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Abteilung Neurologie mit Schwerpunkt neurovaskuläre Erkrankungen und Neuroonkologie

The role of GABAAergic neurotransmission in the human brain probed by paired-pulse TMS-EEG

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(Meinen Eltern)

Agenda

I. List of Figures

II. Abbreviations

AEP	auditory evoked potential
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA	analysis of variance
APB	M. abductor pollicis brevis
CNS	central nervous system
CS	conditioning stimulus
CSP	cortical silent period
DLPFC	dorsolateral prefrontal cortex
EEG	electroencephalography
EMG	electromyography
ENG	electronystagmography
EOG	electrooculography
EP	evoked potential
EPSP	excitatory postsynaptic potential
ERP	event-related potentials
fMRI	functional magnetic resonance imaging
GABA	γ-aminobutyric acid
GIRK	G-protein coupled inwardly rectifying K+ channels
ICA	independent component analysis
IHI	interhemispheric inhibition
IPSP	inhibitory postsynaptic potential
ISI	interstimulus interval
ITI	intertrial interval
LICI	long-interval intracortical inhibition
MEP	motor-evoked potential
MSO	maximum stimulator output
NIBS	non-invasive brain stimulation
NIRS	near-infrared spectroscopy
NMDA	N-methyl-D-aspartat
PCA	principal component analysis
PET	positron emission tomography
RMT	motor threshold at rest
SEM	standard error of the mean
SEP	somatosensory-evoked potential
SICI	short-interval intracortical inhibition
SPV	saccade peak velocity
tDCS	transcranial Direct Current Stimulation
TMS	transcranial magnetic stimulation
TOI	time of interest
TS	test stimulus

1 Introduction

1.1 *Transcranial magnetic stimulation*

Neuronal activity can be modulated by various brain stimulation techniques (Fig. 1). The most frequently used non-invasive brain stimulation (NIBS) methods are Transcranial Magnetic Stimulation (TMS) and transcranial Direct Current Stimulation (tDCS). Differently from tDCS, TMS allows both stimulation and modulation of neuronal activity in the human brain (Wagner et al. 2007). Introduced by Barker in 1985, TMS enables to stimulate the human cortex painlessly (Barker et al. 1985) and without significant long-term side effects (Ilmoniemi and Kicic 2010). In research, TMS is used to investigate neurophysiological (Daskalakis et al. 2002) and cognitive mechanisms (Pascual-Leone et al. 2000, Cappa et al. 2002) both in the healthy and pathologic brain. In clinical practice, TMS - as a promising biomarker - is used for diagnostics (Chen et al. 2008, Kimiskidis et al. 2014), therapy (Fitzgerald and Daskalakis 2012, Rossini and Rossi 2007) and prognostic purposes (Schlaeger et al. 2012, Simpson and Macdonell 2015), especially in cases of neurological or psychiatric disorders like epilepsy, schizophrenia, depression, multiple sclerosis, stroke and pain syndromes.

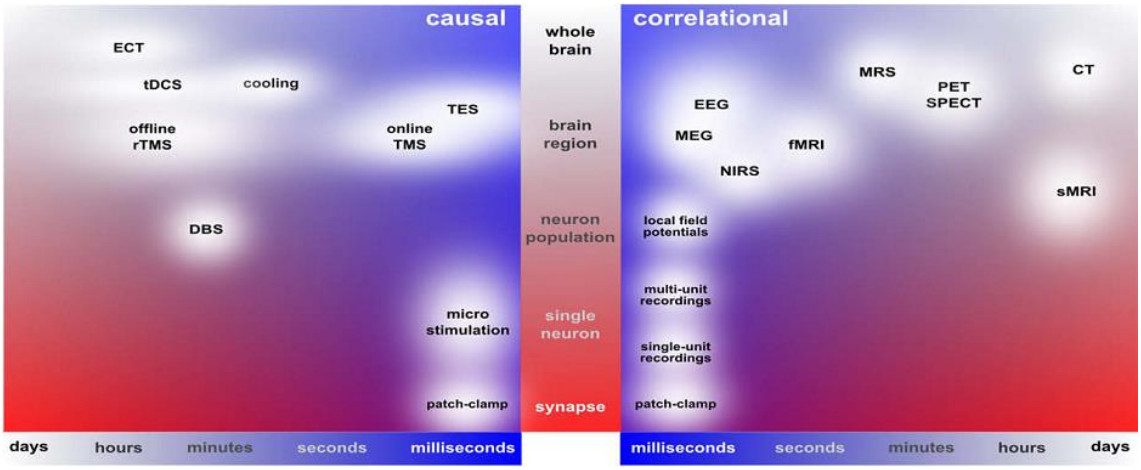

Figure 1: Brain stimulation and brain imaging techniques.
Diverse brain stimulation and neuroimaging techniques are disposed classified according to their spatial and temporal measuring accuracy (source: Siebner et al. (2009)).

TMS is based on Faraday's law of induction (1831): quickly varying currents flowing through circular arranged copper coils placed above the head induce rapidly varying magnetic fields changing in time and rotated by 90°. These result in electrical eddy currents in the intracranial tissue with opposite direction to the original current (Fig. 2) (Rothwell 1997, Ilmoniemi and Kicic 2010, Di Lazzaro et al. 2004, Groppa et al. 2012).

The size of the induced current is proportional to the rate of change of the magnetic field:

$$\nabla \times \mathbf{E} = -\frac{\partial \mathbf{B}}{\partial t}$$

Where a time-varying magnetic field **B** induces a spatially varying, non-conservative electric field **E**, and vice versa. The size of the induced electric field decreases with increasing distance from the surface.

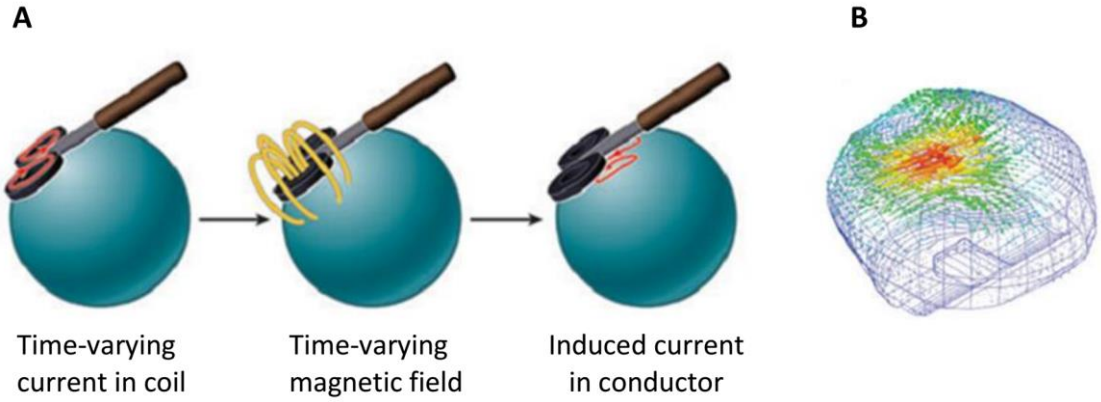

Figure 2: Induction of electric current in the brain by TMS.
(A) Time-varying currents in the coil induce time-varying magnetic fields rotated by 90° leading to electrical eddy currents in the conductor, e.g. intracranial tissue. (B) Figure-of-eight coils produce focal electric fields with a maximum under the center of the coil (source: Wagner et al. (2007)).

The current density is highest in superficial cortical layers (Heller and van Hulsteyn 1992). The induced electric field is affected by location and orientation of the coil relative to the sulci. Structure and conductivity properties of the head also determine the electric diffusion (Ilmoniemi and Kicic 2010, Janssen et al. 2015). To minimize the attenuation of the magnetic field with depth and thus maximizing the strength of the induced electric field, the coil is placed perpendicularly to the scalp (Fox et al. 2004). Figure-of-eight coils produce a more focal electric field (Fig. 2B) than circular coils and reach typically a depth of 2-3 cm (Deng et al. 2014). The shape of the electric field shows a maximum under the center of the coil, so that brain structures near to the center of the coil are stimulated predominantly (Cohen et al. 1990). If the stimulation intensity and the induced current are sufficiently high, voltage-dependent ion channels open and cell membranes get depolarized resulting in an action potential (Ilmoniemi and Kicic 2010). Cortical neurons like pyramidal neurons and interneurons can be activated (Rogasch et al. 2013a). The neuronal activation is not restricted to the point of stimulation. The signal propagates trans-synaptically to further cortical and subcortical regions via cortico-cortical and transcallosal pathways (Groppa et al. 2012,

Voineskos et al. 2010). TMS applied over the motor cortex elicits efferent volleys in the corticospinal tract. Glutamatergic cortico-motoneural synapses are able to transfer the signal and thus evoke action potentials in spinal motoneurons leading to a motor response via peripheral motor axons (Fig. 3) (Groppa et al. 2012, Barker et al. 1985). These motor evoked potentials (MEPs) appear in the targeted muscle contralaterally to the stimulation site and reflect indirectly brain excitability and cortico-muscular connectivity measurable by electromyography (EMG) (Hallett 2000).

However, TMS is not limited to stimulate only the motor cortex by concurrent use of EMG. TMS can be integrated with neuroimaging methods like electroencephalography (EEG) (Cracco et al. 1989), functional magnetic resonance imaging (fMRI) (Bohning et al., 1999) or positron emission tomography (PET) (Fig. 1) (Paus et al. 1997) enabling to gain new insights into brain excitability, plasticity and connectivity of cortical networks (Casali et al. 2010, Casarotto et al. 2010) as well as into excitatory and inhibitory mechanisms.

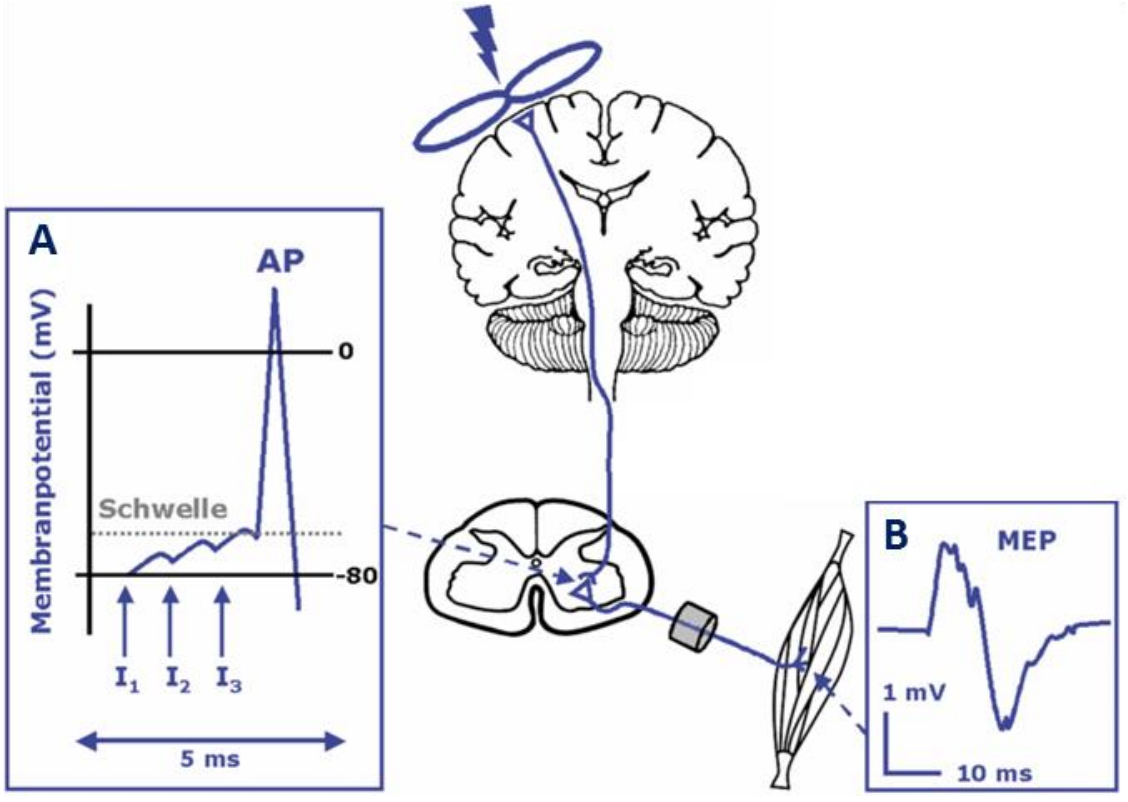

Figure 3: Neurophysiology of motor evoked potentials elicited by TMS.
TMS of primary motor cortex activates corticospinal neurons and results after sufficient temporo-spatial summation (A) at corticomotoneural synapses in a motor response (B) in the target muscle (modified from: Siebner and Ziemann (2007)).

1.2 *Electroencephalography*

As a non-invasive neuroimaging technique (Fig. 1), electroencephalography enables to measure macro-scale electric neuronal activity in the cerebral cortex with an excellent resolution in time domain. Multiple electrodes placed on the scalp record changes of electric potential differences between pairs of electrodes (Buzsáki 2006). Its basic principles subsist since the establishment by Berger in 1923 (Berger 1929). The registered electric potentials derive particularly from the summation of excitatory and inhibitory postsynaptic potentials generated by synchronous activity of underlying neurons (Kirschstein and Köhling 2009).

Neurons convert information into electrical signals. Thus, neuronal activation is measurable as time-varying electrical currents. The intracellular membrane potential at rest is negative (hyperpolarization). Both rapid opening (approx. 1 ms) of voltage-dependent sodium and potassium channels generating action potentials and slower synaptic activation via presynaptic release of neurotransmitter resulting in postsynaptic potentials can depolarize the membrane potential (Lopes da Silva 2010). Whereas action potentials repolarize too fast (about 1 ms) and the small extracellular amplitude would require numerous and synchronous action potentials to be remarkable in EEG, the slower postsynaptic potentials lasting up to several 10 ms are strong enough to be registered by EEG (Kirschstein and Köhling 2009).

Depending on neurotransmitter and receptor, synaptic activation leads to excitation or inhibition of the cell membrane of the dendrites. Entry of cations (e.g. Na^+, Ca^{2+}) across the membrane depolarizes the postsynaptic membrane and generates an excitatory postsynaptic potential (EPSP). The membrane potential is positive (negative extracellular space). Inward current of anions (e.g. Cl^-) or outward current of cations (e.g. K^+) hyperpolarizes the postsynaptic cell and results in an inhibitory postsynaptic potential (IPSP). The membrane potential is negative (positive extracellular space). Among the inhibitory neurotransmitters, the γ-aminobutyric acid (GABA) is the most important one (Lopes da Silva 2010, Kirschstein and Köhling 2009).

EPSPs and IPSPs produce an electric field surrounding the neuron due to the changes in electric potential. Neurons resemble dipoles: in case of an EPSP an active sink is located at the synapse and following the principle of current conservation passive sources are disposed along the soma-dendritic membrane (reversed for IPSPs). The

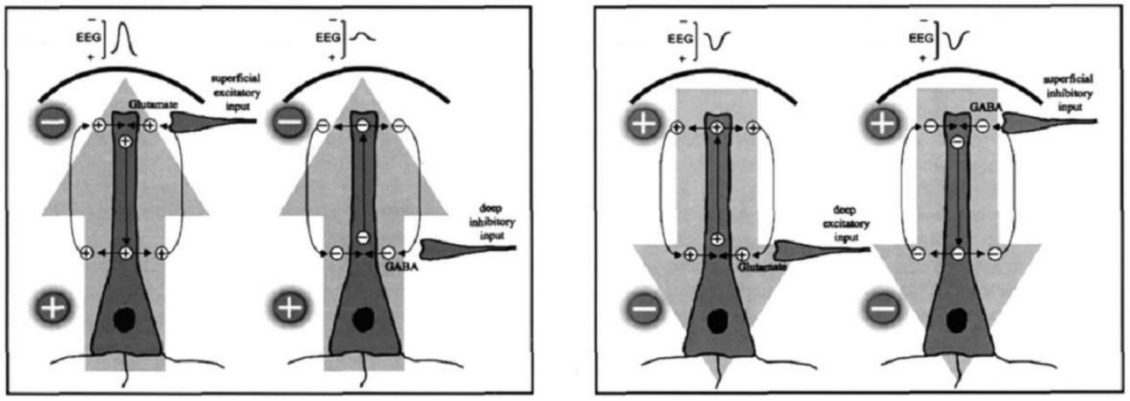

Figure 4: Figure 4: Excitatory and inhibitory potentials generate EEG signals.
(A) Superficial excitatory or deep inhibitory activity in pyramidal neurons causes a negative extracellular polarity near the scalp surface displayed as upward deflection in EEG. (B) Deep excitatory or superficial inhibitory activity in pyramidal neurons leads to a positive extracellular polarity near the scalp surface producing downward deflections in EEG (source: Kirschstein and Köhling (2009)).

summation of the postsynaptically caused extracellular currents generates the EEG signal. Thus, a single electrode contains information of synaptic activation out of tissue masses of a number of neurons between 100 million and 1 billion (Lopes da Silva 2010, Olejniczak 2006, Nunez and Srinivasan 2006a).

