showed in various studies alternating positive (around 13, 30, 60, 190 ms post stimulus) and negative deflections (around 7, 18, 44 and 100 ms post stimulus) (Bonato et al., 2006, Lioumis et al., 2009). The most reproducible TEPs used for analysis in prior studies as well as this one are positive peaks around 25, 70 and 180 ms post stimulus (P25, P70, P180) and negative peaks around 45 and 100 ms post stimulus (N45, N100) (Premoli et al., 2014) (Figure 1.8.).
Studies show a high reproducibility of TMS-evoked EEG deflections in the first 200 ms after stimulation (correlation factor exceeding 0.83) (Lioumis et al., 2009, Casarotto et al., 2010), but these are sensitive to a couple of external factors such as the position and orientation of the coil (Komssi et al., 2002), the state of the cortex (Nikulin et al., 2003) and the vigilance of the subject (Massimini et al., 2005a).

The physiological processes underlying TEPs are not fully understood (Premoli et al., 2014). TEPs need to be further characterized in order to be used as a promising tool for future biological in-vivo markers of brain functioning.

Furthermore, prior studies could identify different locations of the individual TEP components and illustrate them in topographical maps of voltages, so called topoplots. Earlier TEP components are located in the central (P25) and contralateral frontal area (N45). P70 has an activity distribution in the stimulated hemisphere. Later TEP components showed a bilateral distribution over the central (N100) and centro-frontal (P180) cortex (Premoli et al., 2014, Bonato et al., 2006, Ferreri et al., 2011, Rogasch and Fitzgerald, 2013, Komssi et al., 2004).

Combining TMS-EEG and application of CNS active drugs with a well understood mode of action in the brain is a ‘key-tool’ to study drug induced modulation and therewith the involvement of different receptors and neurotransmitters in the physiological underpinnings of TEPs (Ziemann et al., 2015). This work will focus on the role of glutamatergic receptors in the physiology of TEPs.

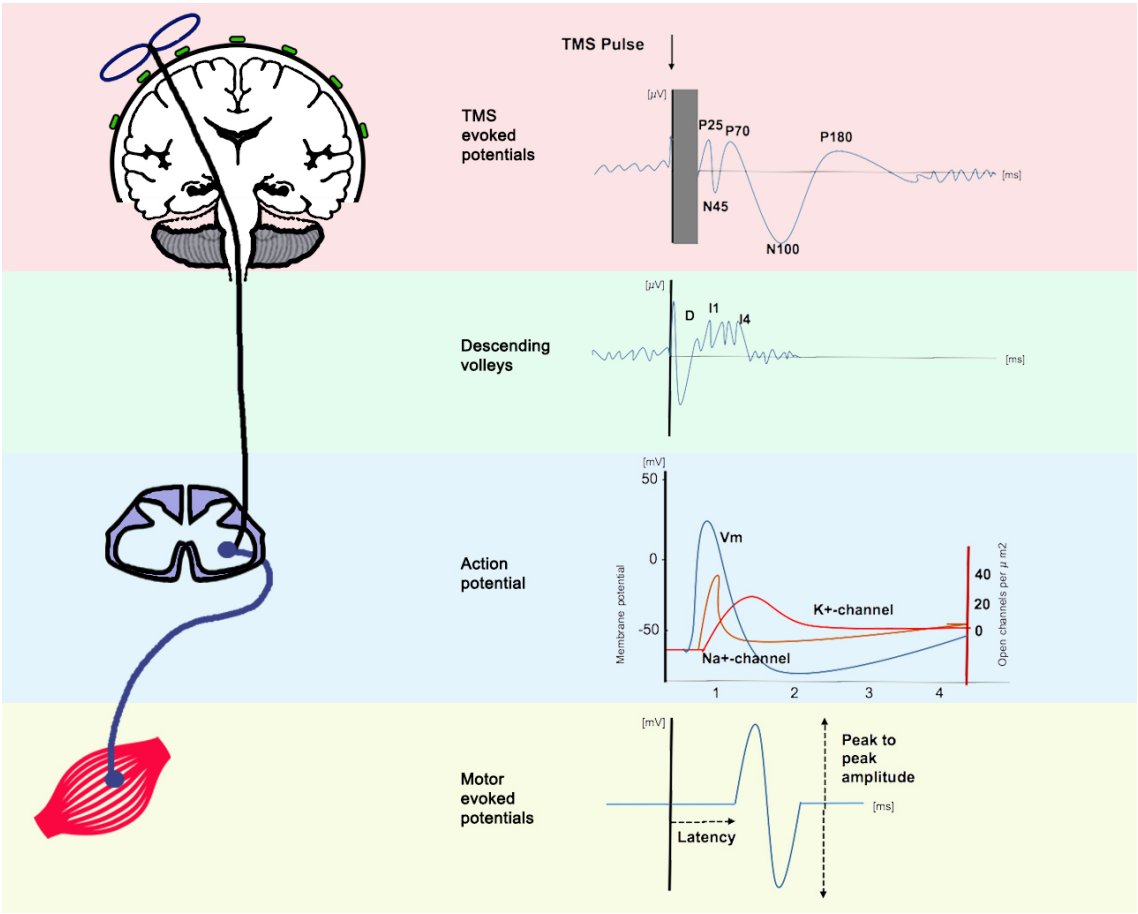

Figure 1.8 Evoked potentials elicited by transcranial magnetic stimulation

The left side of the figure illustrates a simplified sketch of transcranial magnetic stimulation (TMS) of the primary motor cortex via a figure of eight coil. Simultaneously electroencephalography (EEG) is deviated. A stimulus travels through corticospinal pathways down to the anterior horn of the spinal cord. From there the stimulus travels via efferent pathways to the muscle eliciting a contraction. The right side demonstrates sketches of different potentials that can be recorded. Looking at the EEG data simultaneously to stimulation a TMS evoked potentials (TEPs) can be recorded. A TEP consists of five reproducible components as described in literature (Bonato et al., 2006, Lioumis et al., 2009, Premoli et al., 2014): P25, N45, P70, N100, P180 (P = positive deflection, N = negative deflection). The activation travels down the corticospinal pathway eliciting descending volleys. These consist of a D-wave followed by four I-waves.

When the summation of excitatory postsynaptic potentials reaches a threshold an action potential is triggered. At the threshold of -55mV at a cellular membrane, rapid voltage gated sodium channels open up causing a depolarization of the membrane. At around +30mV the sodium channels are closing again while potassium channels open up causing a short hyperpolarization of the membrane. The stimulus travels as an action potential through the pyramidal pathways towards the myoneural junction. The induced muscle activity which can be recorded via electromyography (EMG) and is called motor evoked potential (MEP) (Own figure based on (Siebner and Ziemann, 2007)).

1.5 Study medication

In this study we investigated the impact of three drugs and placebo on TEPs: Dextromethorphan, an antitussive drug; perampanel, an antiepileptic drug (Faulkner, 2017); and nimodipine, a vasodilator.

1.5.1 Dextromethorphan (DM)

Dextromethorphan (DM) functions as an antagonist at the NMDAR. It has been used over 50 years as an antitussive although the molecular mechanism underlying the antitussive effect has not been fully understood (Kamei et al., 1989, Brown et al., 2004, Kim et al., 2009, Canning and Mori, 2010, Young and Smith, 2011).

DM is a prodrug that in vivo is rapidly metabolized to its active form dextrorphan. DM binds as a non-competitive antagonist deeply inside the ion channel pore of the NMDAR (Ferrer-Montiel et al., 1998, Wong et al., 1988).

The activated prodrug shows a very complex pharmacology beyond blockade of NMDARs. For instance, studies have shown a pharmacological interaction with serotonin transporters, noradrenalin transporters, sigma-1-receptors and nicotinergic acetylcholine receptors (Taylor et al., 2016). Although DM shows a wide interaction in the CNS, it has per se very little clinical effects beyond the antitussive effect. This is probably due to its low and variable bioavailability caused by a rapid first-pass metabolism and subsequent elimination (Taylor et al., 2016). It reaches its peak plasma concentration at approximately 1-2 hours post drug intake (PRODUCT INFORMATION Hustenstiller-ratiopharm® Dextromethorphan).

A dosage of 120 mg of DM is assumed safe since in prior studies an even higher dosage (150 mg) showed no or only mild side effects, which did not interfere with the participants' ability to fully comply with the study requirements (Ziemann et al., 1998a).

Beyond its clinical use, DM has shown in human studies an effect on reduction of synaptic plasticity in the human cortex by modulating Ca^{2+} dynamics (Stefan et al., 2002, Wankerl et al., 2010a).

1.5.2 Perampanel / Fycompa

Perampanel (5′-(2-Cyanphenyl)-1′-phenyl-2,3′-bipyridinyl-6′(1′H)-on) functions as a potent non-competitive AMPAR antagonist (Rogawski and Hanada, 2013). It blocks the AMPAR-mediated synaptic glutamatergic transmission (Ceolin et al., 2012). For a better understanding of the induced allosteric blockage further studies investigating the exact binding side are required. Studies suggest that perampanel is highly selective and does not have any effect on NMDAR or kainate receptors (Hanada et al., 2011). After oral application perampanel is rapidly and almost completely absorbed and undergoes a low clearance effect. Overall it has a high bioavailability after oral application. Its half-life in humans adds up to 95-105 hours with a peak plasma concentration at 0.5-4 hours after intake (Rogawski and Hanada, 2013, Xinning Yang, 2012)(Phase I randomized biopharmaceutical study E2007-A001-040 conducted by Eisai) .

Perampanel is an approved add-on therapy for focal epileptic seizures. The therapeutic dosage of perampanel ranges between 4 mg and 12 mg per day (French et al., 2012, Krauss et al., 2012, French et al., 2013). Side effects (e.g. dizziness) and antiepileptic potential are increasing with higher dosages (Rogawski and Hanada, 2013, Krauss et al., 2012). Given the role of AMPARs in EPSP generation, acute motor toxic side effects are not unexpected. However, perampanel has demonstrated in various studies an acceptable tolerability in patients and healthy subjects (French et al., 2012, Krauss et al., 2012, French et al., 2013).

AMPARs play a key role in the evolution of epileptic activity. Therefore, perampanel, as an AMPAR antagonist, has an antiepileptic effect. Even low levels of this antagonist seem to have an antiepileptic effect, underlining the importance of AMPAR in the evolution of epileptic activity (Rogawski, 2013).

1.5.3 Nimodipine

Nimodipine (C21H26N2O7) is a L-type voltage-gated calcium channel (L-VGCC) blocker (Hess et al., 1984). It blocks the L-VGCC in its inactive form inhibiting calcium influx. Due to its lipophilic character it can easily pass the blood-brain barrier and act on cerebral blood vessels (M. Das and Zito, 2018). Nimodipine

can be applied orally or intravenously. A parenteral application has a 100% bioavailability. In contrast, oral application undergoes a high first-pass effect resulting in a bioavailability of only 3-30%. The recommended dosage used after subarachnoid hemorrhage is 60 mg p.o. every 4 hours. It reaches a peak plasma concentration at 0.6-1.6 hours after drug intake (PRODUCT INFORMATION NIMOTOP® Nimodipine Bayer Resources).

Nimodipine was originally introduced as an antihypertensive drug. In 1998 nimodipine was approved by the Food and Drug Administration but with restricted use of preventing vasospasm after subarachnoid hemorrhage (M. Das and Zito, 2018). Nevertheless, nimodipine found off-label use in various other therapies, for instance in the treatment of brain and nerve injury (Sun et al., 2018, Scheller et al., 2016), migraine (Mu and Wang, 2018, Leone et al., 1990) and glaucoma (Maria et al., 2017). Even though the use of nimodipine in these settings still remains controversial (Teasdale et al., 1990, Murray et al., 1996).

Studies indicate a neuroprotective effect of nimodipine by preventing a calcium overload in ischemic neurons (Wahlgren et al., 1994). However, this could not yet have been translated into an efficient clinical setting.