Pyramidal neurons are located in the cortical layers III, V and VI. Due to their long apical dendrite and their perpendicular orientation to the cortex surface, pyramidal cells constitute clear current dipoles and therefore represent the main source of EEG. The polarity of measurable surface potentials depends on the direction of the extracellular dipoles (Fig. 4). The dipoles produced by dendritic postsynaptic potentials are determined by superficial or deep synaptic inputs. Superficial excitatory input leads to an influx of positive ions (EPSP) and a local negative extracellular space (Fig. 4A) with a distant positive extracellular space. This dipole has its negative pole at the scalp surface displayed by convention as upward deflection in EEG (opposite for IPSP, Fig. 4B). Similar but smaller dipoles arise from deep inhibitory inputs (Fig. 4A). Charge carriers of IPSPs own a smaller electrochemical gradient, so that generated EPSPs dominate the source of EEG as well as superficial postsynaptic potentials from nonspecific thalamocortical afferents (Fig. 5A) (Kirschstein and Köhling 2009).

Dipoles reveal different orientations due to pyramidal cells organized in sulci and gyri (Fig. 5B). EEG registers particularly radial voltage sources, with higher amplitude on top of the gyri (Fig. 5B.(a)) than in the depth of a sulcus (Fig. 5B.(d)). Horizontal dipoles (Fig. 5B.(c)) are likely to cancel each other. Some tangential dipole sources in the sulci walls (Fig. 5B.(b)) are better detectable by MEG (Cohen 1968, Kirschstein and Köhling 2009).

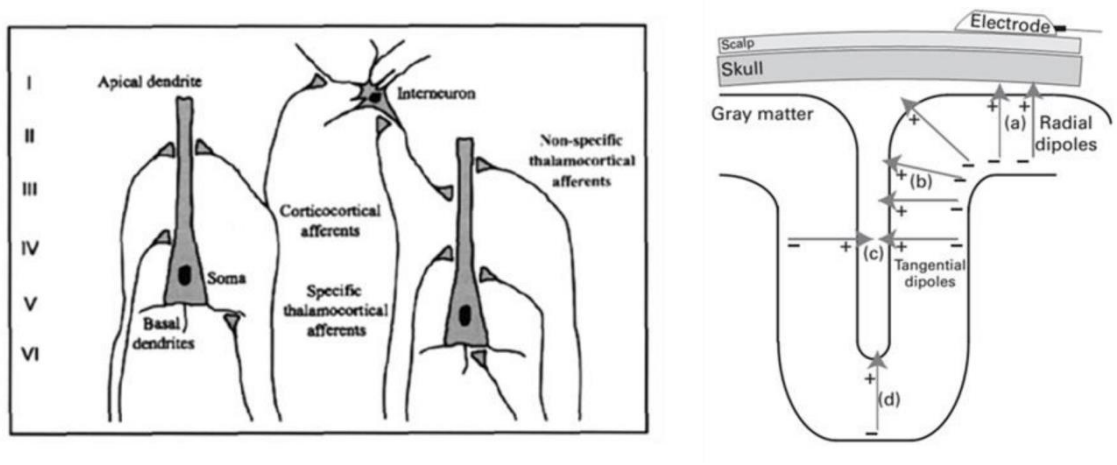

Figure 5: Pyramidal neurons (A) and orientation of dipoles in the cerebral cortex (B).
(A) Cortical pyramidal neurons own basal dendrites and long apical dendrites. Synaptic input of other neurons on pyramidal neurons occurs in different cortical layers. Synapses of cortico-cortical afferents and interneurons can be found in all cortical layers I-VI (source: (A) Kirschstein and Köhling (2009)).
(B) Pyramidal cells show different orientations of dipoles due to the organization in sulci and gyri. Radial poles (a) reveal the strongest signal measurable in EEG, while dipoles in inner segments (d) contribute less as they are further away from surface electrodes. Horizontal dipoles (c) are likely to cancel each other (source: Cohen (2014)).

EEG waves represent the summation of simultaneous postsynaptic potentials. The more synchronous the neuronal activity, the higher the measured amplitude and the less the measured frequency (Kirschstein and Köhling 2009). EEG has a relatively low spatial resolution of centimeters defined by volume conduction spread, the position and number of electrodes. Its temporal resolution of milliseconds is limited only by sampling rate (Taylor et al. 2008, Ilmoniemi et al. 1997). The process of reconstructing a three-dimensional activation map from a two-dimensional sensor map is a basic problem of EEG and MEG, called the inverse problem. There is no unique solution to reconstruct the neuronal source based on EEG potentials (Lopes da Silva 2010, Olejniczak 2006, Niedermeyer and Lopes da Silva 2005).

Dynamic brain behavior is thought to evolve from interaction of large pools of neurons (Nunez 2000) measurable by EEG. Clinical operators and cognitive scientists use this link to gain large-scale insights into the brain activity. Electrical brain activity can occur as spontaneous potentials and evoked potentials (EP) or event-related potentials (ERP) (Nunez and Srinivasan 2006a).

Clinical applications mostly involve spontaneous EEG recordings. In this case, no specific sensory stimuli are presented. Recordings conducted at rest can be used as biomarkers of neuropsychiatric disorders like schizophrenia (Sponheim et al. 2000) or Alzheimer's disease, e.g. with the aim to enable non-invasive screenings for at-risk

populations (Babiloni et al. 2011). It is also useful for diagnostics of epilepsy (Renzel et al. 2016), stroke, tumors - e.g. in order to develop automated tumor detection systems (Selvam and Shenbagadevi 2011) – and surgical monitoring (Nunez and Srinivasan 2006a).

Evoked and event-related potentials are involved in diagnostics of central nerve diseases (e.g. multiple sclerosis) and in exploring the neurophysiological mechanisms of processing emotional or cognitive stimuli (Daskalakis et al. 2012). Evoked potentials are triggered by specific sensory stimuli as light flashes or magnetic stimuli; event-related stimuli are combined with a cognitive task. Usually, they are averaged over a large number of stimuli to eliminate EEG activity not time-locked to the stimulus and background noise.

However, anomalous EEG responses do not refer obligatorily to specific brain abnormalities and EEG alterations get often apparent in late stages of disease merely confirming already clinically evident diseases (Nunez and Srinivasan 2006b).

To enlarge the information value provided by EEG, analysis methods have been refined and combinations of EEG and further neuroimaging or neurostimulation techniques are established complementing existing methods.

1.3 *Combining TMS and EEG*

Several functional neuroimaging techniques (Fig. 1) are used in combination with TMS, each providing complementary temporal and spatial information of cortical reactivity (Siebner et al. 2009). TMS-EEG is considered as a sensitive approach to directly examine and modulate cortical excitability, connectivity and plasticity as well as facilitation and inhibition (Di Lazzaro et al. 2010, Farzan et al. 2013). EEG measurements provide an excellent temporal resolution (Ilmoniemi et al. 1997) in contrast to other combined TMS-neuroimaging modalities as functional magnetic resonance imaging (fMRI) (Bohning et al. 1998), positron emission tomography (PET) (Fox et al. 1997), near-infrared spectroscopy (NIRS) (Noguchi et al. 2003).

The most explored brain area by means of TMS is the primary motor hand area (M1). TMS applied to primary motor cortex activates the corticomotoneural system (Barker et al. 1985, Hallett 2007) and results in motor evoked potentials. Electromyography can record this muscle activity indirectly reflecting cortical excitability (Daskalakis et al. 2002). Whereas TMS-EMG is limited to motor areas, TMS-EEG can be applied to both motor and non-motor regions (Ilmoniemi et al. 1997). Thus, TMS-EEG measurements enable to record cortical neuronal activity bypassing motor as well as sensory

pathways and subcortical structures (Siebner et al. 2009). As a powerful a tool in basic neuroscience and clinical research, TMS-EEG broadens the fields of research, diagnostic and therapy, especially with regard to neuropsychiatric disorders (Platz and Rothwell 2010).

Combining TMS with EEG online emerges as technically challenging. Numerous artefacts have to be monitored. The TMS pulse induces huge electromagnetic discharges that can saturate conventional EEG amplifiers (Virtanen et al. 1999, Veniero et al. 2009). TMS-compatible EEG equipment utilizes gain-control and sample-and-hold circuits locking the EEG signal instantly before the TMS pulse (Ilmoniemi et al. 1997) or DC-coupled amplifiers allowing to record data continuously (Ilmoniemi et al. 1997, Fitzgerald et al. 2008). Despite the rapid recovery from the TMS pulse, electrodes near to the stimulation site show residual related artefacts. Electrical currents at the electrode-electrolyte interface can result in electrode polarization (Ilmoniemi and Kicic 2010) noticeable as EEG baseline shift. An electrode moved with respect to the electrolyte can cause an electrode motion artefact inducing high and low-frequency noise. Eddy-currents on motor cortex do not only result in movement, but also in heating of the electrodes (Virtanen et al. 1999). To alleviate electrode artefacts, the use of small Ag/AgCl sintered pellet electrodes is recommended (Roth et al. 1992, Ives et al. 2006, Virtanen et al. 1999), whereby direct contact of electrode-skin interface and coil should be avoided (Ilmoniemi and Kicic 2010).

TMS can elicit various non-specific or indirect responses affecting the EEG signals. Discharge of the TMS coil causes a loud click resulting in an auditory-evoked potential visible as EEG response at the latency of the N100/P190 peak (Nikouline et al. 1999, Tiitinen et al. 1999). Applying white noise (ter Braack et al. 2015, Massimini et al. 2005, Paus et al. 2001) reduces activation of the auditory system. Stimulation of trigeminal nerve afferents, scalp or facial muscle nerves and muscle movement after motor cortex stimulation can contribute to somatosensory-evoked potentials (SEPs) (Nikulin et al. 2003, Ilmoniemi and Kicic 2010, Rogasch et al. 2013b). Trigeminal stimulation occurs mechanically by vibration of the coil or directly by depolarization (Ilmoniemi and Kicic 2010) and can be minimized by a layer of foam between scalp and coil (Massimini et al. 2005). Muscle fiber activation produces an artefact in the first 30 ms post-stimulus and is minimal stimulating the motor cortex. Residual artefacts can be removed by principal component analysis (PCA) or independent component analysis (ICA) (Korhonen et al. 2011, Maki and Ilmoniemi 2011). SEPs due to peripheral muscle activation do not affect early EEG responses (<40 ms) (Paus et al. 2001), neither later

EEG components (N45, N100) occurring independently of MEP amplitude and being visible at sub-threshold intensities (Komssi et al. 2007, Nikulin et al. 2003, Paus et al. 2001). Reorienting the coil or decreasing the stimulation intensity can minimize the artefacts on the EEG (Ilmoniemi and Kicic 2010). Monitored eye movement artefacts mostly contaminate EEG recordings in frontal electrodes and can be eliminated by discarding affected data (Komssi et al. 2004). Despite the development of TMS-compatible EEG systems, it is necessary to control design, performance and data analysis of experiments for potential artefact sources (Ilmoniemi and Kicic 2010, Rogasch et al. 2014).

The conduction of magnetic stimulation is repeated several times to improve signal-to-noise ratio. In order to obtain brain activity arising from experiment related events, artefactual signal and spontaneous background activity have to be minimized by methods like averaging, independent component analysis cleaning and subtraction methods (Ilmoniemi and Kicic 2010). EEG recordings averaged over trials and time-locked to the stimulus show evoked potentials.

TMS-evoked potentials (TEPs) and their spatio-temporal distribution enable to evaluate local excitability and connectivity in the brain (Ilmoniemi and Kicic 2010, Komssi et al. 2002, Komssi et al. 2004, Kahkonen et al. 2005) also elucidating the link between brain activity and function. The effects depend on the momentary neuronal state (Silvanto and Pascual-Leone 2008, Schurmann et al. 2001), the morphology of stimulated cortex, stimulus intensity, task performance (Nikulin et al. 2003) and subject's vigilance (Massimini et al. 2005, Huber et al. 2013). Differences are observable between patient groups and healthy subjects (Miniussi and Thut 2010). Various brain states like sensory processing (Kahkonen et al. 2001, Massimini et al. 2005), cognition (Bonnard et al. 2009) or motor control (Nikulin et al. 2003, Kicic et al. 2008, Ferreri et al. 2011) can be studied. Stimulating one hemisphere, neuronal activation spreads bilaterally to anatomically and functionally interconnected regions (Ilmoniemi et al. 1997, Komssi et al. 2002, Siebner et al. 2009). TMS at low intensities is thought to stimulate cortico-cortical connections, whereas higher intensities may stimulate cortico-thalamic connections influencing the activation-to-inhibition ratio (Miniussi and Thut 2010).

Averaged TMS-evoked EEG responses are stated to be highly reproducible (Komssi et al. 2004, Lioumis et al. 2009), while MEP recordings suffer from high variability. The TMS pulse generates sequences of positive and negative peaks for a time span of about 300 ms (Ilmoniemi et al. 1997).

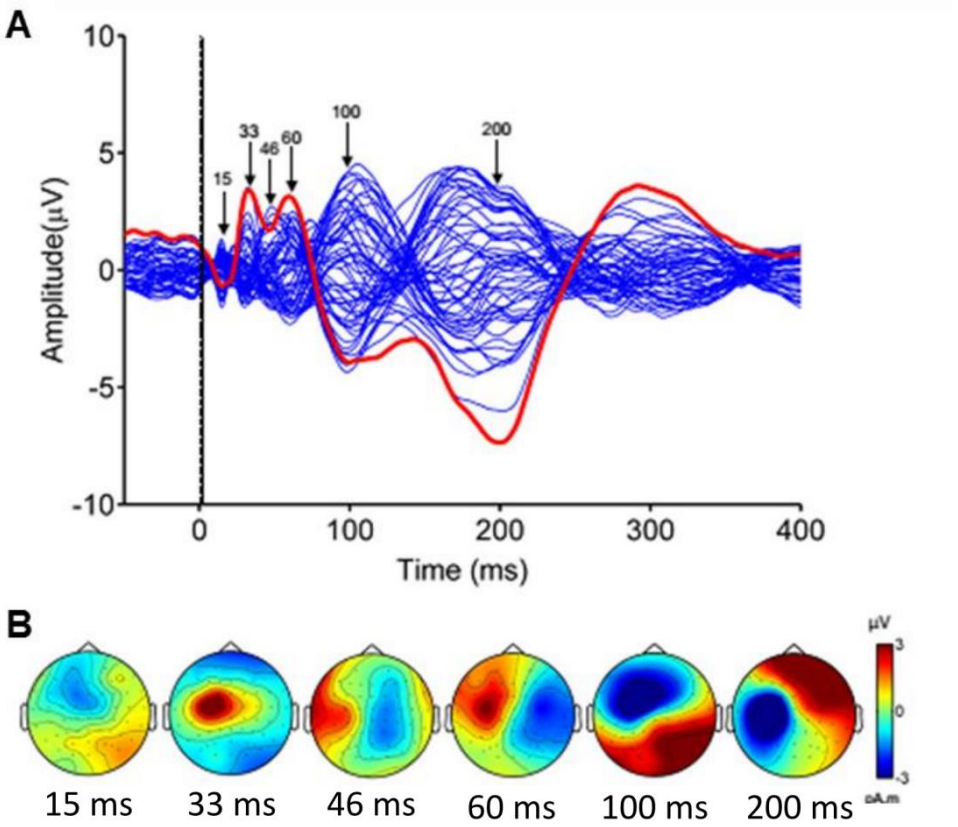

Figure 6: TMS-evoked potentials after stimulation of left M1 (A) and topographical distribution of surface voltages.
The butterfly plot (A, blue) shows characteristic TEPs peaking at specific time points indicated by arrows. The electrode near to the TMS coil (C3) is highlighted as red line. Topographical head maps (B) depict the voltage distribution for each peak (modified from: (Rogasch et al. 2014)).

Single pulse stimulation of primary motor cortex generates EEG responses with clearly identified amplitude, latency and topography (Fig. 6) (Komssi and Kahkonen 2006, Ilmoniemi and Kicic 2010). Following deflections are generally detected and labeled as: N7, P13, N18, P30, N44, P60, N100, P180 and N280 (Ilmoniemi et al. 1997, Paus et al. 2001, Komssi et al. 2002, Nikulin et al. 2003, Kahkonen and Ilmoniemi 2004, Bonato et al. 2006, Daskalakis et al. 2008, Ferreri et al. 2011, Farzan et al. 2009, Veniero et al. 2013, Rogasch et al. 2014, Premoli et al. 2014a, Darmani et al. 2016).

Early TEPs (e.g. N7, P13, N18) are mostly due to the activation of the stimulated cortex. Amplitudes of early peaks are correlated with markers of cortical excitability like MEPs (Maki and Ilmoniemi 2010). The spatiotemporal spreading reflects the existence and the activation of intra- and interhemispheric corticocortical connections (Lee et al. 2003a, Komssi and Kahkonen 2006, Ferreri and Rossini 2013, Ziemann and Rothwell 2000). Later responses originate partially from axonal conducted signals (Ilmoniemi and Kicic 2010).

TMS-evoked EEG responses originate from fast and slow excitatory postsynaptic potentials (EPSPs) and fast and slow inhibitory postsynaptic potentials (IPSPs) (Rosenthal et al. 1967). Whereas fast EPSPs are mediated by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, N-methyl-D-aspartate (NMDA) receptors are proposed to generate and/ or modulate slow EPSPs (N7). GABAA postsynaptic receptors might generate and/ or modulate fast IPSPs as P13, N18, P30 and N44 (Ferreri et al. 2011, Davies et al. 1990, Deisz 1999) and the presynaptic and postsynaptic GABAB receptors might generate and/ or modulate slow IPSPs as P60, N100, P180 and N280 (Nikulin et al. 2003, Bender et al. 2005, Bonato et al. 2006, Ferreri et al. 2011, McDonnell et al. 2006). Premoli et al. (2014a) showed in a pharmaco-TMS-EEG study that early TEPs (N45) are mediated by α1-subunit-containing GABAA receptors. Further pharmacological (Premoli et al. 2014a), functional (Nikulin et al. 2003) and paired-pulse (Rogasch et al. 2013a, Rogasch et al. 2015) studies linked later TEPs (N100) to GABAB receptor mediated inhibitory mechanisms.

The recording of TEPs supplies important information of cortical reactivity, connectivity and drug effects on neurotransmitter pathways. Methodological improvements expanded the knowledge of physiological and pathological brain state mechanisms and opened a broad field of diagnostic and therapeutic research perspectives.

1.4 *Cortical inhibition*

Cortical inhibition describes the attenuating effect of GABAergic inhibitory interneurons on the cortical output by suppressing the activity of other cortical neurons (Iversen et al. 1971, McCormick 1989, Krnjevic 1997).

1.4.1 The GABAergic system

GABA is the main inhibitory neurotransmitter in the vertebrate central nervous system (Sivilotti and Nistri 1991). Out of the three GABA receptor types (GABAA, -B and -C), the ionotropic GABAA and metabotropic GABAB receptors are the predominant ones (Fig. 7) (Watanabe et al. 2002).

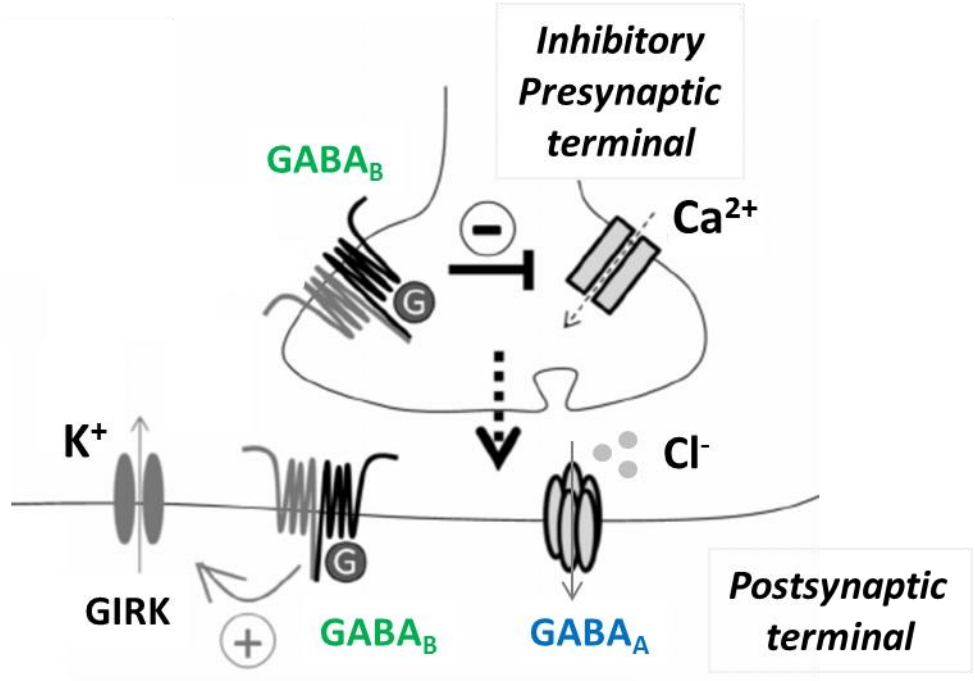

Figure 7: Localization and effect of GABAergic receptors.
The main inhibitory neurotransmitter GABA binds to GABAA and GABAB receptors. Ionotropic GABAA receptors mediate a Cl^- influx via postsynaptic chloride channels. Metabotropic G protein coupled GABAB receptors are linked to inwardly rectifying K+ (GIRK) channels inducing a K+ efflux at postsynapses and a reduction of neurotransmitter release by inhibition of presynaptic calcium channels (modified from: Takahashi et al. (2010)).

The GABAAR (Fig. 8) is a postsynaptic transmitter-gated chloride channel consisting out of five subunit proteins belonging to different classes (α1-6, β1-4, γ1-4, δ, ε and π). An activation of GABAAR leads to a fast hyperpolarization (fast IPSP <20 ms poststimulus) of pyramidal cells due to an influx of Cl^- ions. The channel function can be modulated through different binding sites for benzodiazepines, barbiturates, neurosteroids and ethanol, and can be suppressed competitively by bicuculline and

noncompetitively by pictrotoxinin. The named drugs can only act in presence of GABA and are selective for the GABAAR, e.g. benzodiazepines bind only to GABAA complexes containing a γ-subunit (Bormann 1988, Macdonald and Olsen 1994). Obviously, the subunit isoforms determine specific properties of the receptor (Möhler et al. 2002, Möhler et al. 2004). The α1-subunit-bearing subtype causes sedative, amnestic and anticonvulsant effects while the α2-subunit-bearing subtype act anxiolytic both activated by benzodiazepines (Nusser et al. 1996, Fritschy et al. 1998, Nyiri et al. 2001, Klausberger et al. 2002). Benzodiazepines (e.g. diazepam) are furthermore known as allosteric positive modulators of the GABAR increasing its opening frequency (Haefely 1984).

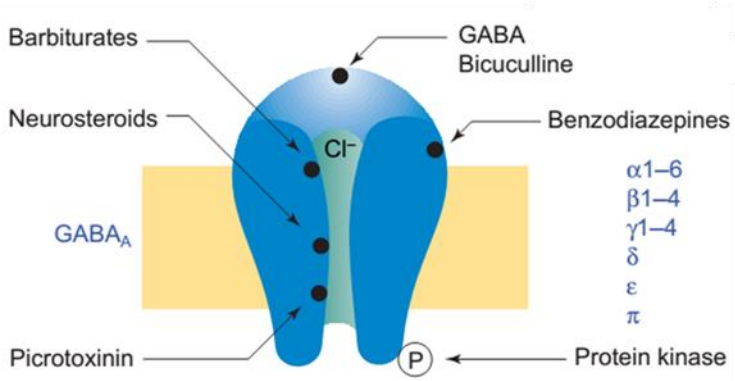

Figure 8: Structure and modulatory binding sites of the GABAA receptor.
The GABAA receptor is a transmitter-gated Cl^- pore mediating fast IPSPs. It is built out of five subunit proteins consisting of different subunit classes (α1-6, β1-4, γ1-4, δ, ε and π). Benzodiazepines, barbiturates and neurosteroids bind to a site on the GABAA receptor leading to an increased response of the receptor to GABA. Picrotoxinin and bicuculline suppress the activation by GABA. Protein kinases located intracellularly are able to modulate GABA responses. (modified from: Bormann (2000)).

GABAB receptors are obligatory heterodimers consisting of two guanine nucleotide-binding (G) protein-coupled receptor subunits, named GABAB1 and GABAB2 (Jones et al. 1998, Kaupmann et al. 1998). GABAB receptors are built up of seven transmembrane helices. The agonist binds at the GABAB1 subunit and the GABAB2 subunit is linked to the effector G-protein (Galvez et al. 2001). Postsynaptical GABAB receptors produce a K^+ efflux via activation of G-protein coupled inwardly rectifying K^+ channels (GIRK) resulting in a slow hyperpolarization (slow IPSP 50-300 ms poststimulus) (Dutar and Nicoll 1988, Luscher et al. 1997). GABAB receptors localized presynaptically reduce the neurotransmitter release by inhibition of calcium channels (Bowery et al. 1980). The presynaptical receptors enable GABA-mediated autoinhibition of inhibitory activity (Davies et al. 1990). The neuroactive substrate baclofen, with its anti-spastic effect, is classified as selective agonist for GABAB

receptors, while GABAB receptors are resistant to GABAA receptor modulating drugs (Bormann 1988, Bowery 1989, Bowery 1993).

GABAergic inhibitory mechanisms are involved in the etiology of several disorders. Malfunction of GABA receptors are linked to the generation of epileptiform activity causing an abnormal synchronous neuronal activity in the brain. Specific GABA agonists (e.g. tiagabine) are promising targets for the treatment of seizures by elevating the amount of synaptic GABA (Bradford 1995, Meldrum 1989, Schousboe et al. 2014, Kohl and Paulsen 2010).

The interaction between GABAergic interneurons and pyramidal cells generates network oscillations in diverse brain regions in the range of theta, gamma (30-90 Hz) and ultrafast (200 Hz) frequency (Buzsáki and Eidelberg 1983, Bragin et al. 1995, Buzsáki and Wang 2012). Psychiatric disorders like schizophrenia show reduced gamma band oscillation in frontal regions connected with a defect of GABA function and working memory impairment (Chen et al. 2014, Fries 2005, Ferrarelli et al. 2008, Uhlhaas and Singer 2010). Therefore, neuroactive substrates modulating the activity of GABA receptors (e.g. GABAB receptor agonists) have therapeutic potential e.g. in schizophrenia (Kantrowitz et al. 2009).

Abnormal activity of the GABAergic system was further related to anxiety (Lydiard 2003) and sleep disorders (Lancel 1999), depression (Cryan and Slattery 2010), spasticity (Davidoff 1985), stiff-person syndrome and autism spectrum disorders (Cellot and Cherubini 2014). More detailed understanding of cellular and molecular mechanisms linked to function and dysfunction of GABAergic neurotransmission offers new therapeutic perspectives and treatment possibilities.

1.4.2 The SICI paradigm

Cortical inhibitory processes, primarily GABAA and GABAB receptor-mediated neurotransmission, can be measured employing paired-pulse TMS. Inhibitory TMS paradigms include short-interval intracortical inhibition (SICI) (Kujirai et al. 1993), long-interval intracortical inhibition (LICI) (Valls-Sole et al. 1992) and cortical silent period (Cantello et al. 1992, Ziemann et al. 1997).

SICI occurs by delivering a subthreshold conditioning stimulus (CS) 1-6 ms before a suprathreshold test stimulus (TS) (Kujirai et al. 1993); its effects are measurable via both EMG and EEG recordings.

In first experiments, TMS application of SICI over the primary motor cortex resulted in suppression of motor evoked potentials (MEPs) in the target muscle compared to TS alone (Kujirai et al. 1993). The alteration of the MEP amplitude is considered as a

measure of corticospinal excitability (Di Lazzaro et al. 2008, Ferreri et al. 2003). Whereas a suprathreshold stimulus leads via corticospinal delivered signals to measurable MEPs, a subthreshold stimulus excites only local, cortical neurons. SICI can thus explore the impact of interneuronal activity on cortical output (Ziemann et al. 1998), where the inhibitory output of the preceding CS on the test response is suggested to be cortical in origin (Kujirai et al. 1993, Nakamura et al. 1997, Rothwell 1997). Pharmacological trials emphasized the enhancing effect of benzodiazepines on SICI (Fig. 9), thus it is thought to reflect GABAAR mediated inhibitory mechanisms, specifically those subtypes with α2 or α3 subunits (Ziemann et al. 1996a, Ziemann et al. 1996b, Chen et al. 1997, Ziemann 2004, Paulus et al. 2008, Ilic et al. 2002).

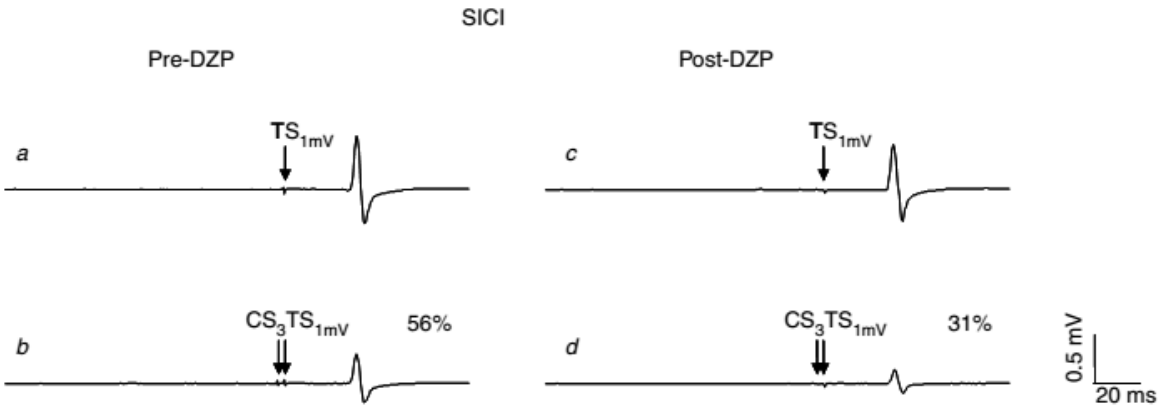

Figure 9: Pharmaco-TMS-EMG measurements and the effect of diazepam on SICI
Each line demonstrates EMG recordings (averaged over eight trials) from the right FDI muscle after TMS stimulation of the left primary cortex before and after drug-intake. SICI suppresses the MEP amplitude to 56% (b) of the unconditioned value (a). The application of diazepam lead to a significant increase of SICI with a MEP amplitude of 31% in the conditioned situation (d) compared to the unconditioned situation (c) (source: Müller-Dahlhaus et al. (2008)).

The limitations of EMG measurements which quantify indirectly the cortical excitability by means of MEP amplitude modulation depending on inhibitory pathways at spinal level and which are restricted to the motor cortex can be overcome by measuring the cortical output to SICI directly with EEG.

Paired-pulse TMS protocols and concurrent EEG measurements make it possible to record neuronal activity over cortical regions associated with certain neurological or psychiatric pathophysiologies closer than the motor cortex, e.g. the dorsolateral prefrontal cortex (DLPFC) in schizophrenia (Daskalakis et al. 2008), and enable new insights into cortical network properties like cortical connectivity and oscillatory frequencies (Rogasch and Fitzgerald 2013). Resulting TEP components include information of excitatory and inhibitory brain activity (Komssi et al. 2004, Nikulin et al. 2003).

With additional use of CNS active drugs specific neuronal circuits can be investigated dependent on activated neurotransmitter systems targeted by the drugs. TMS-EEG and the application of neuroactive substrates with well-known pharmacological properties can characterize cortical inhibition reconstructed spatio-temporally as changes of neuronal activity due to neurochemical modulation (Ziemann 2004).
The pharmacological challenge of TMS-EEG measurements permits a rather articulated understanding of the physiological and pathological mechanisms of cortical excitability down to the level of receptor subtypes.