Even though L-VGCCs are not directly involved in glutamate release from the presynaptic nerve terminals (Catterall, 2011), nimodipine does have an effect on cortex plasticity similar to DM. Nimodipine reduces plasticity of the human cortex probably through inhibiting calcium influx (Wankerl et al., 2010a, Weise et al., 2017, Igelmund et al., 1996).

1.6 Summary of research goals

The physiology underlying TEPs is still largely unknown and for this reason their utility for clinical applications is as of yet limited. In this study we take a closer look at the underpinning physiology of TEPs as in vivo bio-markers for brain functioning and cortical connectivity.

So far there have been studies investigating the role of GABAR / inhibitory neurotransmission on TEPs. Whereas, the role of excitatory neurotransmission and calcium for TEPs remains elusive. To the best of our knowledge there have not been any pharmacological studies in human subjects in regard to this topic.

Indirect evidence from former studies suggest an AMPAR involvement in the early TEP components up to 18 ms post stimulation (Komssi et al., 2004, Bonato et al., 2006, Maki and Ilmoniemi, 2010, Ferreri et al., 2011, Rogasch and Fitzgerald, 2013). In addition, it suggests a glutamatergic involvement in the evolution of the negative deflection around 44 ms post stimulation. Also study results suggest that AMPAR- and/or NMDAR-mediated neurotransmission is a crucial factor for cortico-cortical and/or cortico-thalamo-cortical propagation of evoked neural activity (Premoli et al., 2014).

In order to extend the currently available limited evidence, we use a pharmaco-TMS-EEG approach to investigate the role of AMPAR- and NMDAR-mediated neurotransmission and L-VGCCs for TMS-evoked EEG responses. Therefore, we use three drugs with clear modes of action - perampanel (AMPAR antagonist), dextromethorphan (NMDAR antagonist) and nimodipine (L-VGCC blocker) – in comparison to placebo in a pseudorandomized double-blinded study design.

Our hypotheses were:

1. Perampanel decreases the early and late TEP amplitudes in comparison to placebo
2. Dextromethorphan decreases the later TEP amplitudes in comparison to placebo
3. Nimodipine decreases the amplitude and propagation of TEPs in comparison to placebo
4. Perampanel and/or dextromethorphan respectively will constrain the topographical distribution of early and late TEPs in comparison to placebo.

2 Material and Methods

2.1 Participants

We included eighteen healthy subjects between the age of 22-36 years (mean age 26.0 ± 3.5) in the study. Two of these subjects dropped out during the experiment due to unrelated medical and personal reasons, leaving us with sixteen full data sets that were included in the data analysis.

All subjects underwent a screening procedure before being included in the study. This involved a physical and neurological exam as well as a test TMS trial with RMT determination. Anyone experiencing any discomfort during stimulation or having RMT higher than 60% was not enrolled because these are indicators for higher artifact vulnerability (Ilmoniemi and Kicic, 2010).

Everyone was screened for contraindications for TMS (e.g. a history of seizure or head injury, other neurological or psychiatric diseases among others) (Rossi et al., 2009) and the study medication (e.g. chronic liver- or kidney disease, low blood pressure).

Additionally, only male participants were included to avoid effects on cortical excitability by the female menstrual cycle (Smith et al., 1999).

Furthermore, the use of any CNS active drugs or any substance abuse (including nicotine and alcohol) were excluding factors.

To avoid possible result distortion by different functional network properties in left handers (Kirsch et al., 2018, De Gennaro et al., 2004), only right-handed subjects were included. To determine their right-handedness, a questionnaire according to the Edinburgh Inventory was performed (laterality score 88 ± 15) (Oldfield, 1971).

All participants were native to the drugs that were applied in the study.

Each subject received a magnetic resonance imaging in order to exclude any structural brain anomalies.

This study design was approved by the local Ethics Review Committee of the Medical Faculty of Eberhard-Karls-Universität Tübingen, Germany (Reg.-No.: 536/2014BO1). All subjects provided written informed consent prior to study

inclusion, and the study was conducted in accordance with the latest version of the declaration of Helsinki.

2.2 Experimental design

For a further characterization of pharmaco-physiological properties of brain functioning we used a pharmaco-TMS-EEG approach modulating the glutamate system and L-VGCC to extend previous pharmaco-TMS-EEG investigations on the physiology of TEPs (Premoli et al., 2014, Darmani et al., 2016). To do so we performed a pseudorandomized, placebo-controlled, double-blind crossover study design. Three drugs were used as study medications: perampanel, an AMPAR antagonist, dextromethorphan, a NMDAR antagonist, and nimodipine, a L-VGCC blocker. As a control condition placebo was administered.

Each subject underwent four sessions, each with the same procedure but a different drug condition. Each session consisted of a pre-drug measurement to evaluate a baseline and a post-drug measurement to investigate the effects of the drug. Both, pre and post drug measurements, were performed with the same protocol. In the beginning the RMT was determined. To avoid effects on TEPs due to a varied excitability at the post-drug measurement, stimulation intensity was adjusted in the post-drug measurements if needed. The determination of RMT was performed via the relative frequency method (Groppa et al., 2012), detecting the minimum intensity to elicit MEPs with an amplitude of at least 50 µV peak-to-peak in at least five out of ten trials. RMT determination was followed by a measurement of the resting state EEG (RS-EEG). This consisted of two parts: a 3-min measurement of RS-EEG with eyes closed and a 3-min measurement of RS-EEG with eyes open, fixation on one spot. Afterwards the TMS-EEG measurements took place. 150 single monophasic stimuli were applied. To avoid habituation, we used a randomized interstimulus interval of 5 s $\pm$ 1 s. Stimulation-target was the hand area in the primary motor cortex of the left hemisphere (M1). Between the pre- and post-drug measurements a two-hour medication break took place. Therein, one of the three different drugs or placebo was administered. To account for different drug pharmacokinetics the drugs where applied at two

different times. This allowed each medication to reach its estimated peak plasma concentration when post-drug measurements were performed (see table 2.1.). The participants received either 12 mg/6 mg of perampanel or 120 mg of dextromethorphan or 30 mg of nimodipine or placebo. In order to keep a double blinded study design even with different drug applications, two points in time were defined. At one time point the participant received a drug/placebo and at the respectively another time point placebo was administered (see table 2.2.). Thus, latencies between drug intake and post-drug measurements was 120 min for dextromethorphan and 60 min for perampanel and nimodipine.

All applied dosages were previously approved for medical use and are standard dosages in the clinical use. The dosage of perampanel had to be reduced from 12 mg to 6 mg after three subjects had been tested, due to side effects. Therefore, three participants received 12 mg perampanel and the remaining 13 participants received only 6 mg perampanel.

All sessions where at least two weeks apart from each other to avoid any carry-over effects of the drugs.

Due to nimodipine's effect on lowering the blood pressure we performed a blood pressure screening at five time points during the session.

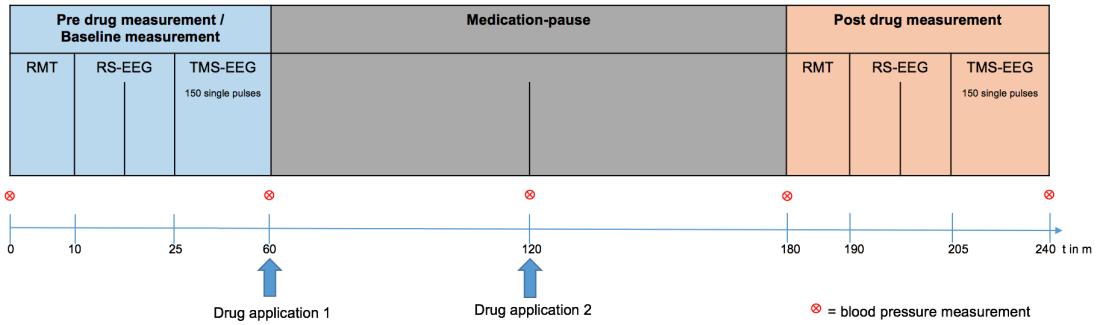

Figure 2.1 Timetable of one session

Each session is composed of a pre drug measurement / baseline measurement and a post drug measurement separated by a two-hour medication break. The measurements consisted of the determination of the resting motor threshold (RMT), followed by a resting state electroencephalography (RS-EEG) 3 min with eyes closed and 3 min with eyes open. To complete the measurement 150 single monophasic stimuli were applied via transcranial magnetic stimulation while simultaneously recording the electroencephalography (TMS-EEG).

During the medication pause two drug application time spots were defined. One in the beginning of the break and one after one hour of break.Throughout the session blood pressure was measured at 5 different time points.

Table 2.1 Study medication with their pharmacokinetics

Drug	Brand name	Peak plasma concentration [h]	Half-live time [h]
Perampanel	Fycompa®	0.5-4[1,2]	95[2]
Dextromethorphan	Hustenstiller-ratiopharm® Dextromethorphan	1-2[3]	1.2-2.2 (CYP2D6-EM) <45 (CYP2D6-PM)[4]
Nimodipine	Nimodipin-Hexal®	0.6-1.6[5]	1.1-1.7[5]
Placebo	P-Tabletten Lichtenstein & 7,8,100mm Winthrop	n.a.	n.a.

[1]Yang, 2012, CLINICAL PHARMACOLOGY REVIEW FDA, [2] Phase I randomized biopharmaceutical study E2007-A001-040 conducted by Eisai, [3] PRODUCT INFORMATION Hustenstiller-ratiopharm® Dextromethorphan, [4](Vetticaden et al., 1989), [5] PRODUCT INFORMATION NIMOTOP® Nimodipine Bayer Resources

Table 2.2 Drug applications during the session

Due to different pharmacokinetics two times of drug application were defined. Drug application 1 at the beginning of the medication pause, two hours prior to the post drug measurement. And drug application 2 one hour later, one hour before post drug measurements. During sessions including drug application at one application the verum was administered and at the respective other application placebo was administered. During the placebo session placebo was administered at both application times.

Drug	Drug application 1	Drug application 2
Perampanel	4 capsules placebo	1 tablet Fycompa® 12mg/6mg
Dextromethorphan	4 capsules Hustenstiller-ratiopharm® Dextromethorphan 30mg	1 tablet Placebo
Nimodipine	4 capsules placebo	1 tablet Nimodipin-HEXAL® 30 mg
Placebo	4 capsules placebo	1 tablet Placebo

2.3 Data recording

2.3.1 Transcranial magnetic stimulation (TMS) measurements

The participants positioning throughout the measurement is important for valid data retrieval. In order to have a good stimulation position and minimize movements of the coil and the participant, all subjects where placed in a comfortable reclining chair and were instructed to find a comfortable position they could hold throughout the measurement. Moreover, to avoid pre-activation of the stimulated hand muscle their hand was placed comfortably on an arm rack. Because muscle pre-activity would increase MEP amplitudes the participant's hand needed to be completely relaxed (Hess et al., 1987).

The vigilance of the participant has a major effect on the required EEG data (Massimini et al., 2005a). Therefore, to ensure wakefulness the subjects were instructed to keep their eyes open. On the other hand, to decrease any eye movement activity the subjects were asked to fixated their view on one specific spot on the wall.

In order to achieve a focal stimulation of the targeted area we used a figure-of-eight (external diameter 90 mm) coil. The coil was linked through a BiStim module with a Magstim 200^2 magnetic stimulator (Magstim Co, Whitland, Dyfed, UK). This system created monophasic current waveforms.

The target of stimulation was the left M1 aiming for the stimulation of the musculus abductor pollicis brevis (APB) of the right thumb. For an optimal stimulation of the M1 hand area the figure of eight coil was placed 6-7 cm lateral from the vertex and held with a 45° angle to the sagittal line (Figure 2.2.). To achieve the lowest attenuation of the magnetic field the coil is positioned tangentially to the scalp (Fox et al., 2004). The coil orientation results in a posterior-anterior and lateral-medial current. This current direction has the highest capability to activate pyramidal neurons. Therefore, this coil location and orientation is associated with the lowest RMT to elicit MEPs (Mills et al., 1992). To maintain an optimal coil position throughout the experiment, the coil position was marked on the EEG-cap.