1.5 *Methodological limitations of previous SICI studies*

Short-interval intracortical inhibition (SICI) is a paired-pulse TMS paradigm indexing intracortical inhibition mediated by GABAAR-mediated neurotransmission (Kujirai et al. 1993). Until recently, SICI was quantified indirectly by EMG measurements. However, after the introduction of TMS-EEG recordings, intracortical inhibitory mechanisms were directly measurable from motor and non-motor regions. Three previous studies have characterized SICI using EEG (Cash et al. 2017, Ferreri et al. 2011, Paus et al. 2001). These studies either revealed no modulation of TEPs by SICI (Paus et al. 2001), an increase of the N100 and a reduction of the P70 and the P180 in the stimulated hemisphere (Ferreri et al. 2011) or a suppression of early TEPs (Cash et al. 2017). Several methodological challenges have not been addressed yet, resulting in an inconsistent interpretation of SICI-induced modulation of TEPs across different studies.
To analyze the interaction between CS and TS in paired-pulse studies, a subtraction of the conditioning pulse and testing pulse alone aligned to their time of application from the paired-pulse EEG waveform is required (Premoli et al. 2014b). The aforementioned studies (Cash, Ferreri and Paus) did not correct for the interfering long-lasting EEG responses evoked by the single stimuli alone.
The diversity of results in previous studies may also derive from smaller sample sizes (5, 8 or 12 subjects), a different number of EEG channels (60, 32 or 64 channels) as well as the use of different interstimulus intervals (2 or 3 ms) and stimulation intensities (Paus et al. 2001, Ferreri et al. 2011, Cash et al. 2017). Furthermore, in two studies, possible contamination by somatosensory feedback was not taken into account (Cash et al. 2017, Ferreri et al. 2011). In a third study, only a few of currently known and reproducible TMS-evoked EEG components were extracted from the EEG signal (Paus et al. 2001).

These factors might have affected the results and led to missing information and inconsistent interpretation of SICI-evoked modulation of EEG responses in the present literature.

To overcome the aforementioned challenges, we analyzed the amplitude modulation of the EEG signal by SICI in specific non-overlapping time windows of interest beginning at 15 ms continuously to 205 ms after the second TMS pulse by means of a non-parametric, cluster-corrected permutation analysis – a well-established method (Maris and Oostenveld 2007) – over all 64 EEG electrodes. We controlled for the late evoked responses evoked by the single pulses alone in the paired-pulse paradigm (Premoli et al. 2014b). Furthermore, we minimized the somatosensory feedback due to the TMS coil click by applying a masking noise through earphones (Massimini et al. 2005). In contrast to previous experiments applying suprathreshold TS intensities, we set the intensity at 100% of RMT to overcome the contamination of the EEG components by somatosensory afferences. One previous TMS-EMG study has shown that SICI can be reliably elicited with the CS and TS intensities used in our study (Ilic et al. 2002).

In sum, our study aims to assess the effects of SICI on TEPs following a valid and reproducible methodological approach and examines for the first time the pharmacological properties of SICI over the EEG traces.

1.6 *Hypotheses and aims*

The present study aims to investigate the physiological underpinnings of TMS-induced EEG potentials modulated by the paired-pulse paradigm SICI. As there is preexisting strong evidence that SICI is mediated by the a2 and a3-subunit bearing subtype of the GABAAR (Kujirai et al. 1993, Di Lazzaro et al. 2006), we expect a suppressive effect primarily on early TEPs (P25 or N45) generated by fast IPSPs. We also hypothesize a GABAAR-mediated effect on GABABergic circuits (Premoli et al. 2014a) resulting in a decrease of the N100, a marker of GABAB-mediated cortical inhibition.

To further broaden the knowledge of the underlying mechanisms of cortical inhibition, we applied two GABAergic agonists enhancing the activity of the predominant GABA receptor types (GABAA and –B) in the vertebrate central nervous system. We state that the application of diazepam will enhance and baclofen will reduce the suppressive effects of SICI on early TEPs as previous pharmacological SICI-EMG studies have revealed comparable effects on MEP amplitudes (McDonnell et al. 2006, Müller-Dahlhaus et al. 2008).

Finally, we compared two different paired-pulse paradigms, SICI and LICI, on the cortical level measured via TMS-EEG. As previous findings based on TMS-EMG

measurements have suggested that both paradigms originate from different cell populations (Sanger et al. 2001), we expect differing EEG patterns.

2 Materials and Methods

2.1 *Subjects*

Sixteen healthy male subjects with an age range of 19 to 35 years (mean age 25.4 ± 4.1 years) assented to participate in the study by giving written informed consent previously. Female participants were excluded a priori in order to avoid menstrual cycle-dependent effects on the cortical excitability, which can occur potential distortion of results in TMS studies (Smith et al. 1999). All subjects were evaluated as right-handed according to the Edinburgh Handedness Inventory (laterality score ≥ 75%) (Oldfield 1971) to prevent differing functional network properties due to left-handedness (De Gennaro et al. 2004). Physical and neurological examinations were performed before and after each trial. Subjects were screened for contraindications to TMS (Groppa et al. 2012). A pre-existing history of neurological or psychiatric disease, the use of CNS active drugs, the abuse of any drugs (including nicotine and alcohol) or a contraindication to study medication (baclofen and diazepam) were considered as exclusion criteria. All subjects were naive to the study medication.

The study was approved by the local Ethics Review Committee of the Medical Faculty of the Eberhard Karls University Tübingen, Germany (protocol number 199/2014B02) in concordance with the Declaration of Helsinki (2013).

2.2 *Experimental design*

In line to further characterize the origin and pharmacological properties of intracortical inhibitory circuits, drugs modulating the GABAergic neurotransmission and TMS paired-pulses provoking short-interval intracortical inhibition (SICI) were applied and recorded concurrently via EEG in a pseudo-randomized, placebo-controlled, double-blinded crossover study.

Subjects were requested to attend three sessions at least one week apart to prevent carry-over effects. In every single trial, the hotspot and the motor threshold at rest (RMT) were defined. Subsequently, saccade movements and TMS-EEG (125 to 150 stimuli per stimulation protocol and drug-condition) were recorded. All measurements were performed at baseline and 90 min after drug-intake (Fig. 10). Drug application included a single oral dose of baclofen (50 mg Lioresal®, Novartis Pharma), diazepam (20 mg Diazepam-ratiopharm®, ratiopharm GmbH) or placebo (P-Tabletten Lichtenstein). Baclofen is known as a specific GABABR agonist. Diazepam is a classical benzodiazepine targeting α1, α2, α3 and α5 subunit-containing GABARs. The dose of drugs and the time point of post-drug measurements were adjusted to previous

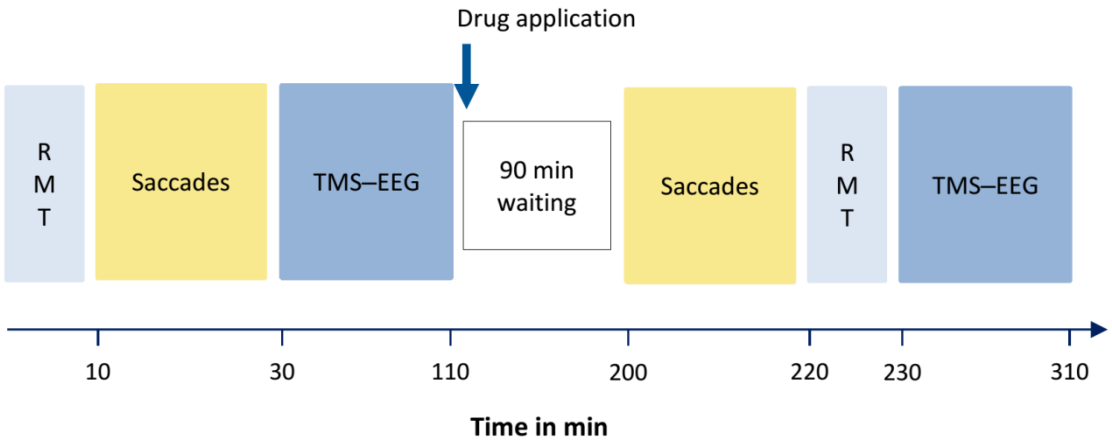

Figure 10: Study design.
In each session, the motor threshold at rest, saccade velocity measurements and TMS-evoked EEG responses were performed at baseline and 90 min after drug-intake.

experiments indicating a significant modulation of TMS parameters in EMG- as well as in EEG-recordings for motor cortical inhibition 90 min after drug application (Müller-Dahlhaus et al. 2008) and to pharmacokinetic studies reporting peak plasma concentrations 90 min after drug application (McDonnell et al. 2006, Shader et al. 1984).

2.3 *Data recording*

2.3.1 Transcranial magnetic stimulation and electromyography

TMS pulses were applied over the hand area of left primary motor cortex (M1) (Fig. 11). A figure-of-eight coil (external diameter of each wing, 90 mm) was used connected to a Magstim 200^2 magnetic stimulator (Magstim Company, Carmarthenshire, Wales, UK) inducing a focal current with monophasic waveform. The optimal location of the coil was identified as the position where the largest MEPs in the right abductor pollicis brevis muscle (APB) were consistently elicited by giving marginally subthreshold TMS pulses. Surface electromyography arranged in a belly-tendon montage and with a ground electrode over the styloid process of the radius allowed to measure MEPs while complete relaxation of the contralateral hand using Ag-AgCl cup electrodes. Its raw signal was amplified and bandpass filtered (20 Hz to 2 kHz; D360 amplifier, Digitimer, Hertfordshire, UK) and digitized at an A/D rate of 10 kHz per channel (CED Micro 1401; Cambridge Electronic Design, Cambridge, UK). Intending to maintain a constant coil orientation and thus to avoid diverse stimulation conditions throughout the experiment, the APB hotspot was marked on the EEG cap with a felt pen. The coil held

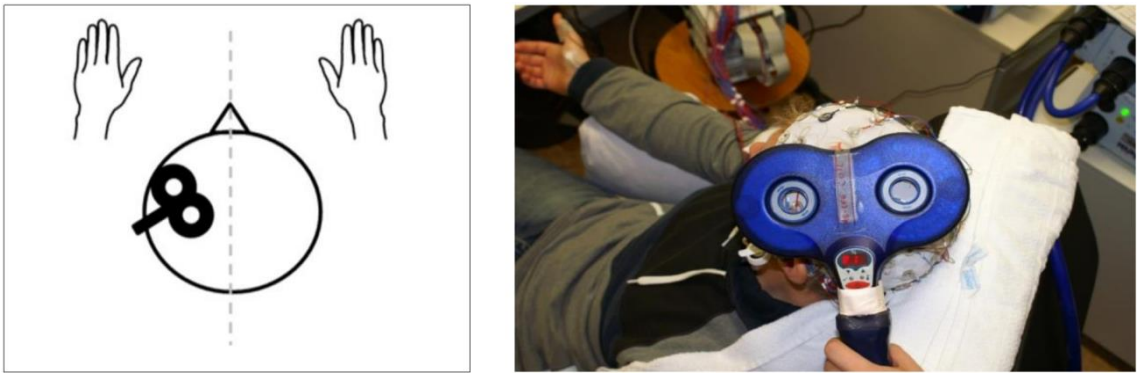

Figure 11: Placement of the TMS coil.
The figure-of-eight coil was placed over the left primary motor cortex tangentially over the scalp with the handle pointing backwards 45° laterally to the midline evoking MEPs in the right APB measured by EMG recordings (source left picture: Morishita et al. (2014).

tangentially to the scalp with the handle pointing backwards and 45° laterally from the midline generates a current in the underlying cortex from posterior-lateral to anterior-medial and is considered as optimal orientation to elicit motor evoked potentials (MEPs) over the motor cortex (Thielscher et al. 2011, Kammer et al. 2001). The stimulation intensity was adjusted at baseline and after drug intake to the resting motor threshold (RMT) according to the relative frequency method (Groppa et al. 2012) which equals the minimal stimulus intensity (in percentage of the maximum stimulator output [MSO]) that was adequate to elicit MEPs larger than 50 µV peak-to-peak amplitude in at least five out of ten subsequent trials.

2.3.2 Electroencephalography recordings with concurrent TMS

EEG recordings were conducted concomitantly with TMS stimulation providing information of TMS-induced cortical excitability. The use of a TMS-compatible EEG system (BrainAmp DC, Brain Products GmbH, Munich, Germany) allowed continuous data recording during application of TMS, whereas saturation of the EEG amplifier was obviated (Virtanen et al. 1999). The EEG signal derived from 62 sintered Ag/AgCl pellet pin electrodes attached to an elastic cap which were arranged in the standard layout based on the international 10-20 EEG system (BrainCap-Fast'n Easy 64Ch, Brain Products GmbH, Munich, Germany; Fig. 12). The ground electrode was placed on the forehead (channel AFz). During the registration, all electrodes were referenced to channel FCz. The resulting signal was amplified, low pass filtered and digitized at a sampling frequency of 5 kHz and stored for further offline analysis. Each stimulation was registered as event by placing a specific marker (TS70 as R128, TS100 as S132

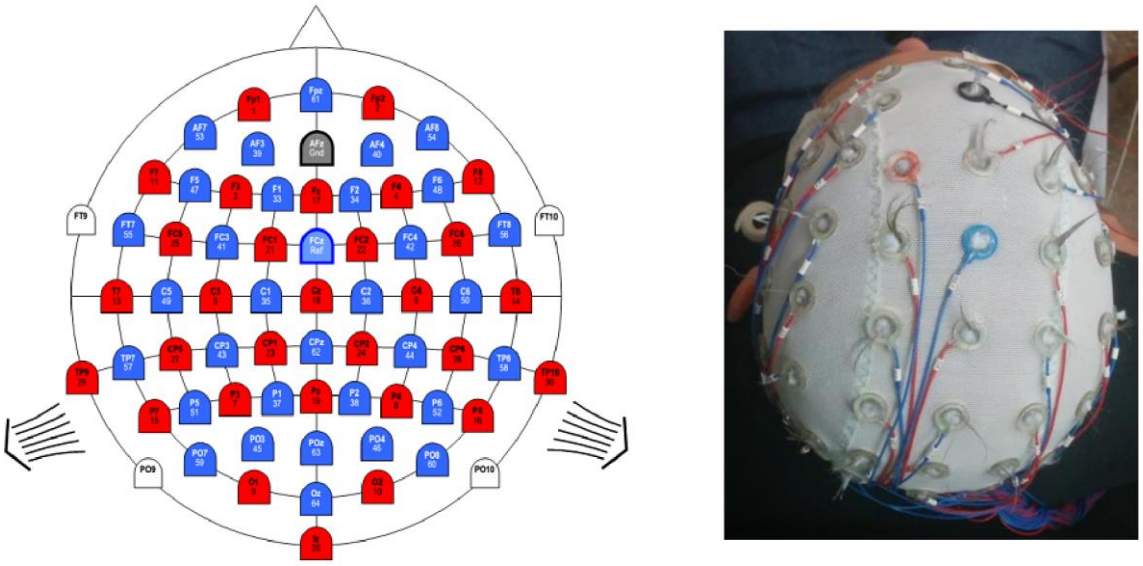

Figure 12: Electrode placement of the EEG cap.

The EEG signal was derived from 62 electrodes arranged according to the international 10-20 EEG system. Two additionally electrodes around the right eye were used to track vertical and horizontal eye movements. The ground electrode AFz was placed on the forehead (source left picture: brainproducts.com).

and CS-TS as S131) at the onset of TMS pulses. To minimize TMS- induced artefacts, the lead wires were rotated radially away from the coil (Sekiguchi et al. 2011). The impedance of the electrodes was kept below 5 kΩ by means of an appropriate preparation of the skin. In order to reduce the resistance, the scalp was cleaned by alcohol and scrubbed slightly with a wooden stick having a cotton tip. A mild abrasive gel (Nuprep Skin Prep Gel, Weaver and Company, Colorado, USA) and an electrode paste (GE GmbH, Freiburg, Germany) were applied directly at the electrode-skin contact. To avoid 'bridging' between the electrodes the paste was used sparingly. Before each EEG recording, the impedance of the electrodes was controlled.

Electrooculography (EOG) recordings identified both vertical and horizontal eye movements (Amassian et al. 1992) by two additional sensors (named 31 and 32). An electrode located outside the outer canthus of the eye detected horizontal eye movements, whereas the vertical eye movements and blinks were detected by an electrode above the right eye.

The subjects were seated in a comfortable reclining chair placing their head in a stable position. To ensure wakefulness, the participants were requested to keep eyes open and to fix a target to avoid eye movement artefacts.

Auditory evoked potentials caused by current discharge of the TMS coil can contaminate the EEG signal between 100 and 200 ms (Nikouline et al. 1999). The

auditory click artefacts were eliminated by applying a colored masking sound through earphones. The time-varying frequencies included the specific frequency spectrum of the TMS clicks (ter Braack et al. 2015, Massimini et al. 2005). The sound volume was adapted to that level subjects did not hear any TMS clicks.

In this study, a paired-pulse paradigm provoking short-interval intracortical inhibition (SICI) was applied in order to investigate intracortical inhibitory circuits based on an established protocol (Kujirai et al. 1993, Ziemann et al. 1996c). The paired-pulse consisted of a subthreshold conditioning pulse with an intensity of 70% of the RMT (CS70) followed by a suprathreshold testing stimulus after 2 ms with an intensity of 100% (TS100). Additionally, one single-pulse was given at 70% (TS70) and one at 100% of the RMT (TS100). The maximum intensity was set at 100% RMT to reduce re-afferent somatosensory feedback which can contaminate the EEG recording (Paus et al. 2001).

To obtain the EEG signature of SICI without the confounding factor of potentials evoked by the CS stimulus, a two-step subtraction was applied to the EEG responses, in line with previous studies (Premoli et al. 2014b). Specifically, our aim was to eliminate all late CS-evoked EEG responses overlapping with early TS-evoked EEG potentials in the paired-pulse condition. Therefore, the EEG signal of the average single-pulse TS70 was subtracted from those of the average paired-pulse uncorrected aligned to the time of application of CS70. The first subtraction resulted in the EEG signature of paired-pulse corrected. The effect of CS70 on TS-evoked TEPs, depicted as the effect of SICI, was determined by subtracting average single-pulse TEPs of TS 100 from paired-pulse corrected TEPs aligned to the time of application of the paired-pulse TS100 (Premoli et al. 2014b).

During the experiment, at least 125 randomly distributed single- and paired-pulses, for each stimulation protocol and drug-condition were delivered over the left M1 APB hotspot at an intertrial interval (ITI) of 5 s on average with a random ITI variation of 25% to avoid subject's anticipation of the following trial.

2.3.3 Saccade peak velocity measurements

Peak velocity of visually guided saccades is reckoned as biomarker of sedation mediated by α1 subunit-containing GABAARs (de Visser et al. 2003, de Haas et al. 2008, de Haas et al. 2009). Saccade peak velocity (SPV) measurements were conducted before and after drug-intake (Fig. 10). Subjects were seated in front of a screen adhering to an eye-screen distance of 67.5 cm and were instructed to follow a jumping white dot on a black screen by performing visually guided saccades (enclosing

an angle of view of 1°). The position of the head was held straightly. The dot appeared at the horizontal lateral edges of the screen alternately at random intertrial intervals of 2-3 s preventing anticipation of the next occurrence (subtending an angle of view of 40°). 50 trials were shown before and after drug-intake respectively. The saccadic eye movements were recorded by electronystagmography (ENG) using AgCl surface electrodes placed at the outer canthus of each eye. The resistance of the skin was kept below 5 kΩ. The raw signals of the ENG were amplified and bandpass filtered (0.5-70 Hz; Digitimer D360), then digitized at an A/D rate of 10 kHz per channel (CED Micro 1401; Cambridge Electronic Design) and preserved for online visual control and later offline analysis using customized data collection and conditional averaging software. ENG data was exported into MATLAB (version 2012b; MathWorks). The saccade onset and offset of artefact-free samples were identified manually using in-house-written software (Velázquez-Perez et al. 2004). Third-order polynomial fits of the ENG raw signal were performed in order to receive the SPV (in °/s). Conditional SPV averages were computed for leftward and rightward saccades.

2.4 *Data analysis*

2.4.1.1 Preprocessing of TMS-EEG data

"Preprocessing and analysis of obtained EEG recordings were performed by using BrainVision Analyzer (version 2, BrainProducts GmbH, Munich, Germany), MATLAB 2012b (version 8.0, The MathWorks, Natick, Mass) and the Fieldtrip open source MATLAB toolbox (version 2013, www.ru.nl/fcdonders/fieldtrip/) (Oostenveld et al. 2011).

The EEG data was re-referenced to the linked mastoids (channels TP9 and TP10) and down-sampled to 1 kHz" (Premoli et al. 2018). The continuously recorded EEG time series were segmented each for single- and paired-pulse trials considering the TS and CS respectively. Epochs were considered starting 500 ms pre-stimulus at baseline and ending 600 ms post-stimulus. To eliminate TMS-related artefacts, the EEG signal enclosing an interval of 12 ms before and after the TMS pulse with respect to TS (Fig. 13) was replaced by NaNs (MATLAB numeric value, "not-a-number"). The data was linearly interpolated between the last sample before and the first after the removed artefact. Linear interpolation enables to smooth artefacts by creating a linear connection between a start- and endpoint not affecting the raw signal (Thut et al. 2011). Visual data inspection of single trials was conducted to detect and reject trials containing artefacts (e.g. eye movements as blinks or saccades, muscle activation or

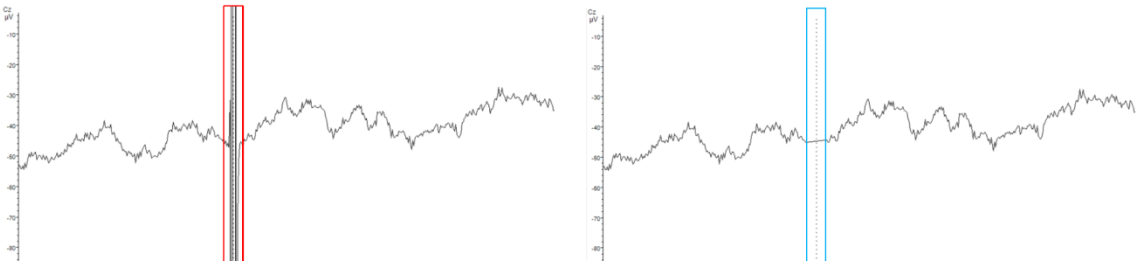

Figure 13: Linear interpolation of EEG raw signal
Pronounced TMS-related artefacts (left, red) in the EEG recording were removed by linear interpolation (right, blue) of the signal during an interval of -12 to 12 ms with respect to the testing stimulus.

decay artefacts). After having exported the data to MATLAB, the artefact-free EEG signals from 62 channels for single-pulse TS70 (averaged number of trials across subjects ± SEM before and after diazepam: 114 ± 11 and 108 ± 16; baclofen: 108 ± 18 and 107 ± 13; placebo: 109 ± 18 and 111 ± 14), single-pulse TS100 (averaged number of trials across subjects before and after diazepam: 109 ± 6 and 97 ± 14; baclofen: 103 ± 11 and 107 ± 8; placebo: 105 ± 8 and 105 ± 9) and paired-pulse TMS (averaged number of trials across subjects before and after diazepam: 103 ± 12 and 97 ± 15; baclofen: 102 ± 13 and 103 ± 9; placebo: 105 ± 10 and 98 ± 13) were baseline corrected by subtracting the mean amplitude -500 ms to -100 ms prior to TMS application (i.e. before CS in the paired-pulse condition and TS alone in the single pulse-conditions). "*The trials were linearly detrended and bandpass filtered between 2 and 80 kHz* (48 dB/octave)" (Premoli et al. 2018). The line noise contamination was reduced by applying a 50 Hz notch filter.

An independent component analysis (ICA) decomposition was performed separating the components of various brain signals to detect and remove artefacts in EEG recordings. "*Components were visually inspected considering time course, topographical distribution and amplitude allowing to remove residual muscle activity"* (Korhonen et al. 2011, Premoli et al. 2018). This was detectable mostly in the fronto-temporal region ipsilateral to the stimulation site: TMS-related artefacts showing exponential decays (Fig. 14a) (Ilmoniemi and Kicic 2010), saccades (Fig. 14b) and recharge artefacts around 200 ms after TMS pulse (Fig. 14c) were removed as well (Mutanen et al. 2013, Rogasch et al. 2014, Veniero et al. 2009).

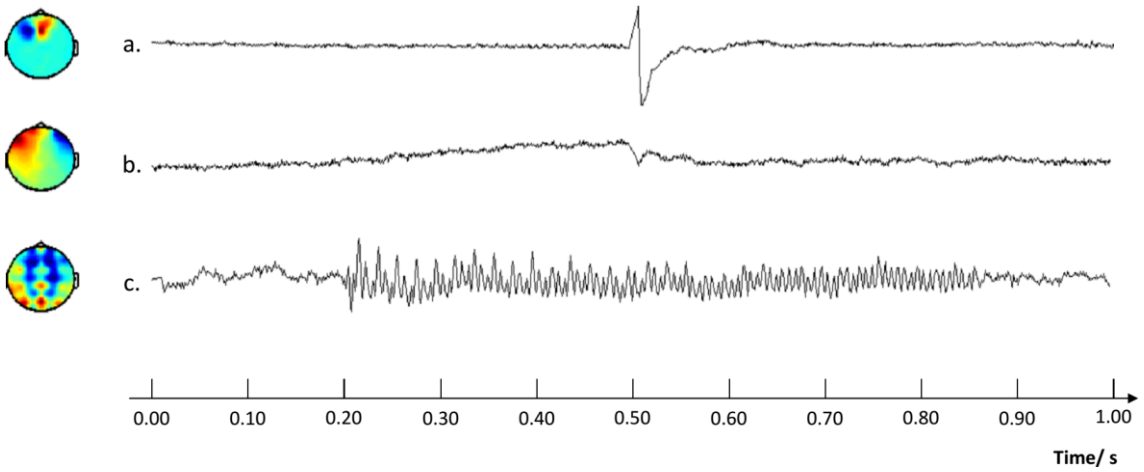

Figure 14: Independent component analysis
Artefactual components were manually identified. Independent components are shown from a representative individual illustrating decay artefacts (a), saccades (b) and recharge artefacts (c).

2.4.1.2 Analysis of TMS-evoked potentials

TEPs elicited by single- and paired pulses were determined by averaging the EEG signal over all remaining trials and all channels. A filter between 1 and 45 Hz was applied in order to smooth the TEPs. "*According to the literature (Lioumis et al. 2009, Premoli et al. 2014a), five reproducible TEP components (P, positive deflection; N, negative deflection) were defined depicting non-overlapping time windows of interest (TOI): P25 (15-35 ms), N45 (36-57 ms), P70 (58-80 ms), N100 (81-144 ms) and P180 (145-205 ms), based on the grand averaged butterfly plot of the single-pulse TS100%. TOIs were kept identical irrespectively of TMS (single- or paired-pulse) or drug condition" (Premoli et al. 2018).* In general, the peak to baseline amplitude of TEP components was identified before and after drug-intake considering the specified TOIs. To evaluate the effects of SICI at baseline, single- and paired-pulse corrected TEPs were compared for all TEP components. The drug-effects on SICI were obtained contrasting the modulation of SICI in the post-drug to the pre-drug condition for all TEP components.

2.4.1.3 Statistics

Drug effects on SPV were evaluated performing a repeated-measures ANOVA. Considered within-subject factors were DRUG (3 levels: baclofen, diazepam and placebo) and TIME (2 levels: predrug and postdrug).

RMT measurements were tested for consistence and reproducibility at baseline across the three drug conditions by applying a one way repeated measures ANOVA including the within-subject factor DRUG (3 levels: baclofen, diazepam and placebo). The test-

retest reliability for each TEP component of single- and paired-pulses respectively was quantified by computing Cronbach's alpha.

"TEP amplitude modulations were analyzed both for single- and paired-pulse conditions. Multiple dependent sample t-tests were conducted separately for different conditions and TOIs" (Premoli et al. 2018). Drug-induced changes of TEPs and alteration of TEPs provoked by SICI at baseline were scrutinized. Accordingly, to analyze drug-induced changes in the single-pulse conditions and in the SICI paradigm, the corresponding values were contrasted pre- versus post-drug intake. "*The effect of SICI at baseline was obtained by comparing the paired-pulse corrected with the single-pulse TS100%" (Premoli et al. 2018).* A cluster-based permutation analysis (Maris and Oostenveld 2007) was performed as established in FieldTrip (http://fieldtrip.fcdonders.nl/) to correct for multiple comparisons. Thus, a paired t-test was used to compare the different conditions respecting each electrode at each time bin within the five TOIs. t-values exceeding a threshold of $p < 0.05$ were clustered referring to adjacent time bins and contiguous electrodes. The sum of the t-values within each cluster was utilized to calculate cluster-level statistics. The statistical comparisons were executed considering the maximum values of summed t-values. A permutation test randomizing the data across post- versus pre-drug conditions or paired- versus single-pulse and recalculating the statistical test 1500 times provided a reference distribution of the maximum of summed cluster t-values to assess the statistic of the actual data. Clusters in the original data set achieved statistical significance at an α-level of 5% once less than 5% of the permutations used to construct the reference distribution resulting in a maximum cluster-level statistic larger than the cluster-level value in the original data. If not declared otherwise, all data are presented as means ± SEM.

3 Results

The study protocol was completed by sixteen out of nineteen subjects. All included participants were compliant to the experimental design. In general, the study medication was tolerated well. As main adverse effects drowsiness (diazepam n = 11; baclofen n = 7) and dizziness (diazepam n = 2; baclofen n = 4) were reported. Nevertheless, the ability of the subjects to fulfill the study requirements was not impaired by any side effect.

The section “Results” is based on the work of Premoli et al. (2018).

3.1 *Resting motor threshold*

Interindividual and intraindividual variations of baseline RMT values were observable most likely due to natural alteration of excitability and the subjects’ vigilance (Fig. 15).

The mean of the RMT in %MSO for each drug condition pre- and post-drug respectively was 37.31 ± 5.2 and 40.75 ± 7.44 for diazepam, 37.94 ± 5.21 and 39 ± 5.47 for baclofen and 39.31 ± 6.76 and 39.5 ± 7.44 for placebo.

A repeated-measure ANOVA of drug effects on RMT was performed including the within-subject factor drug (3 levels: diazepam, baclofen and placebo) and time (2 levels: pre-drug and post-drug). The statistics resulted in significant main effects of time ($F_{(1, 15)}$ = 15.27, p = 0.02) and an interaction effect of drug and time ($F_{(2, 30)}$ = 7.06, p = 0.03).

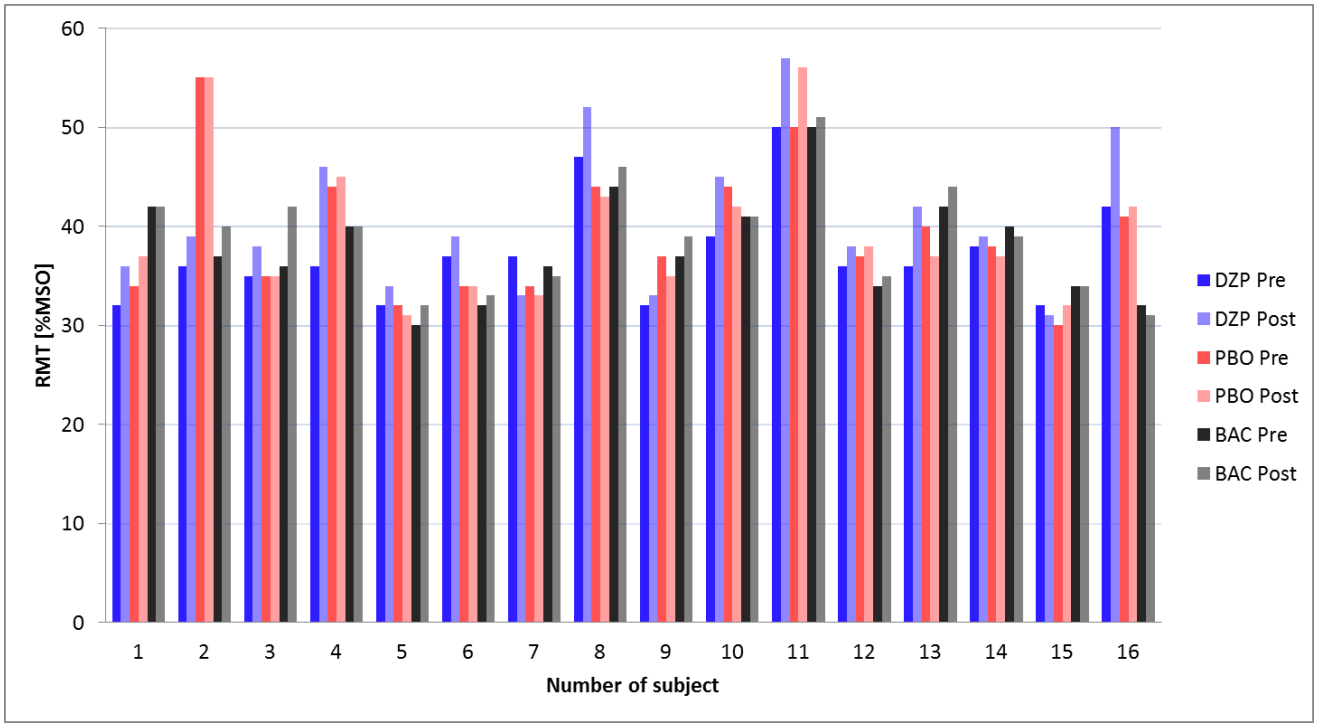

Figure 15: Bar diagram depicting RMT values on individual level for each session pre- and post-drug.
For each of the 16 subjects the RMT (in % MSO) in all three sessions with pre- and post-drug conditions are represented as colored bar to visualize the intra-subject and inter-subject variability.