The discharge of the coil results in a clicking noise. This can induce an auditory EEG-artifact represented by a wave component between 100 and 200 ms (Nikouline et al., 1999). In order to minimize auditory artifacts each subject received headphones and was listening to a masking noise during TMS application (Massimini et al., 2005a). This masking noise has the same frequency spectrum as the click of the discharging coil and can therefore reduce the auditory response (ter Braack et al., 2015). The masking noise was regulated as loud as possible for the subjects, at least as loud until they were indicating not to hear the TMS-click anymore.

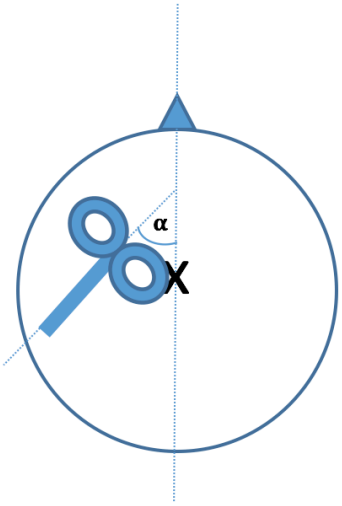

Figure 2.2 TMS coil positioning

The figure of eight coil was held over the left primary motor cortex. To stimulate the APB hotspot, the coil was pointing backwards. It was placed 6-7 cm lateral and 2 cm anterior from the vertex (x) and held with a 45° angle (α) to the midline.

2.3.2 Electroencephalography (EEG) data recording

In order to measure brain activity while stimulating via TMS various technical features need to be taken into account.

For the EEG-data recording we used a setting of 62 high-density TMS-compatible EEG-electrodes (Ag-AgCl-electrodes) (Roth et al., 1992, Ives et al., 2006) attached to an elastic cap in an international 10-20 system (EASYCAP, Germany) (Dmochowski et al., 2017). The international 10-20 system is an international standard method describing the EEG-electrode montage based on 4 main positions easily transferable between patients (nasion, inion and two pre-auricular points). An imaginary line is drawn between the nasion and the inion and divided in 10% and 20% increments. The same procedure is applied between the right and left pre-auricular points. Similarly, electrodes are applied around the circumferences of the head in 10% increments and paracentral with 20% increments and last transversal putting electrodes at the crossing points of the paracentral and transversal lines (Figure 2.3.). The different electrodes are labeled with a letter for its location in regard to the cranium (f=frontal, c=central, p=parietal, o=occipital) and a number indicating the side (odd number are on the

left and even numbers are on the right hemisphere). Lower numbers are closer to the midline, which is labeled z for zero.
Additionally, two electrodes were attached to monitor eye movements. One above the right eye and one at the lateral canthus of the right eye.
The EEG-Cap was attached to a TMS-compatible amplifier (BrainAmp DC, BrainProducts GmbH, Munich, Germany) allowing a continuous EEG recording throughout stimulation. The data was required at a sampling rate of 5 Hz.
In order to receive valid EEG-signal and reduce artifact due to thermal voltage noise and electrode movements or polarization throughout the measurements the impedances were kept under 5 kΩ (Rogasch et al., 2017). To reach these required low impedances a careful EEG cap preparation is necessary. Therefore, a three step procedure was performed: first the skin beneath the electrodes was cleaned with an alcoholic solution, then peeling was applied followed by the application of an EEG-paste which reduces the resistance (Rogasch et al., 2017).

2.3.3 Electromyography (EMG) data recording

MEPs were recorded using surface electromyography (EMG). Therefore, three electrodes (Ag-AgCl cup electrodes) including one ground electrode were placed in a belly-tendon montage on the right APB muscle, i.e. one electrode was placed on the muscle belly, one was placed on the tendon and the ground electrode was placed at the forearm between the electrodes and the point of stimulation. All three EMG electrodes underwent the same preparation procedure as the EEG electrodes described earlier.
MEP data was recoded with the Spike2 software (Cambridge Electronic Design). The EMG raw data underwent a three step processing whilst recorded: it was amplified (Digitimer D360 8-channel amplifier), bandpass filtered (20 Hz – 2000 Hz) and digitized at an A/D rate of 10.000 Hz (CED Micro 1401; Cambridge Electronic Design).
To minimize contamination of TEP data by MEP-induced re-afferent signals from muscle twitching (Fecchio et al., 2017), the stimulation took place with a 100% RMT intensity inducing just minimum MEPs. Additionally, MEPs were visually monitored by the experimenter throughout the measurement.

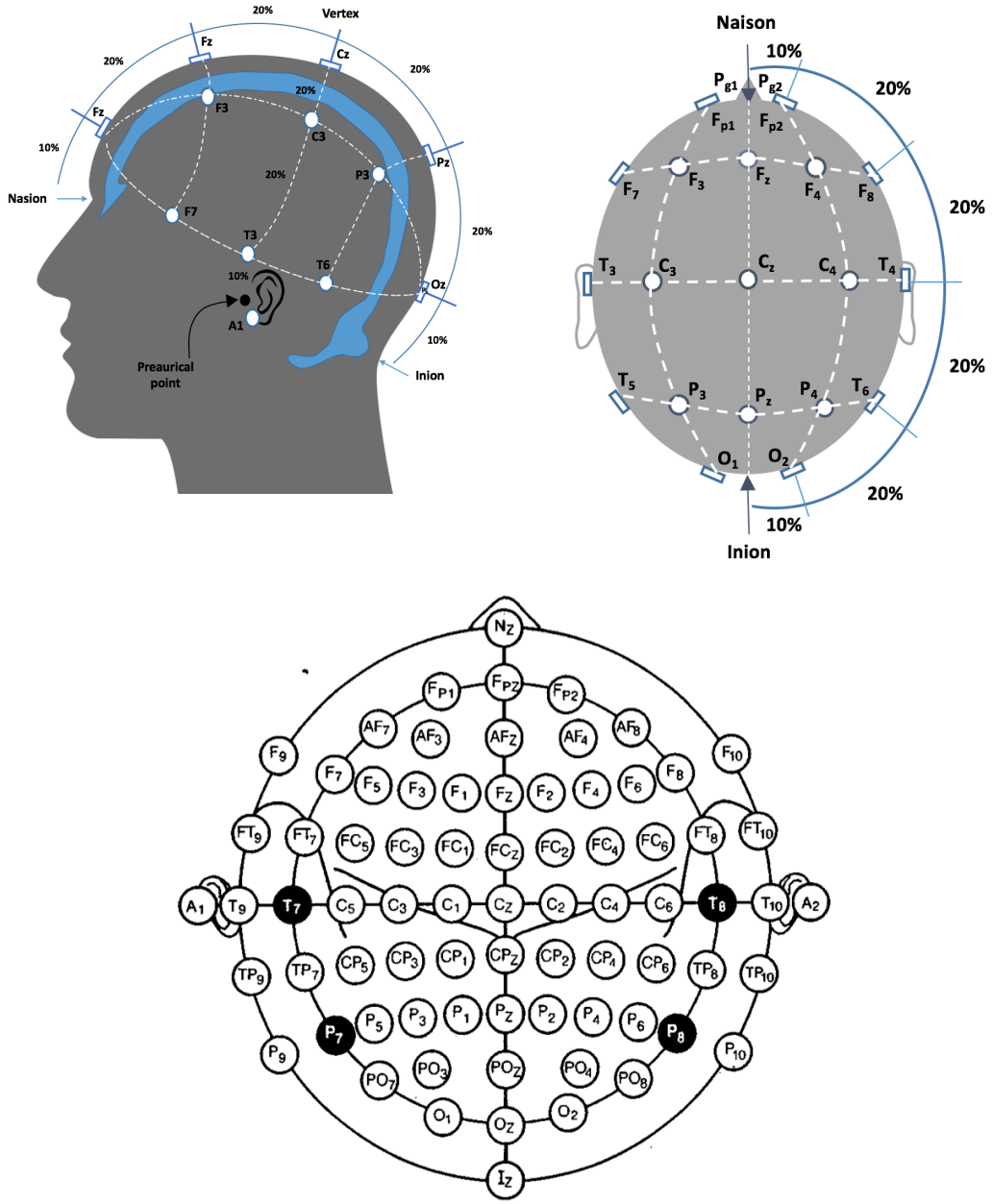

Figure 2.3 The international 10-20 system of EEG-electrodes organization

The international 10-20 system describes a EEG electrode organization that is easily transferable between patients because it is based on 4 points: Nasion, inion, periauricular point left and right. Between these four points imaginary lines are drawn and divided into 10% and 20% increments. This results into a grid where the electrodes are placed. Each electrode is named with a letter-number combination based on their location. (Upper figure: own figure based on (Malmivuo and Plonsey, 1995)) (lower figure: source (Sharbrough et al., 1991)).

2.4 Data analysis and preprocessing

For data processing and analysis we used customized analysis scripts on MATLAB R2016a and the Fieldtrip open source MATLAB toolbox (Oostenveld et al., 2011).

To filter out the time points of interest the required EEG data was epoched in sequences of 600 ms pre-stimulus to 600 ms post-stimulus (-600 ms to +600 ms). The TMS artifact itself (-1 ms until +15 ms) was removed and spline interpolated (Thut et al., 2011), followed by downsampling the data to 1000 Hz. Bad trials and channels were removed via visual inspection of the EEG epochs (removed epochs 25.4 $\pm$ 12.0%; removed channels 4.5 $\pm$ 2.5).

Afterwards a two-step procedure of independent component analysis (ICA) was performed (Rogasch et al., 2014). ICA is a blind source technique. It enables us to divide the required data into statistically independent components from linear mixed signals. Therewith, artifacts and neural signaling in the EEG data can be separated (Hyvarinen and Oja, 2000, Makeig et al., 1997). Large artifacts (e.g. TMS evoked muscle artifacts) can interfere with the accuracy of ICA separation. To insure an accurate recovering of neural activity and removal of smaller artifacts we used a two-step ICA approach (Hernandez-Pavon et al., 2012, Rogasch et al., 2014). During the first round of ICA the focus was on detecting and removing TMS-related artifacts (number of removed components 4.3 $\pm$ 2.6). Between the two ICA sessions the data was filtered via 1-80 Hz Butterworth zero phase band filter (3rd order). Also a 49-51 Hz notch filter was applied to reduce line noise of nearby voltages (e.g. electric socket). At the second round of ICA the focus was on detecting and removing physiological artifacts such as eye blinking or movement and muscle artifacts (number of removed components 13.6 $\pm$ 6.2). All removed channels were interpolated with the data of their neighboring channels (Perrin et al., 1989).

All electrodes underwent re-referencing to the linked mastoids. The linked mastoids are the average of the auricular electrodes TP9 and TP10.

In order to baseline correct the retained data, we subtracted the average data from -600 to -100 ms. Finally, the data was smoothened by using a 45 Hz low-pass filter (Butterworth zero phase band pass filter, 3rd order).

After undergoing the described preprocessing procedure, the analyisis-focus of this work lies on the analysis of the five most reproducible TEP components: P25, N45, P70, N100, P180 (P = positive deflection, N = negative deflection) (Lioumis et al., 2009, Premoli et al., 2014).

Additionally, the corresponding topographical distribution of these five TEP components was visualized with topoplots (Komssi et al., 2004, Bonato et al., 2006, Litvak et al., 2007, Rogasch and Fitzgerald, 2013, Premoli et al., 2014, Ferreri et al., 2011, Veniero et al., 2013). Therefore, the average of the signal of each time window of interest (Figure 2.4) was obtained. The data are based on a range of voltages at sensor level. The colors are adapted with respect to minimum and maximum of the voltages (Figure 3.1).

2.5 Statistical analysis

2.5.1 Transcranial evoked potential (TEP) analysis

We identified time windows of interest (TOIs) of the five TEP components for further analysis. Therefore, we averaged the data from all sessions and all EEG channels. All TOIs are non-overlapping and were centered around their peak at the known five TEP components (P25, N45, P70, N100 and P180) from earlier studies (Komssi et al., 2004, Bonato et al., 2006, Premoli et al., 2014). More precisely, in our study TOIs were set at the epochs 16-34 ms (P25), 38-55 ms (N45), 56-82 ms (P70), 89-133 ms (N100) and 173-262 ms (P180) post stimulus (Figure 2.4).

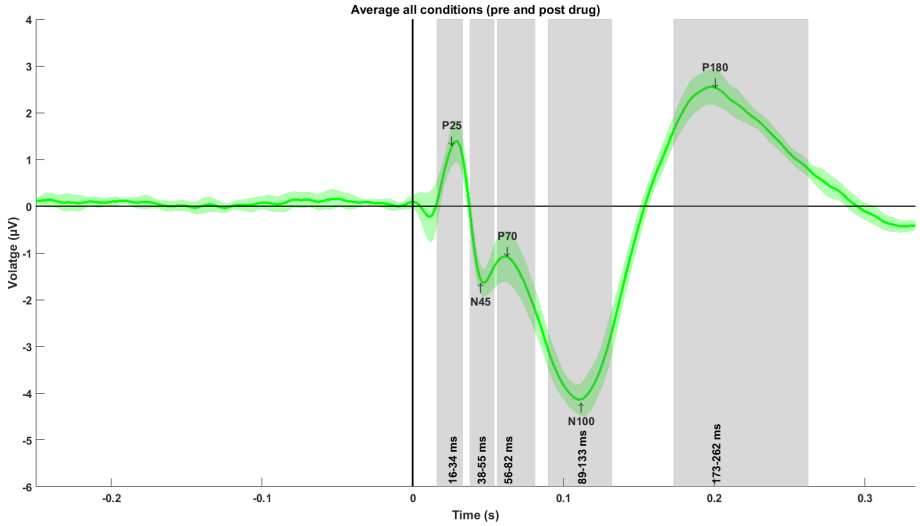

Figure 2.4 Definition of the time windows of interest (TOIs)

To identify the significant time windows to analyze we defined five non-overlapping TOIs. Therefore, we took the average of all sessions and channels and centered the TOIs around the peak of the known TEP components from earlier studies. This led to five TOIs at the epochs 16-34 ms (P25), 38-55 ms (N45), 56-82 ms (P70), 89-133 ms (N100) and 173-262ms (P180) post stimulus.

Each TOI of each drug condition served as the area to be analyzed for drug induced modulations. We were aiming to compare pre-drug measurements with post-drug TEP amplitudes retrieved from the same subjects. These are dependent and paired data. Therefore, we performed a channel-wise paired-sample t-test for each TOI.

The EEG data poses a statistical challenge because the data has a two-dimensional structure: sample observations are gathered from multiple channels (spatial dimension) and multiple time points (temporal dimension). This results in a large number of channel-time pairs and therewith in a large number of statistical comparisons (for each pair). This problem is known as the “multiple comparison problem” (MCP). Standard statistical procedures use the familywise error rate (FWER) at a single-pair level, but this approach is invalid as a consequence of the MCP. The FWER describes the probability of making false discoveries when performing multiple tests. To apply the same procedure in a case with the MCP, Fieldtrip offers a cluster-based permutation approach (Maris and Oostenveld, 2007). We used this approach for our analysis. Therefore, we grouped the t-

values lower/higher than an a priori defined threshold (p = 0.05) into clusters based on temporal and spatial proximity with a minimum of two channels each. Afterwards, we summed all t-values within each cluster. To compute the t-statistics at a cluster level we compared the maximum of the calculated t-values. In order to receive a reference distribution of the maximum cluster t-values we performed the same procedure repeatedly on the randomized data across pre- vs post-drug measurements. Therefore, we conducted 1500 randomizations. Following, we rejected the null hypotheses ($p < 0.05$) on the base that < 5% of the constructed permutations showed a larger maximum cluster level t-value than the cluster level t-value of the originally observed data. With this approach we tested the null hypothesis ($p > 0.05$) that the data from the drug conditions are drawn from the same probability distribution. The same procedure was applied to test possible differences among the TEPs of the pre-drug measurements to test reproducibility.

In order to avoid an alpha error of multiple comparison we applied the Bonferroni correction to the obtained p-values. Therefore, we divided the critical α by the number of comparison in order to achieve a new critical α that is now determined for a statistical significance of p ($\frac{\alpha}{\text{number of comparison}} = new\ critical\ \alpha$).

2.5.2 Resting motor threshold (RMT) analysis

RMT is an indicator for cortical excitability (Darmani et al., 2016, Ziemann et al., 1996a). Therefore, the effect of our study medication on RMT plays an important role in the understanding of the underlying physiology of excitability including TEPs.

For assessing possible RMT changes due to drug intake we performed a two-step statistical analysis of the RMT data including an analysis of variance (ANOVA) followed by paired t-tests.

In order to analyze the possible effects of DRUG (perampanel, nimodipine, dextromethorphan, placebo) and TIME (pre-drug measurements, post-drug measurements) on RMT data we performed a repeated measures ANOVA. TIME was treated as within-subject effect and DRUG as between-subject effect.

When performing a repeated measures ANOVA sphericity is assumed. Thus, the variances of the differences between all possible pairs of within-subject conditions are equal. A Mauchly's test was performed to evaluate if the sphericity assumption had been violated. In case of violation of sphericity (significant Mauchly's test) a Greenhouse-Geisser correction was applied. The Greenhouse-Geisser correction adjusts the degrees of freedom, resulting in a reduced type I error rate.

In case of significant effects in the repeated measures ANOVA, post hoc two-sided paired t-tests were performed for each drug condition to test drug-induced changes in the RMT data (significance level $p < 0.05$).

2.5.3 Motor evoked potential (MEP) analysis

MEP amplitudes as indicator for cortical excitability are also worth taking a closer look at. In order to analyze the significant parts of the EMG data sequences from 100 ms before stimulation until 100 ms after stimulation were epoched. If the time interval before stimulation showed a pre-innervation of the APB defined as an amplitude ≥ 0.02 mV the segment was discarded because pre-innervation causes an augmentation of the MEP amplitude (Hess et al., 1987). Due to this error source we discarded 11.0% $\pm$ 17.9% of the MEP-epochs. The required MEP data then underwent a statistical analysis via two-way repeated measure ANOVA as described in the previous chapter for RMT. Finally, to achieve a normal distribution we performed a log-transformation on the MEP data.

3 Results

The following presented results are based on our preprint published paper Koenig et al 2019.

3.1 Tolerance of the study procedure

The study protocol was completed by sixteen out of eighteen subjects. All subjects reported a good tolerance of the TMS procedure. Every included participant showed good compliance throughout the measurements.

The application of 12 mg perampanel led to side effects such as dizziness, nausea and ataxia. Since the side effects of perampanel increase with dosage (Krauss et al., 2012), the dosage of perampanel was reduced from 12 mg to 6 mg for the remaining 13 participants. Otherwise, all study medications were tolerated well, besides some minor side effects (slight nausea and dizziness) after the intake of dextromethorphan and perampanel. Therefore, the participants' ability to fulfill all study requirements was not impaired at any time.

3.2 Drug effects on RMT

We adjusted the stimulation intensity for post drug measurements to insure an equal excitability for pre- and post-drug measurements. Since the intensity of stimulation is an indicator for cortical excitability it is very interesting to see if our study medication had an effect thereon (table 3.1).

The performed repeated measurement ANOVA revealed a significant DRUG*TIME interaction ($F_{3.45} = 8.993$, $p < 0.001$). Therefore, we performed post hoc paired t-tests post- vs. pre-drug in each drug condition and compared it to post- vs. pre-drug changes in the placebo condition. This revealed a significant increase of RMT in the drug conditions perampanel (mean RMT increase (post-drug/pre-drug) $\pm$ SD = 1.09 $\pm$ 0.08; $t_{15} = 4.11$; $p < 0.001$) and nimodipine (mean RMT change (post-drug/pre-drug) $\pm$ SD = 1.04 $\pm$ 0.04; $t_{15} = 2.91$; $p = 0.004$). There were now significant RMT changes (post-drug/pre-drug) in the conditions dextromethorphan (mean RMT change $\pm$ SD = 0.99 $\pm$ 0.07; $t_{15} = 0.94$; $p = 0.36$) and placebo itself (mean RMT change $\pm$ SD = 0.97 $\pm$ 0.07).

Table 3.1 Mean RMT for pre- and post-drug measurements in all four conditions

Conditions with significant RMT differences between pre- and post-drug measurements are marked yellow.

Drug	RMT (%) pre ± SD	RMT (%) post ± SD
Perampanel	40.0 ± 6.4	43.6 ± 7.6
Dextromethorphan	40.9 ± 7.5	40.4 ± 6.7
Nimodipine	40.4 ± 7.2	41.8 ± 7.1
Placebo	40.8 ± 5.8	38.7 ± 8.3

3.3 Drug effects on MEPs

The repeated measurement ANOVA we performed on the MEP data (after correction of post-drug stimulus intensity for RMT changes) did not show any significant differences in the MEP amplitudes regarding DRUG or TIME and no DRUG*TIME interaction was found (table 3.2). This suggests an equal excitation of motor cortex by TMS for pre- and post-drug measurements.

Table 3.2 Mean MEP amplitudes for pre- and post-drug measurements in the different conditions

Drug	MEP (mV) pre ± SD	MEP (mV) post ± SD
Perampanel	0.19 ± 0.31	0.16 ± 0.16
Dextromethorphan	0.17 ± 0.35	0.18 ± 0.20
Nimodipine	0.18 ± 0.07	0.19 ± 0.37
Placebo	0.18 ± 0.21	0.16 ± 0.16

3.4 Drug effects on TEPs

In comparison to previous studies the retrieved TEP components were consistent and reproducible throughout our measurements (Komssi et al., 2004, Bonato et al., 2006, Premoli et al., 2014, Darmani et al., 2016) (Figure 3.1.).

Furthermore, the topoplots of the five TEP components were consistent with previous studies (Darmani et al., 2016, Komssi et al., 2004, Bonato et al., 2006, Premoli et al., 2014) (Figure 3.1.).

A pairwise comparison of the TEPs retrieved during pre-drug measurements in all drug conditions did not show any significant differences. This demonstrates a good reproducibility of the TEP components when measured at baseline, making them potentially an excellent bio-marker for studying drug effects.

After evaluating TEP reproducibility, we performed further analysis of the pre- vs. post-drug measurements to discover possible drug effects on TEPs. The placebo condition and the nimodipine condition showed no significant differences in any of the five TOIs comparing pre- with post-drug measurements (all $p > 0.05$). In contrast, perampanel showed a decreasing effect of the amplitude of the positive deflection at 70 ms post stimulation (P70) ($p = 0.002$). In the according topoplot this difference was expressed in the contralateral hemisphere with regard to the stimulation site (Figure 3.2, Figure 3.3 and Figure 3.4).

After the intake of dextromethorphan a significant increase of the negative deflection around 45 ms post stimulation (N45) was observed ($p = 0.027$). The according topoplot of this TEP component expressed this difference in the pericentral bilateral brain area (Figure 3.2, Figure 3.3 and Figure 3.4).