3.2 *Single-pulse TEPs*

Firstly, the single-pulse TEPs at baseline were analyzed. Both single-pulse stimulation protocols using an intensity of 100% and 70% of RMT respectively, showed typical TEPs (P25, N45, P70, N100 and P180) (Fig. 16.AB) and typical distribution of scalp voltages. There were no significant differences detectable between different pre-drug conditions by cluster-based permutation analysis considering the TEPs elicited by TS-100% and TS-70% (all $p > 0.05$).

The application of diazepam lead to an increase of the N45 ($p = 0.03$) and to a suppression of the N100 and P180 ($p < 0.001$ and $p = 0.01$, respectively) over channels contralateral to the stimulated site at TS-100%. This confirms previous findings of our group (Premoli et al. 2014a). At subthreshold intensities (TS-70%) the same effects were detected: diazepam increased the N45 ($p = 0.005$) and decreased the N100 ($p = 0.01$; Fig. 16.CD).

In contrast, baclofen enhanced the TS-100% elicited N100 ($p = 0.04$) over channels close to the stimulated left M1 as previously shown by Premoli et al. (2014a), as well. The TS-70% evoked TEPs showed no significant alteration by baclofen (Fig. 16.EF).

3.3 *The effect of short-interval intracortical inhibition on TEPs*

The EEG waveform of SICI is obtained by subtracting the single pulse TS-100% responses from the paired-pulse corrected responses (Fig. 17.A) - which is corrected for the late CS effects. The scalp voltages of the paired-pulse corrected components showed similar topographical distribution compared to single-pulse scalp potentials (Fig. 17.BC). SICI affects the late TEPs by suppressing significantly the N100 and the P180 (both $p < 0.001$; Fig. 17.DE). Values of SICI effects on the N100 and P180 at single subject level demonstrated a limited variability with a small amount of subjects showing effects contrary to the group average (Fig. 18).

3.4 *Drug effects on SICI evoked EEG responses*

The modulation of the drugs diazepam and baclofen on the EEG waveform of SICI paired-pulse corrected minus single-pulse TS-100%) was provided by the comparison between pre- and post-drug intake. Cluster-based permutation analysis resulted in a reduced SICI of the N100 over contralateral sites under application of diazepam representing a decrease of the suppressive effect of CS-70% on the TS-100% stimulus of the N100 ($p = 0.03$; Fig. 19.A). The intake of baclofen, however, lead to an increase of SICI of the N100 over frontal sites ($p = 0.070$; Fig. 19.B). As no significant differences were found between all three baseline conditions SICI pre-drug intake (all p

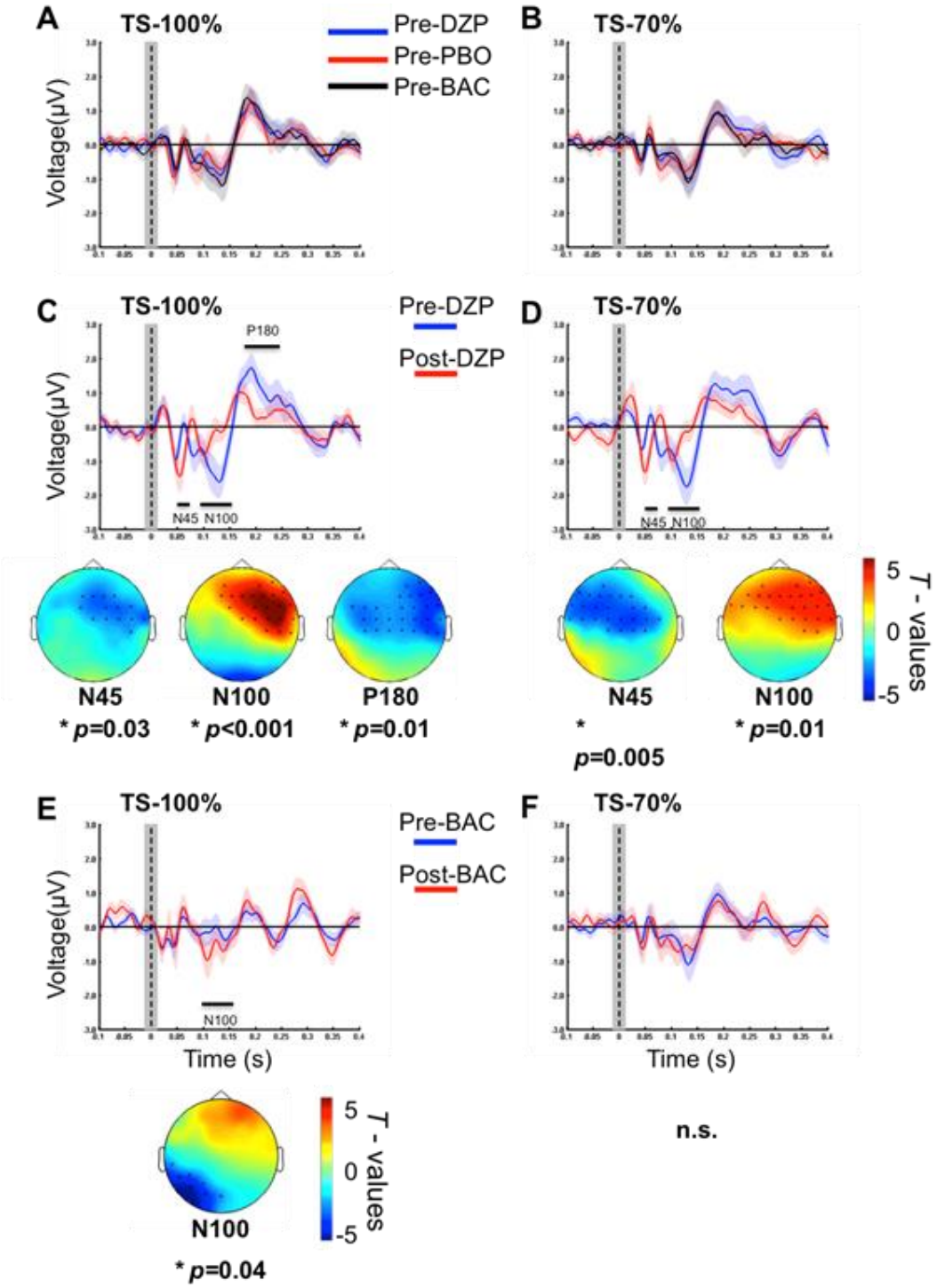

Figure 16: Single-pulse TMS-evoked EEG potentials pre and post drug-intake

Grand-average TMS-evoked potentials (TEPs) elicited by single-pulse TMS at 100% RMT (**A**, TS-100%) and 70% RMT (**B**, CS-70%) over left primary motor cortex before intake of diazepam (DZP, blue), placebo (PBO, red) and baclofen (BAC, black). DZP increased the N45 and suppressed the N100 and P180 primarily contralateral to the stimulation site (**C**). BAC increased the N100 over the stimulated hemisphere (**E**). DZP increased the N45 and suppressed the N100, whereas BAC had no effects on TEPs at subthreshold intensities (**F**, CS-70%). Black bars underneath indicate significant drug-induced changes in TEPs. Shades illustrate ± 1 SEM. T-statistic maps of the TEP amplitude show post- versus pre-drug differences. Blue colors in topoplots represent an increase in surface voltage negativity or a reduction in positivity. **C**, **D**, **E** are grand-averages across significant channels indicated by black dots in the t-statistic maps (source: Premoli et al. (2018)).

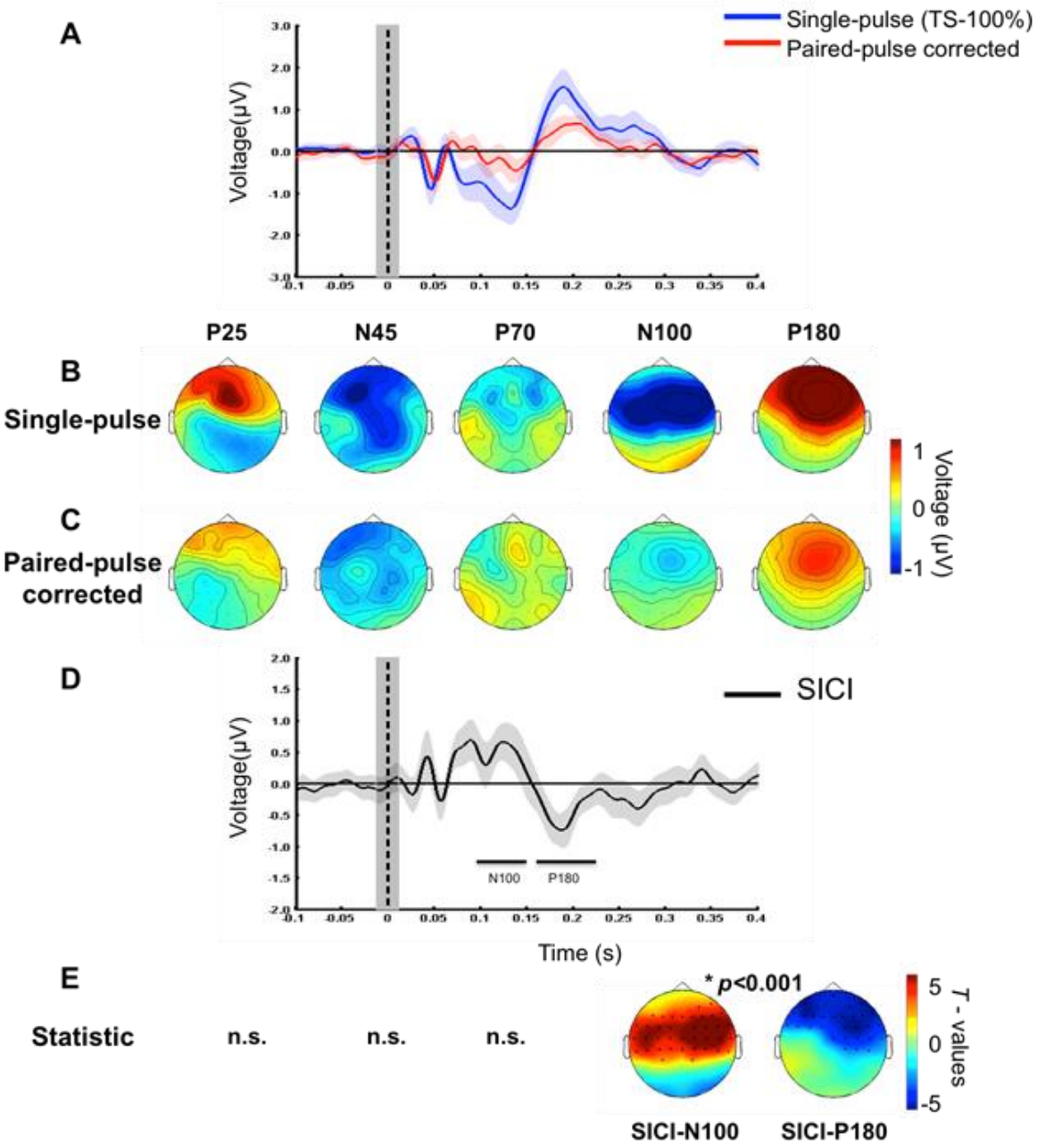

Figure 17: Spatiotemporal EEG characteristics of SICI before drug-intake

(**A**) Grand-averaged TEPs at baseline before drug intake elicited by single-pulse (TS-100%, blue) and paired-pulse TMS (CS70-TS100, corrected for late CS effect, red). Topographical distribution of surface voltages (μV) of each TEP component (P25, N45, P70, N100 and P180) for single-pulse (**B**) and paired-pulse (**C**) stimulation. (**D**) Grand-average of SICI at baseline determined as paired-pulse minus single-pulse TS-100% TEPs. Black bars underneath indicate significant CS-induced changes in TS-evoked TEPs. Shades illustrate ± 1 SEM. (**E**) T-statistic maps of the TEP amplitude show post-pulse minus single pulse TS-100% differences for the significant components N100 and P180. Black dots represent channels with significant differences between paired-pulse and single-pulse conditions. Blue colors indicate a decrease in positivity and red colors a decrease in negativity (source: Premoli et al. (2018)).

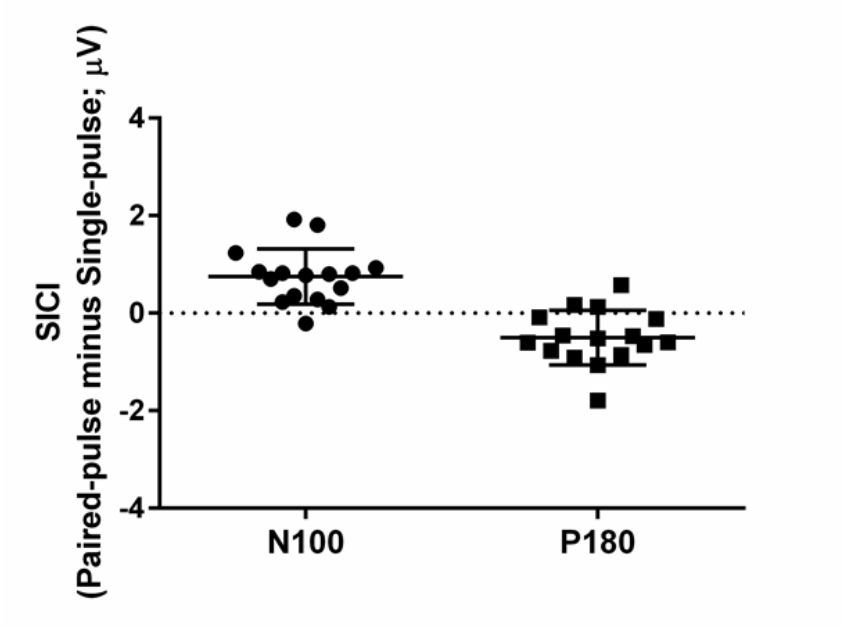

Figure 18: Scatter plot of individual SICI of significant TEP components
Single subject data of SICI at baseline before drug-intake. Grand-averaged across the three pre-drug condition and illustrate consistent suppression of the N100 and P180 components (paired-pulse minus single-pulse TS-100%) across subjects. Error bars represent group mean ± SEM (source: Premoli et al. (2018)).

> 0.05), the contrary effects of diazepam and baclofen on SICI are not explainable with varying baseline measurements.

3.5 Comparison between EEG responses of SICI and LICI

For the comparative analysis between the baseline EEG waveform of SICI and LICI, we employed data from a previous study of our group measuring long-interval intracortical inhibition (LICI) by subtracting single-pulse from paired-pulse corrected responses - corrected for CS-70% effects (Premoli et al 2014b). Fig. 20 presents the SICI and LICI curves averaged across channels and the topoplots of TEPs within the respective TOI as well as the results of statistical comparison of the TEPs. Cluster-based permutation analysis showed no significant effects of all TEP components (all $p < 0.05$) apart from the P70 which presented a higher amplitude in the EEG signatures of LICI ($p = 0.02$; Fig. 20.D).