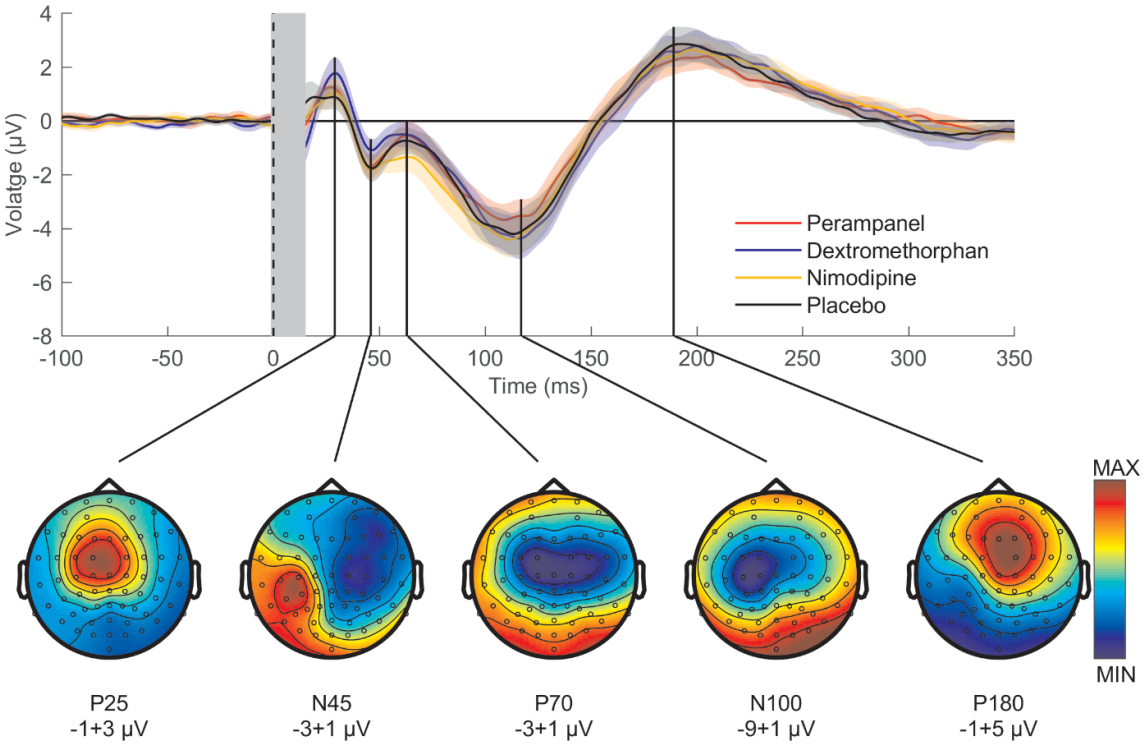

Figure 3.1 Group average of TEPs and the according topoplot of pre drug measurements in all four drug conditions

The top panel shows the average of the pre drug TEP components across all completed 16 datasets and all EEG electrodes. The different colored curves indicate for the four different conditions: perampanel (red), dextromethorphan (blue), nimodipine (yellow), placebo (black). The grey shadow surrounding the curves represents ± SEM. The removal and interpolation of the TMS artifact is indicated by the vertical gray bar. The bottom panel shows the according topographical distribution of the five TEP components as an average of all 16 subjects in all four conditions. The analyzed time windows of interest were defined as follows: P25: 16-34 ms, N45: 38-55 ms, P70: 56-82 ms, N100: 89-133 ms, P180: 173-262 ms. The data are voltages at sensor level (areas indicated below the plots). The colors are normalized to maximum/minimum voltage. (source (Koenig et al., 2019))

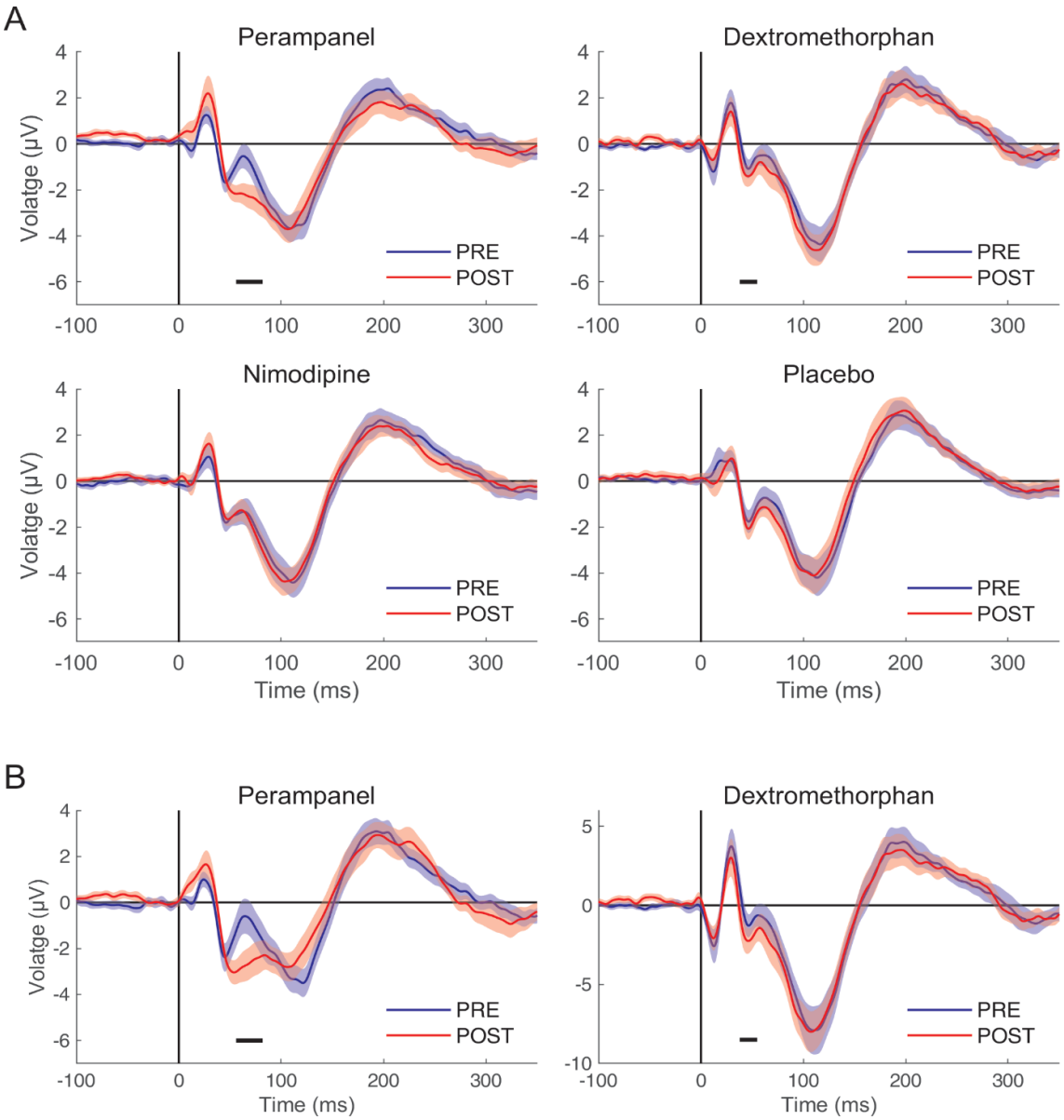

Figure 3.2 Group average of TEPs of the pre and post drug measurements

(A) Each window shoes the average TEP across all subjects (n=16) and all EEG channels of the pre drug measurement (blue curve) and the post drug measurement (red curve). The grey shadow surrounding the curves represents ± SEM. Significant differences between pre and post drug measurements are underlined with a horizontal black bar.

The bottom row (B) illustrates the same average time course but only for significant electrodes for the two significant drug conditions. This was done to emphasize drug induced changes of TEP components. (source (Koenig et al., 2019))

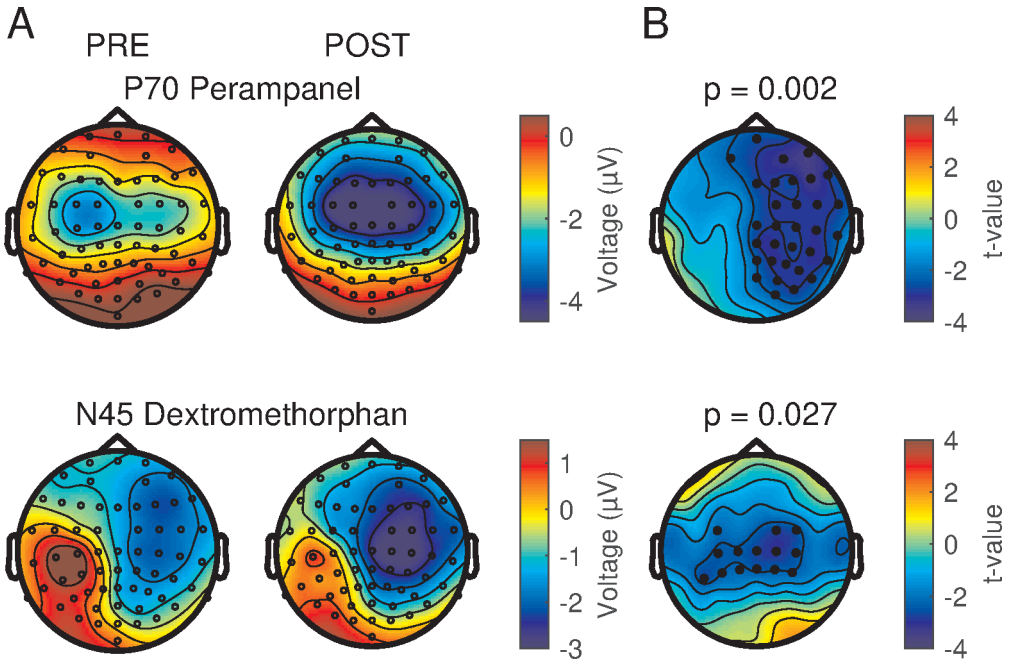

Figure 3.3 Topographical voltage distribution maps of the two significant TEP components

(A) shows the topographical distribution of P70 in the drug condition perampanel and N45 in the drug condition dextromethorphan. It is illustrated the topographical distributions of these two before (left) and after (middle) drug intake. The topoplots on the right (B) show a t-value statistical map of significant clusters with the corresponding significant EEG channels represented by black dots. The respective p-values are reported above. For P70 in the perampanel condition a significant topography change in the contralateral hemisphere to stimulation is shown. For N45 in the dextromethorphan condition a pericentral bilateral topographical change can be illustrated. (source (Koenig et al., 2019))

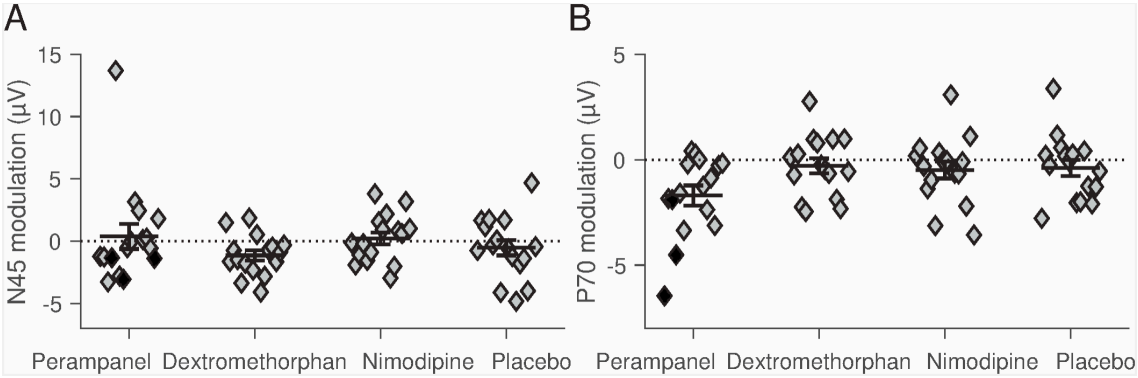

Figure 3.4 Scatter plots of drug induced TEP changes in N45 and P70 across single subjects

The scatter plots show amplitude variations (post drug minus pre drug) for the two TEP components N45 (A) and P70 (B) in all four conditions. The amplitudes were computed by taking the average voltages from the identified significant channels in N45 of the dextromethorphan condition and P70 of the perampanel condition. The error bar stands for mean ± SEM. The 3 subjects who took 12mg of perampanel (instead of 6mg) are illustrated by black rhombs. (modified from (Koenig et al., 2019))

4 Discussion

4.1 Methodological opportunities and limitations

Even though TMS-EEG gives us a great opportunity to study in vivo and non-invasively how brain areas interact during various processes, it remains challenging to acquire valid data. Therefore, a close supervision of the data collection as well as a constant revision for potential improvements is still crucial for significant research.

In the following our methodical approach will be discussed and challenged in comparison to other studies.

4.1.1 TMS-EEG data recording

Even though TMS-EEG offers us the great opportunity to measure brain activity online with a very good time resolution, the spatial resolution remains a general shortcoming of this technique. Via scalp electrodes it is only possible to measure neuronal activity of cortical neurons, whereas subcortical structures are not available for scalp electrode measurements. To overcome this limitation, an invasive procedure with implanted electrodes would have to be applied. But this makes the procedure much more intrusive and potentially unsafe. In the end the many advantages of TMS-EEG (i.e. easy application, temporal resolution and low costs) make it the best method for wide range studies in health and disease.

The accuracy of the acquired EEG data increases with the number of applied stimuli (Ilmoniemi and Kicic, 2010). Therefore, we chose a relatively high number of 150 stimuli per measurement to keep the noise ratio low.

4.1.2 RMT determination and adaption

First step in the study procedure and a crucial factor to receive interpretable data is the determination of the individual participant's resting motor threshold.

The RMT was determined via the relative frequency method (Groppa et al., 2012), in order to receive a reproducible and reliable activation of the cortico-

subcortical pathways and at the same time elicit minimal MEPs. Therewith, we guaranteed a high enough intensity to induce motor responses and at the same time kept the potential re-afferent interferences by muscle activation to a minimum.
The cortex to muscle conduction time is approximately 20-25 ms for hand muscles (Paus et al., 2001). Prior studies showed an afferent somatosensory response caused by TMS-induced muscle activation at around 50-60ms post stimulation (Bonato et al., 2006, Maki and Ilmoniemi, 2010). Whereas Fecchio et al. (2017) have shown that a desynchronization appears around 300 ms after stimulating the M1 hand area. This desynchronization correlates with MEP amplitudes. Therefore, it is suggested that the excitation of M1 via TMS results in a proprioceptive feedback of the targeted muscle activation (Fecchio et al., 2017).

Not just re-afferent sensorimotor potentials may contaminate TEP components but a higher RMT also results in a higher likelihood of somatosensory evoked potentials from activation of trigeminal nerve afferents in the scalp.

RMT is an indicator for cortical excitability. In particular, it mirrors the excitability state of corticospinal neurons. Cortical excitability can be influenced by various factors. For example vigilance (Massimini et al., 2005a) and alcohol (Ziemann et al., 1995, Kahkonen et al., 2003) have an influence thereon. But also drugs can reversibly modulate cortical excitability and therewith RMT. For instance, various voltage-gated sodium channel blockers and ketamine, a NMDAR antagonist, have shown a modulation of the motor threshold (Ziemann et al., 1996a, Lazzaro et al., 2003). Both, voltage-gated sodium channels and NMDARs, are crucial factors for cortical excitability. Dextromethorphan itself has not shown any RMT modulations in prior studies (Ziemann et al., 1998b).
In our study design we adapted the TMS intensity for the post-drug measurements in order to guarantee roughly the same degree of cortical excitation. This makes the TEPs in the pre- and post-drug measurements comparable. But Premoli et al (2017b) discovered that adapting RMT may

influence the effect of drugs on TEPs. Hence it is suggested for future studies to use a range of stimulation intensities for the pre- and post-drug measurements in order to provide comparable TEP data on an absolute and relative level (Premoli et al., 2017).

4.1.3 Coil-positioning

It has been demonstrated that the coil position has an important impact on the motor threshold (Conforto et al., 2004). Once the optimal stimulation site has been determined, it is crucial to keep this position stable because even small changes in the positioning (10 mm) may modulate EEG responses (Komssi et al., 2002). To ensure a constant coil position, we marked the determined "hot-spot" on the EEG-cap. But one might argue that this method does not guarantee a stable position especially in the tilt-dimension. Furthermore, it does not ensure the same coil positioning in a test re-test design between the different sessions.
The neuronavigation technique based on the participant's MRI allows an accurate online supervision of the coil positioning (Sparing et al., 2008, Wagner et al., 2007, Schonfeldt-Lecuona et al., 2005). The online use of this technique in each session enhances the accuracy of the coil positioning and therewith also the reproducibility and comparability of the acquired EEG data. In addition, due to an optimal coil position over the "hot-spot" a lower stimulation intensity can be used which minimizes induction of muscle artifacts (Ilmoniemi and Kicic, 2010) and sensory evoked potentials.
Therefore, an online monitoring of the coil position via neuronavigation presents clear advantages. At the same time, it does need to be taken into account that the state of the brain may influence the "hot-spot" coordinates and therewith the TMS outcome (Farzan et al., 2016).
In addition to the coil position monitoring, some of the neuronavigation systems are able to estimate the induced electrical field in the brain by TMS. This offers an alternative for RMT determination. Especially for the stimulation of non-motor brain areas this technique seems promising. The accuracy for this RMT determination depends on the analytic software, in particular the ability to

compartmentalize different tissue layers according to their conductivity (Wagner et al., 2007).

4.1.4 Auditory and somatosensory evoked potentials/artifacts by TMS

The charging of the coil leads to a coil-shape deformation which results in a clicking noise (approx. 100-120 dB and a rise time of <0.5 ms) (Starck et al., 1996). The clicking noise travels through air and bone transmission to the participant's auditory organ (Nikouline et al., 1999). This may trigger an auditory evoked potential around 100 to 200 ms after stimulation in the participant's EEG data (Nikouline et al., 1999, Rogasch et al., 2014). Appling a masking noise, with the same frequency spectrum as the coil click, reduces induced auditory potentials (ter Braack et al., 2015, Ferrarelli et al., 2010). Studies by Paus and colleagues showed the effectiveness of the masking noise using positron emission tomography. Therewith, they could show that applying a masking noise effectively suppresses an increase of blood flow in the auditory cortex (Paus et al., 1996, Paus et al., 1997).

During TMS the scalp surface underneath the coil is reached by an electric current. This may give rise to a somatosensory potential. As already described, this phenomenon increases with stimulus intensity. Therefore, we did not stimulate with intensities higher then 100% RMT.

Both the auditory evoked potentials as well as the somatosensory evoked potentials can also be reduced by using a foam underneath the coil (Massimini et al., 2005a). This technique not only lowers the bone conduction of the clicking noise, but also weakens the direct electrical stimulation of the scalp surface. A similar effect has the use of an adjusted coil position, located 2 cm above the scalp. As a result there is no direct contact between the coil and the scalp, which reduces bone transmission and direct electric scalp excitation (Nikouline et al., 1999).

The described artifact can be removed in an offline procedure if the online avoidance was not possible. Therefore, TMS induced potentials from a control

group can be subtracted from the active condition (Daskalakis et al., 2008). Or the artifacts can be separated and removed via ICA (Rogasch et al., 2014).

4.1.5 Data analysis and processing

The analysis of TMS-EEG data can focus on numerous markers. The latency of induced potentials (TEPs) has been replicated and shown as reproducible among various studies (Komssi and Kahkonen, 2006). Further analysis of the TEPs was executed for example by looking at the slope of the TEP wave-components (Vyazovskiy et al., 2008, Huber et al., 2013). To characterize a global brain response a global main field amplitude analysis was performed (Komssi and Kahkonen, 2006). Moreover, the frequency domain of the TEP components was further analyzed either with fixed-length time windows (Paus et al., 2001, Farzan et al., 2009) or with sliding time windows (Rosanova et al., 2009, Frantseva et al., 2014).

For all TMS-EEG data, analysis of a clear dataset with minimized artifacts is a crucial requirement. Despite a meticulous attempt of online noise reduction, not all EEG artifacts can be avoided. Therefore, a dedicated data processing including artifact removal is necessary. But removing recorded data whilst preserving physiological brain activity and TMS responses poses many challenges. In particular the early TEP components (<50 ms) are very vulnerable for contamination by the TMS artifact.

EEG recordings generally include two artifact categories: environmental and physiological. A great deal of environmental noise is created by power lines (50-60 Hz). This can rather easily be filtered but the filtering procedure includes the risk of eliminating physiological information as well. In order to keep a close monitoring of physiological noise, an electrooculogram and/or electrocardiogram may be performed during EEG measurements. This allows an offline assessment followed a manual artifact removal.

More recently, another possibility of noise separation was developed: ICA. This method allows to separate artifacts with typical characteristics for independent removal of artifacts.

Further development of ICA has shown that a two-step procedure is superior. This is due to the possibility that large artifacts, such as the TMS artifact, affects the separation via ICA. Therefore, those large artifacts should be removed during the first round followed by the main ICA (Rogasch et al., 2014).

The removal of the TMS artifact can be performed in different ways. A simple approach is the removal of contaminated sensors (Kahkonen et al., 2001). Alternatively, contaminated trials can be removed (Reichenbach et al., 2011). Both approaches may create a bias because it is more likely to remove sensors close to the stimulation site. This creates an asymmetry that can be partially addressed with later interpolation from non-contaminated neighboring channels. Therefore, we performed another procedure, the removal of the contaminated time area. This technique creates a discontinuity which should be interpolated for the missing data (Reichenbach et al., 2011, Garcia Dominguez et al., 2014). But this approach does not imply the danger of creating an asymmetry.

Even though we already included many features to optimize the data recording and minimize artifacts, there is always room for improvement as the TMS-EEG as a rather new technique that is still being developed. The following data discussion takes place against this background.

4.2 Discussion of the study results

This study aims for a further investigation of the induced modulation of TMS-evoked EEG potentials by our study medication. Particularly, by the drugs dextromethorphan, a NMDAR antagonist, perampanel, an AMPA receptor antagonist, and nimodipine, a L-VGCC blocker. All drugs were tested with respect to placebo.

Our investigation revealed a significant modulation of TEP components by the glutamate receptor antagonists. In particular, the amplitude of N45 was increased by dextromethorphan. Perampanel, on the other hand, decreased the amplitude of P70. No significant TEP modulation were detected in the conditions nimodipine and placebo.

4.2.1 Dextromethorphan modulating N45

The effects of dextromethorphan application on TMS measurements has been subject to various studies before. Dextromethorphan shows a significant suppression of the excitatory drive in the human brain (Ziemann et al., 1998a). It does not only decrease cortical excitability but also decreases intracortical facilitation in a paired-pulse TMS protocol (Ziemann et al., 1998a, Schwenkreis et al., 1999). Intracortical facilitation functions as a marker for glutamatergic neurotransmission. However, RMT or MEP amplitudes remained unchanged after DM intake in prior studies (Ziemann et al., 1998a, Wankerl et al., 2010b).

The effect of dextromethorphan on TEPs has not been studied to date. Our study has revealed that DM has an increasing effect on the amplitude of the negative TEP deflection at 45 ms post stimulation (N45). It did not show any significant modulations of the remaining TEP wave-components.

In previous studies effects of benzodiazepines have been investigated. Through the activation of the GABA-A neurotransmission IPSPs are generated. This leads to an inhibition of intracortical facilitation (Ziemann et al., 1996b). A more recent study investigated the effect of benzodiazepines on TEPs showing an increase of the N45 amplitude as well. The authors argue that the targeted a1-subunit containing GABA-A receptors play a key role in the generation and modulation of N45 (Premoli et al., 2014).

As a synopsis of all available pharmaco-TMS-EEG data, N45 reflects a summation of inhibitory and excitatory neurotransmission resulting in a balance of EPSPs and IPSPs generated by NMDA- and GABA-A-receptors.

The deflection N45 has shown a reproducible expression in the contralateral frontal region (Bonato et al., 2006, Ferreri et al., 2011, Veniero et al., 2013). The changes induced by dextromethorphan take place mainly in the bilateral pericentral region. In slight contrast, the significant electrodes for induced changes of alprazolam and diazepam are placed pericentrally with a clear orientation towards the non-stimulated hemisphere (Figure 4.1).