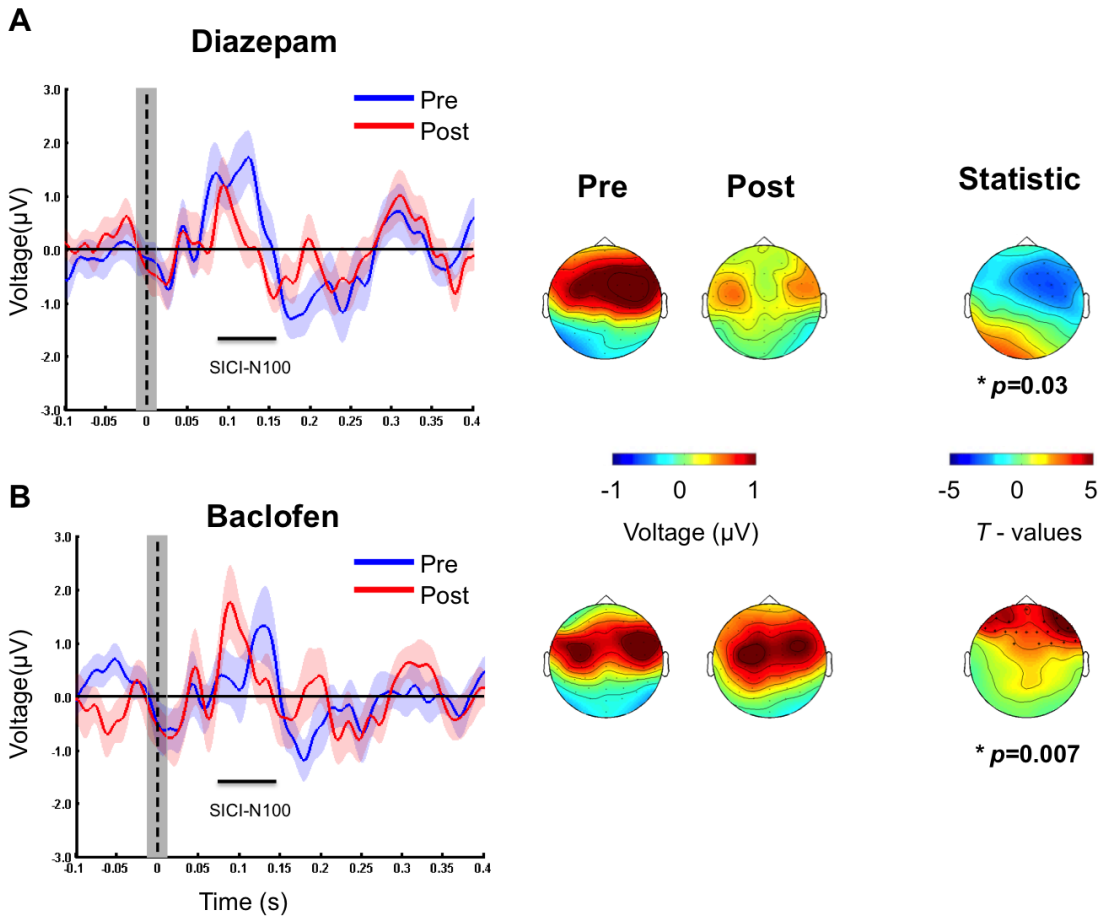

Figure 19: Drug effects on SICI of TEPs

Grand-averaged SICI measured before (blue) and after (red) intake of diazepam (A) and baclofen (B). Shades represent ± 1 SEM. Diazepam suppressed, baclofen increased SICI of the N100. Black bars underneath indicate significant drug effects. Topographical distribution of surface voltages and t-statistic maps show significant drug-induced effects on SICI of the N100. SICI curves are averaged across significant channels, marked with black dots in the t-statistic maps. Blue colors indicate a decrease in positivity and red colors an increase in positivity (source: Premoli et al. (2018)).

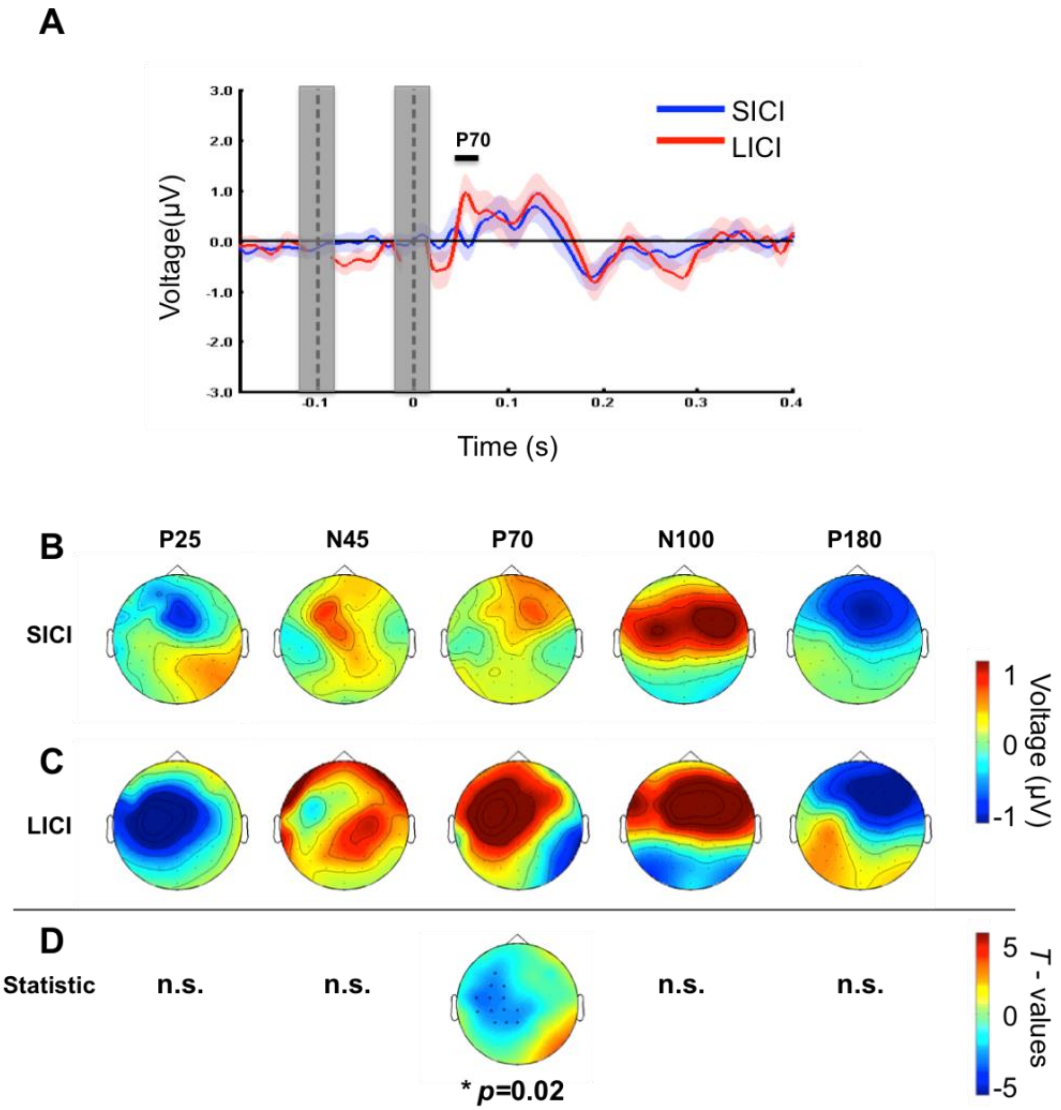

Figure 20: Comparison of spatiotemporal SICI and LICI EEG responses
The grand-average of SICI (blue) and LICI (red) of TMS-evoked components are illustrated in **A**. The LICI data are extracted from a previous publication of our group (Premoli et al., 2014b). Topographical maps of voltage distributions for SICI and LICI (paired-pulse minus single-pulse minus single-pulse TEPs) of each TEP component (P25, N45, P70, N100 and P180) are shown in **B** and **C**. T-statistic maps of the TEP amplitude show SICI minus LICI differences (**D**). LICI significantly increased the P70 (**C**), SICI showed no effect (**D**). Black dots represent channels with significant differences SICI and LICI (source: Premoli et al. (2018)).

4 Discussion

This study presents a novel, systematic approach to elucidate the neurophysiological mechanisms of SICI by an elaborated solid pipeline which focus on the whole brain cortex without apriori assumptions. Paired-pulse TMS-evoked brain responses were recorded by a high-density 64-channel EEG and were modulated by neuroactive drugs acting as agonists on GABAA or GABAB receptors (diazepam, baclofen) probing the pharmacological characteristics of SICI.

SICI at baseline significantly reduced the amplitudes of late TEPs (N100 and P180). Whereas diazepam reduced SICI of the N100, baclofen showed an increase. The comparison of SICI and LICI (from previous findings of our group) showed a largely similar modulation of TEPs.

4.1 *Methodological considerations and limitations*

The effect of SICI on TMS-evoked EEG potentials has been investigated previously by three studies (Paus et al. 2001, Ferreri et al. 2011, Cash et al. 2017). However, the results were inconsistent, also stressing the methodological difficulties to measure paired-pulse TMS paradigms on cortical level via EEG. In the following, our methodological approach will be discussed and compared to recent studies.

In paired-pulse studies, the interstimulus interval between the conditioning and the testing stimulus plays a major role. Depending on the interstimulus interval, inhibitory or facilitatory circuits can be tested (Fig. 21). Here, we applied SICI, a paired-pulse TMS paradigm consisting of a subthreshold conditioning pulse with an intensity of 70% of the RMT followed by a suprathreshold testing stimulus after 2 ms with an intensity of 100% of the RMT (Kujirai et al. 1993, Ziemann et al. 1996c).

Previous research testing SICI effects on EEG traces used differing intervals: 3 ms (Paus et al. 2001, Ferreri et al. 2011) or 2 ms (Cash et al. 2017). TMS-EMG measurements showed that an interstimulus interval of 2 ms avoids contamination by excitatory interneurons (Peurala et al. 2008) and leads to an optimal suppression of late I-waves and MEP amplitude (Fig. 21) (Di Lazzaro et al. 1998, Ilic et al. 2002, Kujirai et al. 1993).

So far, TMS-EMG studies measured the effect of SICI on the MEP amplitude as a function of the interstimulus interval. However, no direct insight into cortical mechanisms was provided. For subsequent paired-pulse TMS-EEG studies, it is advisable to test SICI at various interstimulus intervals to establish a more accurate understanding of inhibitory cortical circuits and to investigate optimal testing conditions.

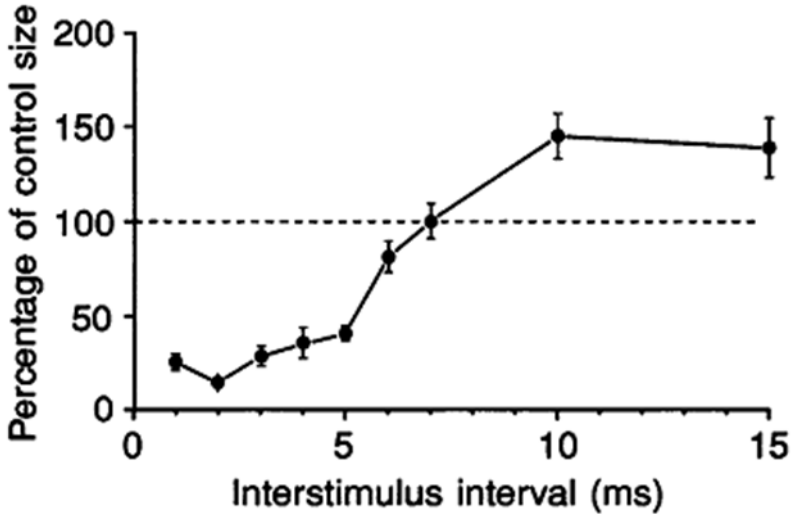

Figure 21: Mean time course of the suppression of the MEP-amplitude of TS after application of CS at short interstimulus intervals (1 – 5 ms)
The effect of SICI on the MEP-amplitude of the TS is shown as function of the interstimulus interval in 10 subjects (mean ± SEM). Amplitudes are shown as a percentage of the size of the test stimulus alone. At an ISI of 1-6 ms, the EMG response was suppressed. At longer interstimulus intervals (here 10 and 15 ms) the inhibition turns into facilitation (source: Kujirai et al. (1993)).

Not only the interstimulus interval but also the stimulation intensities of the paired TMS pulse determine whether the interaction of conditioning and testing pulse results in an inhibitory or facilitatory effect. Here, we set the intensity of CS at a subthreshold level of 70% of the RMT and the TS at a suprathreshold level of 100% of the RMT.

EMG studies revealed that the variation of the CS intensity with a constant TS intensity at short latencies, shows a U-shaped relationship between the intensity of the conditioning pulse and the amount of inhibition (Kujirai et al. 1993, Ziemann et al. 1996c, Schafer et al. 1997, Ilic et al. 2002, Kossev et al. 2003, Orth et al. 2003, Peurala et al. 2008). In previous studies, the reduction of MEPs became significant, when CS was set at 0.6 times threshold and reached its maximum at 70% of the RMT with higher level of confidence ($p < 0.01$) (Kossev et al. 2003). A further increase of stimulation intensity to 75% of the RMT showed less inhibition ($p < 0.05$). Above this intensity, the suppression of MEPs lost significance (Fig. 22) (Kossev et al. 2003) and changed into facilitation at CS intensities greater than RMT (Kujirai et al. 1993). No facilitation at intensities of 60 – 70% RMT suggests that lowest threshold circuits stimulated by TMS over M1 are of inhibitory origin (Davey et al. 1994, Ziemann et al. 1996c, Awiszus et al. 1999).

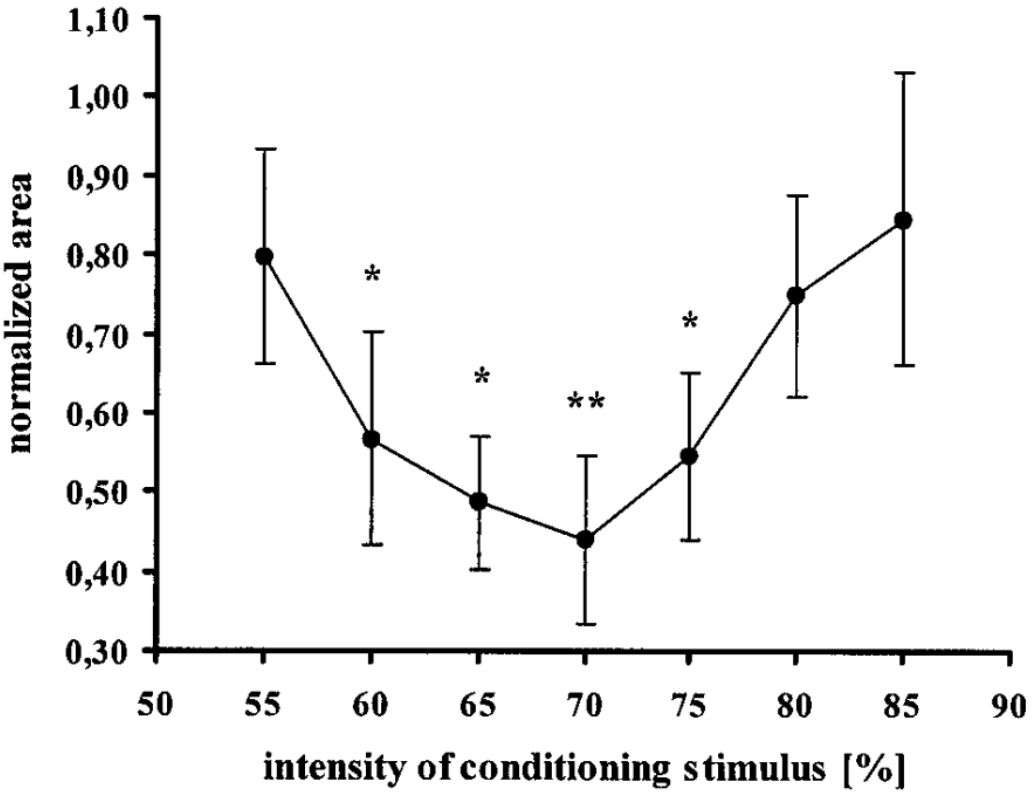

Figure 22: The reduction of MEP areas by SICI depends on the CS intensity
The size of MEP areas of the TS (paired-pulse) is shown as a function of the conditioning intensity at an ISI of 3 ms. The size of MEP areas was determined as total voltage time integral (Siggelkow et al. 1999) and was normalized to unconditioned MEPs. The CS intensity is expressed as percentage of motor threshold. The intensity of the TS was set at 120% of RMT. The asterisks show significant effects of the CS (Wilcoxon's test) relative to control values (*P < 0.05 and **P < 0.01) (source: Kossev et al. (2003)).