In agreement with prior studies, dextromethorphan does not affect cortical excitability or RMT. Ketamine on the other hand, another NMDAR antagonist, does decrease the motor threshold dose-dependently (Lazzaro et al., 2003). This leaves the exact role of NMDARs in the evolution and propagation of brain activity and cortical excitability still elusive.

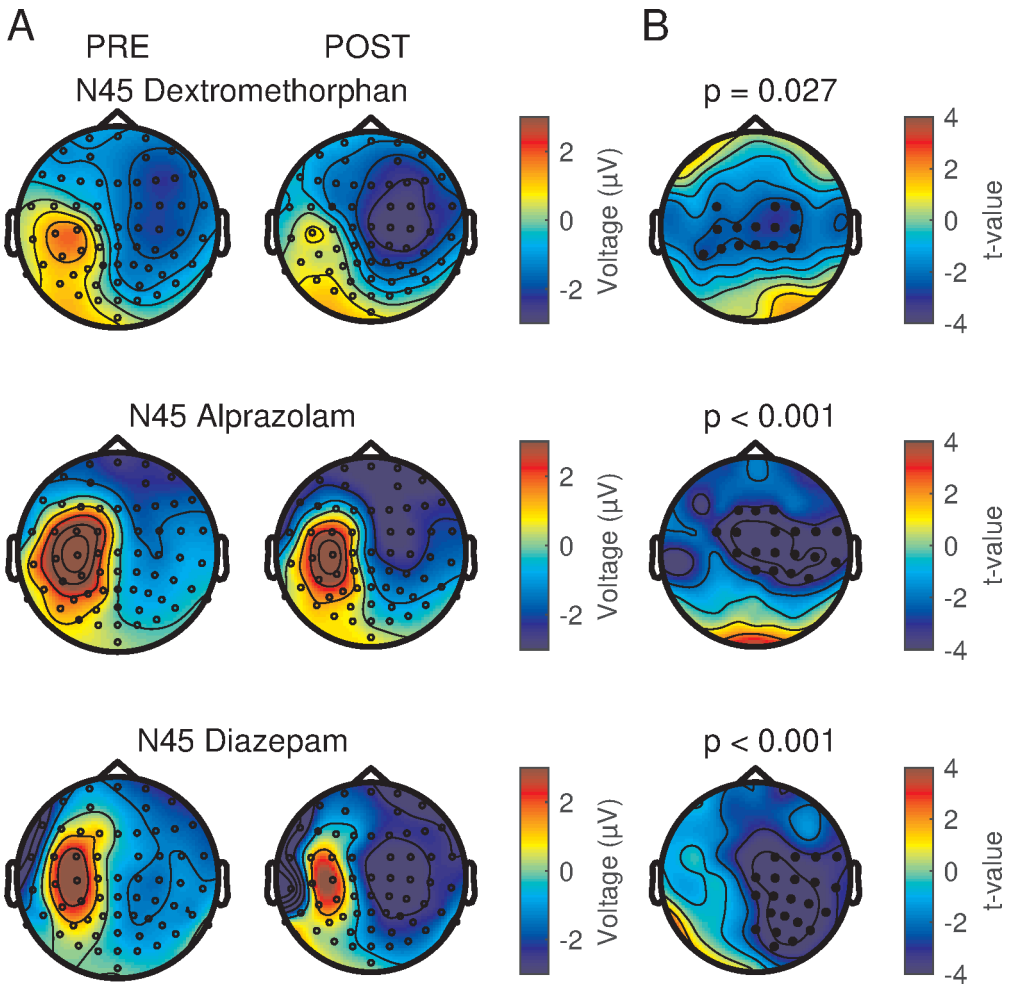

Figure 4.1 Comparison of drug induced changes of N45 by dextromethorphan and two classic benzodiazepines (alprazolam and diazepam)

In the first two columns (A) show a comparison of the voltage gated maps of N45 before (left) and after (middle) drug intake. Whereas the column on the right (B) illustrates the t-statistics of the topoplots pre- vs post drug measurements. The significant electrodes are marked with black dots. (source (Koenig et al., 2019))

4.2.2 Perampanel modulating P70

So far the function of perampanel, an AMPAR antagonist, in the evolution of TEPs remained elusive. This study demonstrates that the application of perampanel decreases the amplitude of the positive deflection at 70 ms post stimulation (P70). It is the first drug that has shown a modulation of P70. Hence, the glutamatergic system, more specifically AMPARs, is responsible for the evolution of P70.

The corresponding topographical distribution showed the main effect in the contralateral hemisphere. This indicates an influence in the interhemispheric propagation after TMS. This theory is for once supported by the fact that AMPARs are responsible for the genesis of fast EPSPs which play a crucial role in the distribution of neural activity (Niciu et al., 2012). Furthermore, perampanel has an antiepileptic effect. It was demonstrated that another antiepileptic drug (CNQX) with the same mode of action (AMPAR antagonist) acts through stopping the spreading of epileptic activity (Rogawski, 2011). This effect was not seen during the use of the NMDAR antagonist D-AP5 (Davies et al., 1986, Hwa and Avoli, 1992, Telfeian and Connors, 1999).

The inhibition of propagation of activity may also be the responsible factor for a higher RMT after drug intake.

In a synopsis, the available information suggests that glutamatergic excitation through AMPARs contributes to P70 expression, in particular in the non-stimulated contralateral hemisphere. If it also is a valid biomarker for epilepsy stays elusive. So far studies including epileptic patients with generalized epilepsies treated with antiepileptic drugs did not detect any P70 modulations. (Ter Braack et al., 2016, Kimiskidis et al., 2017a, Kimiskidis et al., 2017b, Julkunen et al., 2013). However, a very recent study showed an enhancement of P60 in patients with juvenile myoclonic epilepsy with no treatment after the application of single-pulse TMS in comparison to epileptic patients with treatment and the healthy control group (Bauer et al., 2019)

4.2.3 The lack of TEP modulation by nimodipine

In our study nimodipine has shown a significant increase of RMT. This might be due to nimodipine's contribution to cortical excitability. Even though previous

studies did not report a RMT variation due to nimodipine because of its mainly postsynaptic mode of action, other studies suggest also a presynaptic mode of action of the drug (Fourcaudot et al., 2009).

Nimodipine does not have a direct influence on glutamatergic transmission (Catterall, 2011). Accordingly, no glutamatergic suspected modulations were expected. Indeed, nimodipine has no influence on the evolution of TEPs.
Prior studies have suggested a major role of Ca^{2+}-dynamics in the modulation of LTD and LTP as well as metaplasticity (Wankerl et al., 2010a). L-VGCC together with NMDARs are the major pathways for Ca^{2+} ions into the postsynaptic terminal and therewith responsible for the postsynaptic Ca^{2+} concentration. Further it was indicated that the magnitude Ca^{2+} concentration in the postsynaptic terminal is responsible for the outcome of LTD/LTP induced by TMS protocols (Wankerl et al., 2010a, Lisman et al., 1997, Artola et al., 1990).
It is not always easy to find the right dosage for the utilized study medication because it is always a balance between effectiveness and safety for the participants. Many parameters may influence the effectiveness of a drug, such as absorption or passing the blood-brain barrier. Although 30 mg of nimodipine is unlikely to cause a complete blockage of L-VGCCs, it was used as a potent dosage in prior studies. The same dosage led in previous studies to a modulation of LTD and LTP (Weise et al., 2017, Wankerl et al., 2010a, Wolters et al., 2003) making it unlikely that the lack of influence on TEPs arose from the selection of a too low dosage.

4.3 Summary of pharmaco modulations on TEP components

Combining TMS-EEG and CNS active drugs with a well-known mode of action improves our comprehension of the effect of TMS on induced potentials. It helps us to further understand the physiological processes underpinning transcranial evoked potentials. The full understanding of TEP evolution is essential for a further use of TEPs as an in-vivo bio marker, a diagnostic tool or a prognostic instrument.

So far only a few studies investigating TEPs with a pharmaco-TMS-EEG approach have been performed. This chapter gives a brief overview of the suggested processes behind the generation of the different TEP components, in its course focusing on the results of pharmaco-TMS-EEG approaches (table 4.1).

The underpinning physiology of TEPs remain largely unknown. However, the summary of various modelling and animal studies so far suggests the following neural events underlying TMS-evoked EEG potentials:
Evoked potentials in the timespan 10 to 40 ms post stimulation probably reflect activity of the motor system on the ipsilateral and contralateral cortical areas. This is assumed because their latencies are consistent with recordings of the time delay of cortico-cortico potentials measured on the intrahemispheric (Markram et al., 2004) and interhemispheric sites (Terada et al., 2008).
Further the generation of N45 is assumed to relay on either the activation of inhibitory cortical neurons of the cortical or cortico-thalamic circuits (Markram et al., 2004), or on the activation of thalamic reticular nucleus (Guillery et al., 1998).
Finally, N100 is probably generated through an inhibitory phenomenon, e.g. a long-lasting inhibition (100-300 ms) of the pyramidal cells after stimulation (Krnjevic et al., 1966a). A follow up study has suggested that this inhibition is due to GABAergic transmission (Krnjevic et al., 1966b). Another inhibitory process underlying N100 may be inhibitory circuits on the contralateral site of stimulation. Though GABA neurons in the layer V are inhibited by the direct activation of apical dendrites in layer I via TMS (Palmer et al., 2012).

The addition of pharmaco-TMS-EEG measurements gives us further insight of receptor involvement by specifically enhancing or blocking different receptors and channels.

P25:
Prior studies have shown that repetitive TMS and transcranial direct current stimulation modulate early TEP components (Esser et al., 2006, Pellicciari et al., 2013, Veniero et al., 2012). Both techniques have been identified as modulators

for synaptic strength and cortical excitability (Ziemann and Siebner, 2008). Furthermore, a direct correlation between the amplitude of P25 with the amplitude of MEPs was detected (Maki and Ilmoniemi, 2010).
A pharmaco-TMS-EEG approach just recently was able to identify a suppression of P25 after the application of carbamazepine (Darmani et al., 2018). Carbamazepine is an antiepileptic drug which blocks the voltage-gated sodium channels (VGSCs) and therewith decreases cortical excitability.
In summary, it is likely that P25 reflects corticospinal excitability at the site of stimulation.

N45:
As described earlier, evidence indicates that the evolution of N45 is based on a balance of EPSPs and IPSPs generated by NMDA- and GABA-A-receptors, more specifically the α1-subunit containing GABA-A-receptors (Premoli et al., 2014, Koenig et al., 2019).
Additionally, the antiepileptic drugs lamotrigine and levetiracetam showed an increasing effect on the N45 amplitude (Premoli et al., 2016). Lamotrigine is a voltage-gated sodium channel blocker, hence stabilizes the membrane but also leads to inhibition of glutamate release (Cheung et al., 1992). Even though lamotrigine does not directly function on GABARs, various studies suggest a GABAergic like component in lamotrigine's mode of action (Cunningham and Jones, 2000, Kumar et al., 2012, Braga et al., 2002). Therefore, it was argued that this GABAergic like effect contributed to the increase of N45. With the additional information from this study also the decreased glutamate release could be responsible for lamotrigine's influence on N45. Also levetiracetam reduces glutamate release into the synaptic gap through binding to the presynaptic glycoprotein SV2A (Lynch et al., 2004). Since both drugs have an increasing effect on the N45 amplitude an involvement of the neurotransmitter glutamate can be assumed.

P70:

Perampanel is the first drug that has shown an impact on P70. In the topoplot analysis, P70 has always shown a focus on the non-stimulated hemisphere. This suggesting that a fast EPSP generation and interhemispheric propagation via AMPARs is responsible for P70 evolution.

N100:

The results of an animal study showed that slow inhibition after stimulation (50-200 ms) is mediated though GABA-B receptors (Connors et al., 1988, Deisz, 1999). The hypothesis that the N100 might reflect GABABergic inhibition was supported by a pharmaco-TMS-EEG study using baclofen, a GABA-B-R agonist. The study showed an increase in the amplitude of N100 at the site of stimulation (Premoli et al., 2014).

Furthermore, classical benzodiazepines, such as alprazolam and diazepam, additionally modulate N100. They showed a decrease of the N100, predominantly in the non-stimulated hemisphere (Premoli et al., 2014).

All benzodiazepines have a sedative effect, implying that the decrease of N45 may be caused by this effect. But the sedative zolpidem did not have had an effect on N100, making this argument unlikely. Zolpidem has in comparison to classical benzodiazepines a much lower affinity to the α2- and α3-subunit bearing subtypes of the GABA-A-R, making it likely that these subtypes of the GABA-A-R play a role in the evolution of N100 in the non-stimulated hemisphere.

Midazolam is a benzodiazepine used to induce loss of consciousness. A study has shown that midazolam reduces late TEPs, suggesting a breakdown of long-range cortico-cortico connectivity (Ferrarelli et al., 2010).

Moreover, two medications (levetiracetam and brivaracetam) that inhibit presynaptic excitatory neurotransmitter release affect the N100 as well (Premoli et al., 2016, Darmani et al., 2018). Both antiepileptic drugs showed a decreasing effect on the amplitude of N100.

In summary, the evidence so far suggests that N100 is mediated through cortico-subcortical pathways which are positively modulated by GABA-B receptors at the site of stimulation and negatively modulated in the contralateral hemisphere by

GABA-A receptors, containing the α2- and α3-subunits, as well as drugs inhibiting excitatory transmitter release.

P180:

The late deflection P180 was decreased after the intake of the antiepileptic drugs lamotrigine and levetiracetam (Premoli et al., 2016) as well as carbamazepine (Darmani et al., 2018). All three drugs have different and complex modes of functioning, but all have an excitability-lowering effect which may cause the amplitude reduction of P180. Whereas the studied GABAergic and anti-glutamatergic drugs had no effect on P180 so far (Premoli et al., 2014, Koenig et al., 2019). The evidence suggests that cortical excitability in general affects P180, but there is no evidence for the involvement of GABA- or glutamate receptors on P180 evolution to date. Further studies need to be performed to identify the exact physiology behind P180.

Table 4.1 The effect of different drugs on the TEP components

Drug	Mode of action	P25	N45	P70	N100	P180	Reference
Glutamate recepetor antagonists							
Perampanel	AMPAR antagonist	x	x	↓	x	x	Koenig et al., 2019
Dextromethorphan	NMDR antagonist	x	↑	x	x	x	Koenig et al., 2019
GABA-A-R modulators							
Alprazolam	GABA-A-R agonist	x	↑	x	↓	x	Premoli et al., 2014
Diazepam	GABA-A-R agonist	x	↑	x	↓	x	Premoli et al., 2014
Zolpidem	GABA-A-R agonist (mainly α1)	x	↑	x	x	x	Premoli et al., 2014
S44819	α5-GABA-A-R antagonist	x	↓	x	x	x	Darmani et al., 2016
GABA-B-R modulators							
Baclofen	GABA-B-R agonist	x	x	x	↑	x	Premoli et al., 2014
Calcium-channel modulators							
Nimodipine	L-VGCC blocker	x	x	x	x	x	Koenig et al., 2019
Antiepileptic drugs							
Lamotrigine	VGSC blocker	x	↑	x	x	↓	Premoli et al., 2017
Levetiracetam	SV2A binding	x	↑	x	(↓)	↓	Premoli et al., 2017
Carbamazepin	VGSC blocker	↓	x	x	x	↓	Darmani et al., 2018
Brivaracetam	VSA2 binding	x	x	x	↓	x	Darmani et al., 2018
Tiagabine	GABA reuptake inhibitor	x	x	x	x	x	Darmani et al., 2018

4.4 Outlook

Characterizing the underlying processes of TMS-evoked EEG potentials may enable us to better identify neurophysiological aspects in disease. A full understanding of TEP evolution would open many doors for TMS-EEG. With this information TMS-EEG could be used as a non-invasive in vivo bio-marker.

A biomarker is defined as an objective measurement to evaluate physiological and pathological processes and effects thereon through intervention, e.g. through drug application (Biomarkers Definitions Working Group, 2001). Therefore, TMS-EEG could function as an improved diagnostic tool or a prognostic instrument for treatment success. Furthermore, it could be used for designing individualized and target-oriented new treatments.

Adding pharmacology to TMS-EEG has helped to identify some TEP characteristics, but further studies will be necessary for a deeper understanding. Additionally, to the drug use a combination of TMS-EEG with other neuroimaging techniques can enhance our understanding of TEP-physiology.

A summation of future studies hopefully reveals and completes the understanding of causal mechanism of brain-behavior, enhancing the understanding of neurological and psychiatric diseases as well as identifying potential target points for new treatments.

5 Conclusion

In our study we could supplement prior pharmaco-TMS-EEG studies with the effects of glutamatergic inhibition on TEPs. It was shown that N45 was increased by the application of dextromethorphan, a NMDAR antagonist. Hence this deflection (N45) is a summation of activity through α1-subunit containing GABA-A receptors and NMDARs. Furthermore, P70 was decreased by the application of perampanel, an AMPAR antagonist. The effect was mainly expressed in the hemisphere contralateral to stimulation, which indicates that perampanel reduces interhemispheric propagation of neural activity.

Additionally, it was shown that nimodipine and perampanel increased RMT. This indicates an involvement of L-VGCC- and glutamatergic AMPAR-mediated neurotransmission in corticospinal excitability.

6 Summary

Physiological neuronal activity in the brain requires a balance between excitation and inhibition. Glutamate is the most important neurotransmitter of the excitatory system. Therefore, this thesis takes a closer look at the role of the glutamatergic system in brain activity.

The combination of transcranial magnetic stimulation and electroencephalography (TMS-EEG) is a unique method for non-invasive measurements of brain physiological processes. TMS generates an EEG-response with specific positive and negative deflections called TMS evoked potentials (TEPs). TEPs can be used to quantify pharmacological effects on neuronal activity in the human cortex. In this work we tested the influence of two glutamatergic drugs on TEPs. First, perampanel, an AMPA receptor antagonist and second, dextromethorphan, a NMDA receptor antagonist. Additionally, the effect of nimodipine, a voltage-gated L-type calcium channel blocker, on TEPs.

The study was conducted in a pseudorandomized, double-blind, placebo-controlled crossover design. A Total of 16 healthy subjects were included in the study after undergoing a screening protocol. All subjects participated in four measurements, in which we stimulated the hand area of the left motor cortex (M1) with single-pulse TMS and recorded TEPs before and after drug intake. Significant changes of the TEPs were observed after the intake of glutamatergic drugs. Dextromethorphan elevated the amplitude of N45, a negative potential about 45 ms after stimulation. Perampanel reduced the P70 amplitude, a positive potential about 70 ms after stimulation, in the non-stimulated hemisphere. Nimodipine and placebo had no influence on TEPs.

These data complement previous pharmaco-TMS-EEG studies with important insights into the role of the glutamate receptor in the development of TEPs. More precisely, the new evidence indicates that the evolution of N45 is based on a balance of EPSPs and IPSPs generated by NMDARs and GABA-A-Rs.

Whereas fast EPSP generation and interhemispheric propagation via AMPARs is responsible for P70 evolution.

These new data deepen the understanding of the underlying processes of TEPs. This acts as a very important step towards TEPs as non-invasive biomarkers for excitability and propagation of neuronal activity in the human cortex in disease and health.

Ort/Datum Unterschrift Betreuer (Prof. U. Ziemann)

Ort/Datum Unterschrift Doktorandin (Franca S. König)

7 Zusammenfassung

Physiologische neuronale Aktivität im Gehirn setzt eine Balance zwischen dem erregenden und dem hemmenden System voraus. Glutamat ist dabei der wichtigste Neurotransmitter des erregenden Systems. In dieser Arbeit haben wir einen die Rolle des glutamaterge System in neuronaler Aktivität genauer untersucht.

Die Kombination aus transkranieller Magnetstimulation und Elektroenzephalographie (TMS-EEG) ist eine Methode zur nicht-invasiven Messung hirnphysiologischer Prozesse. TMS erzeugt spezifische positive und negative Auslenkungen im EEG, die als TMS evozierte Potentiale (TEPs) bezeichnet werden. Mittels TEPs lassen sich pharmakologische Effekte auf die neuronale Aktivität im menschlichen Kortex quantifizieren. In dieser Arbeit haben wir den Einfluss zweier glutamaterger Medikamente auf besagte TEPs getestet. Erstens, Perampanel, einem AMPA-Rezeptor-Antagonisten und zweitens, Dextromethorphan, einem NMDA-Rezeptor-Antagonisten. Des Weiteren haben wir die Wirkung von Nimodipin, einem spannungsabhängigen Kalziumkanalblocker vom Typ L, auf TEPs getestet.

Die Studie wurde in einem pseudorandomisierten, doppelblinden, placebokontrollierten Crossover-Design durchgeführt. Insgesamt wurden sechzehn gesunde Probanden, nach durchlaufen eines Screening Protokolls, in die Studie integriert. Alle Probanden durchliefen vier Messungen, bei denen wir den Handbereich des linken motorischen Kortex mit Single-Pulse TMS stimulierten und TEPs vor und nach der Medikation aufzeichneten.

Es zeigten sich signifikante Veränderungen der TEPs nach Einnahme der glutaminergen Substanzen. Dextromethorphan erhöhte spezifisch die Amplitude des N45, ein negatives Potential ca. 45 ms nach dem TMS-Puls. Perampanel reduzierte die P70-Amplitude, ein positives Potential ca. 70 ms nach dem TMS-Puls, in der nicht stimulierten Hemisphäre. Nimodipin und Placebo hatten keinen Einfluss auf die TEPs.

Unsere erhobenen Daten ergänzen frühere Pharmako-TMS-EEG-Studien um wichtige Erkenntnisse bezüglich der Rolle des Glutamatrezeptors in der

Entstehung von TEPs. Im Detail, konnten wir zeigen, dass N45 durch ein Gleichgewicht aus GABA-A-R- vermittelter Hemmung und NMDAR-vermittelter Erregung reguliert wird. Im Gegensatz dazu trägt die AMPAR-vermittelte Neurotransmission zu einer interhemisphärischen Verteilung der Aktivität bei, die sich in P70 widerspiegelt. Diese neuen Daten spielen eine wichtige Rolle um die Physiologie der TEPs als Marker für Erregbarkeit und Verteilungsmuster neuronaler Aktivität im menschlichen Kortex bei Gesundheit und Krankheit besser zu verstehen.

Ort/Datum Unterschrift Betreuer (Prof. U. Ziemann)

Ort/Datum Unterschrift Doktorandin (Franca S. König)

8 Literature

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PRODUCT INFORMATION NIMOTOP® Nimodipine Bayer.

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9 Erklärung zum Eigenanteil

Die Arbeit wurde in der Universitätsklinik für Neurologie in der Abteilung Neurologie mit Schwerpunkt Neurovaskuläre Erkrankungen und Neuroonkologie unter der Betreuung von Herrn Prof. Dr. Ulf Ziemann durchgeführt.

Die Konzeption der Studie erfolgte in Zusammenarbeit mit Herrn Prof. Dr. Ziemann, Herrn Dr. med. Florian Müller-Dahlhaus und Herrn Dr. Carl Zipser und Chen Liang.

Die Erhebung der Daten wurde nach Einarbeitung durch Herrn Dr. Carl Zipser und in Zusammenarbeit mit Chen Liang eigenständig durch mich erhoben. Dabei wurden wir von Dr. Carl Zipser und Dr. Christoph Zrenner von ärztlicher Seite aus betreut.

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Ich versichere, das Manuskript selbständig verfasst zu haben und keine weiteren, als die von mir angegebenen, Quellen verwendet zu haben.

Tübingen, den 04.09.2019

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Franca S. König

10 Veröffentlichung

Teile der vorliegenden Dissertationsschrift wurden bereits in der folgenden Preprint Publikation veröffentlicht:

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