The intensity variation of the TS in the paired-pulse at a given subthreshold CS also results in a U-shaped variation of SICI magnitude. SICI peaks when the TS intensity was set to evoke an unconditioned MEP of 1 mV peak-to-peak amplitude (Sanger et al. 2001, Daskalakis et al. 2002, Müller-Dahlhaus et al. 2008). Paus et al. (2001) assumed that suprathreshold intensities applied to the motor cortex activate somatosensory afferences through TMS-elicited muscle twitches. Therefore, in our study, the stimulation intensity for TS was set at 100% of RMT to limit re-afferent somatosensory feedback as possible confounding factor.

However, this point has to be examined more carefully. If the M1 hand area is stimulated, the conduction time between the brain and the small hand muscles takes approximately 20 ms. Thus, it is assumed that somatosensory feedback caused by TMS-induced muscle activation can affect cortical responses occurring 50-60 ms after brain stimulation (Bonato et al. 2006, Maki and Ilmoniemi 2010). Previous studies focusing on the relationship between specific TEP components and MEP amplitudes,

reported inconsistent results. Whereas Paus et al. (2001) stated a significant correlation between the MEP amplitude and the N100, Nikulin et al. (2003) found any effect of peripheral muscle activity on TEPs.

A more recent study tested the impact of somatosensory feedback on cortical activity applying TMS pulses at motor threshold by comparing trials resulting in MEPs to those without. A significant difference in the TMS-evoked response between the MEP and no-MEP condition was shown at 60 ms (Petrichella et al. 2017). We used the same stimulation intensity at RMT arguing to limit possible confounding factors. It is confirmed that the likelihood of generating MEPs increases with higher stimulation intensities (Devanne et al. 1997, Hess et al. 1987), but Petrichella et al. (2017) clarifies that MEPs released at motor threshold do alter the cortical activity in the somatosensory area at a specific latency of 60 ms. Therefore, for future TMS-EEG experiments, it is suggested to measure EMG signals concurrently. The MEP and no-MEP conditions should be separated instead of averaging all trials with differing EMG activity. To a certain extent, this method is limited as suprathreshold intensities evoke MEPs in most trials. Furthermore, the effect of MEPs on cortical responses was not yet tested in paired-pulse paradigms.

Additionally, several studies revealed that the effect of SICI enhances with higher test pulse intensity - in detail, when the test MEP amplitude was increased from 0.2 to 1 mV (Daskalakis et al. 2002, Sanger et al. 2001, Wagle-Shukla et al. 2009). Consequently, the question raises if testing pulses at lower intensities do reliably evoke SICI. Ilic et al. (2002) conducted a systematic investigation of the effects occurring by varying the intensities of CS and TS of the paired-pulse measured at different interstimulus intervals. Their results support the evidence that the paired-pulse settings of our study (CS 70%, TS 100%) at an ISI of 2.1 ms evoked no facilitation but an average inhibition in approximately 50 % of the trials (Fig. 24). To disprove this concern more accurately, MEP measurements need to be conducted with exact the same settings of our experiments showing the amount of inhibition and its reliability. For upcoming TMS-EEG experiments over the primary motor cortex testing inhibitory or facilitatory circuits, it would be once again recommendable to register and analyse the MEP amplitudes.

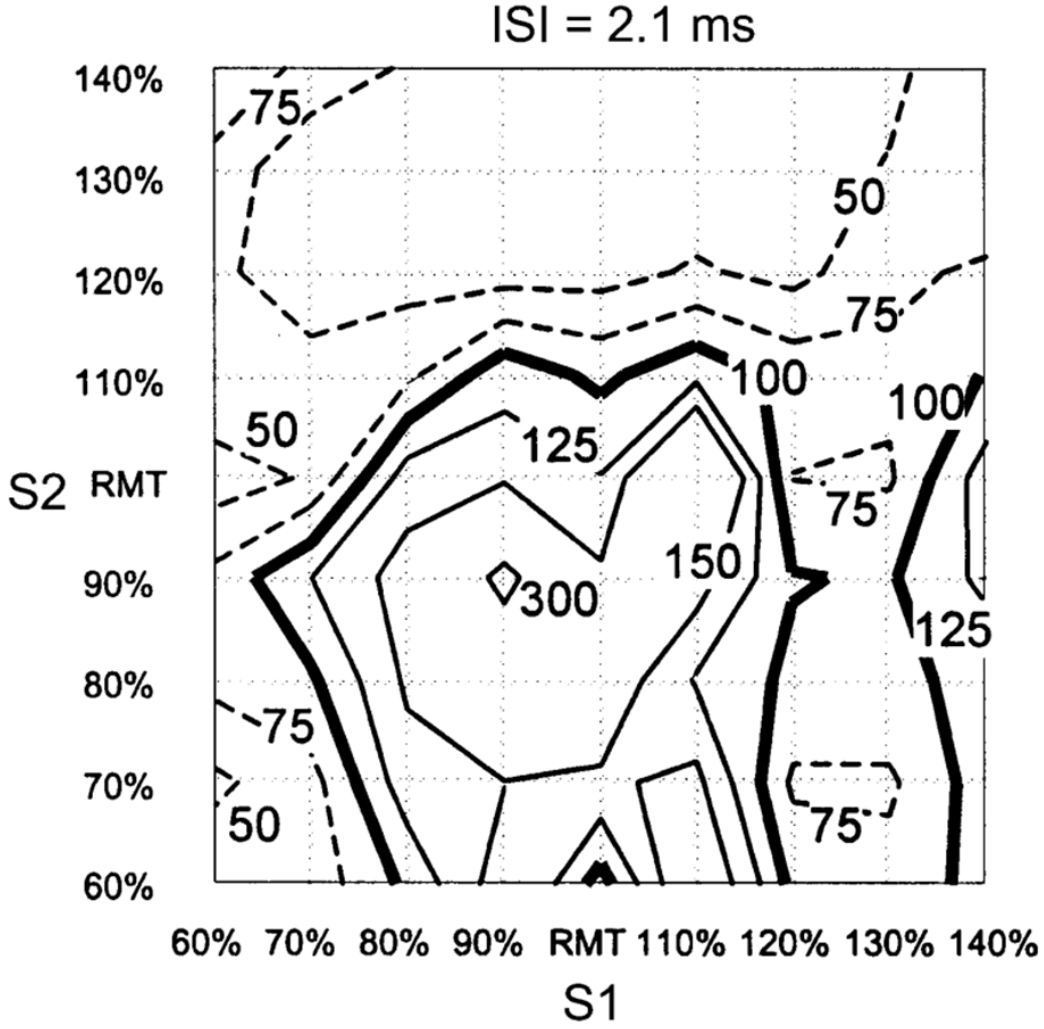

Figure 23: SICI shown as a function of stimulus intensity and interstimulus interval
The stimulus intenstiy of the first pulse (S1 = CS) and the second pulse (S2 = TS) are referred to the RMT. There are 81 conditions as nine different stimulus intensities in steps of 10% RMT were tested each for S1 and S2. The effect of S1 on S2 was assessed as the percentage of the MEP amplitudes elicited by paired-pulse TMS (MEP_{S1+S2}) over the arithmetic sum of the MEP amplitudes elicited by the corresponding single stimulus (MEP_{S1} + MEP_{S2}). The data includes means of six subjects and is illustrated as contour plots. Thick continuous lines mark no interaction (100%), dashed lines show inhibitory (< 100%) and thin continuous lines facilitatory effects (> 100 %); the numbers represent contour line values (modified from: Ilic et al. (2002)).

In this study, the stimulation intensity was adjusted to 100 % RMT, measured before and after drug application respectively (Fig. 10) according to the relative frequency method (Groppa et al. 2012). The RMT reflects the excitability state of corticospinal neurons and depends on intracortical circuits generating MEPs. Drug intake can reversibly alter the RMT (Ziemann et al. 1996b, Di Lazzaro et al. 2003): baclofen and diazepam were shown to produce a significant RMT increase (Premoli et al. 2017a).
No adaptation to the RMT between sessions implies a constant absolute stimulation intensity, but would lead to a different degree of excitation and output of cortical motor

neurons. The neurons may be excited either non-effectively or excessively. Changed network properties, in turn, would limit the interpretation of the TMS-evoked EEG responses. Oppositely, the post-drug readjustment of the stimulus intensity relative to the RMT keeps the TMS-evoked muscle activity constant and assures a comparable excitation of the corticospinal tract. Thus, changed EEG responses are attributable to modified connectivity in the same network or excitability of connected areas (Premoli et al. 2017a, Premoli et al. 2017b). However, disparate intensities stimulate different neuron populations at differing excitation levels and thus generate different EEG responses (Casarotto et al. 2010). Premoli et al. (2017b) examined the effect of increased stimulation intensity due to elevated post-drug RMT on TMS-evoked EEG responses testing anti-epileptic drugs. Their study revealed a difference between calibrating the TMS intensity against the RMT only in the pre-drug session (absolute stimulation intensity) or adjusting the RMT to the post-drug session (relative stimulation intensity). To overcome this limitation, future pharmaco-TMS-EEG experiments should conduct the measurements at multiple stimulation intensities both pre-and post-drug to be able to compare the TEPs between adjusted and unadjusted intensities (Premoli et al. 2017b).

We placed the TMS coil according to the hot spot method producing maximum MEP amplitudes in the target muscle (Conforto et al. 2004, Rossini et al. 1994). With this approach, a functional targeting accuracy in the range of few millimeters has been reported.

Reproducible TMS-evoked EEG responses are required to investigate changes of cortical excitability in a reliable way. The inaccurate positioning of the TMS coil represents one large source of variability. A shift of 10 mm in coil location leads to highly relevant changes in TEPs (Komssi et al. 2002, Casali et al. 2010). Especially in test re-test designs, it is important to control for the correct coil position and orientation to maintain the stimulus location and the applied electric field strength in the cortex (Danner et al. 2008). Image-guided frameless stereotactical neuronavigation of TMS (Sparing et al. 2008, Wagner et al. 2007, Schonfeldt-Lecuona et al. 2005) uses the data of anatomical structures from the subject's individual magnetic resonance image (MRI) for navigation via a subject-image co-registration. This navigation technique enables to monitor the cortical target and allows an online control of the coil location and orientation with possibility of readjustment. In addition, the location and strength of the induced electric field can be estimated for every single TMS pulse (Ruohonen and Ilmoniemi 1999, Bikmullina et al. 2009, Raij et al. 2008, Fox et al. 2004). Navigated

TMS thus provides a high degree of reproducibility as the recorded parameters can be recalled and reproduced even across sessions. Furthermore, the use of neuronavigation minimizes muscle artefacts, because the coil is positioned more accurately and coil misplacement is not compensated anymore by using increased stimulation intensities (Ilmoniemi and Kicic 2010). Spatial accuracy lies within the millimeter range (Schonfeldt-Lecuona et al. 2005). Mechanical automatic assistance may help to improve the accuracy and reproducibility in positioning the stereotactic coil (Lancaster et al. 2004). However, it should be noted that function-guided approaches and thus their coordinates may vary over time as a function of brain state (Farzan et al. 2016).

The work of Sack et al. (2009) points out that between different coil positioning approaches the optimal sample size required to reveal statistical significance can vary. Therefore, the use of MRI-guided frameless stereotactic neuronavigation provides clear advantages: it enhances the reproducibility of TMS-EEG experiments also across sessions, minimizes the sample size and the time needed to retrieve the target area previously found by trial-and-error.

As TMS pulses are accompanied by a clicking sound whose evoked potential is very likely to contaminate at least part of the EGG responses, we applied white noise (Fuggetta et al. 2005, Hamidi et al. 2010, Paus et al. 2001, Veniero et al. 2010) through earphones. White noise is thought to reduce the effect of auditory evoked potentials (AEP) on TEPs.

The TMS clicking sound elicits AEPs modulating the N100-P180 complex and producing a positive deflection around 50 ms in some subjects (ter Braack et al. 2015). Although exposing the subjects to white or adapted noise resulted in a small significant reduction of the N100-P180 amplitude, the largest attenuation of the AEP was obtained by adding a layer of foam between coil and head combined with white or adapted noise. The layer of foam most likely diminishes bone-conduction. However, it has to be considered that the additional layer of foam enlarges the distance between coil and targeted area and increases significantly the motor threshold (ter Braack et al. 2015). In the study of ter Braack et al. (2015), somatosensory evoked potentials were not controlled for which could still influence the EEG signal (Nikouline et al. 1999).

In future TMS-EEG experiments, an additional layer of foam and white or adapted noise should be used to achieve an optimal reduction of the AEP components in the TMS-evoked responses. In case of a non-sufficient masking method, the modulation of

the N100 and the P180 has to be employed carefully as an outcome measure (ter Braack et al. 2015).

In this work, we addressed multiple methodological difficulties measuring the effect of paired-pulse paradigms on cortical level via EEG. Nevertheless, we also point out several methods to further optimize the condition of measurements. Against this background, we want to discuss the following results.

4.2 Discussion of the results

TMS-induced EEG responses originate from the summation of fast and slow excitatory and inhibitory postsynaptic potentials generated by synchronous neuronal activity (Rosenthal et al. 1967). Previous studies hypothesized that GABAA postsynaptic receptors generate fast IPSPs as P13, N18, P30 and N44 (Ferreri et al. 2011, Davies et al. 1990, Deisz 1999, Avoli et al. 1997, Ferrarelli et al. 2010). Pre- and postsynaptic GABAB receptors might contribute to slow IPSPs as P60, N100, P180 and N280 (Nikulin et al. 2003, Bender et al. 2005, Bonato et al. 2006, Ferreri et al. 2011, McDonnell et al. 2006, Premoli et al. 2014a). As pharmacological EMG studies revealed that SICI can be enhanced by potentiation of GABAAergic neurotransmission (Di Lazzaro et al. 2005, Di Lazzaro et al. 2007, Müller-Dahlhaus et al. 2008), SICI may represent short-lasting IPSPs mediated by GABAA receptors. The subthreshold pulse is thought to activate low-threshold cortical inhibitory circuits provoking fast IPSPs in corticospinal neurons, which inhibit the subsequent generation of an action potential in these neurons by the suprathreshold second pulse (Hanajima et al. 1998, Kujirai et al. 1993, Ilic et al. 2002). However, these findings have been derived from the evaluation of peripheral responses. Conversely, SICI depends primarily – not necessarily exclusively - on intracortical mechanisms: as double transcranial electric stimulation (TES) failed to inhibit the conditioned MEP response (Kujirai et al. 1993), the H-reflex was not affected by the CS (Kujirai et al. 1993, Ziemann et al. 1996c) and epidural recordings from the cervical spinal cord showed a decrease in the number and the amplitude of late I-waves after paired-pulse TMS (Di Lazzaro et al. 1998). The physiological and especially the cortical mechanisms of SICI are still barely understood. Additionally, the pharmacological modulation of SICI at cortical level has not been probed yet. Furthermore, this approach allows us, for the first time, to compare different paired-pulse paradigms measured via TMS-EEG providing novel insights into cortical inhibitory mechanisms.

4.2.1 The effect of SICI on TEPs

We investigated the effects of GABAA potentiation provoked by SICI at baseline on the amplitude of TMS-evoked components in the motor cortex.

The effect of SICI was obtained by subtraction of paired- minus single-pulse TEPs: the amplitudes of the N100 and the P180 were significantly reduced (both $p < 0.001$). Topographical surface voltage maps of SICI of late TEPs showed a bilateral distribution over central (N100) and frontal sites (P180) expressed at the same sites as in the single-pulse condition.

The immediate cortical response and the modulation of TEP amplitudes offer a measure of cortical reactivity of the primary motor cortex. The cortical responses also provide information about the spatiotemporal spread of neuronal activation from the stimulated site to connected networks including interneuronal activation of cortical circuits (Ilmoniemi et al. 1997). However, the underlying neurophysiological processes of the TMS-induced responses have not been fully elucidated yet.

Several lines of evidence indicate that SICI is GABAAR-mediated through a cortical circuit of inhibitory interneurons. Apparently, the duration of SICI lasts approximately 20 ms (Hanajima et al. 1998), compatible with the duration of fast IPSPs mediated by GABARs (Avoli et al. 1997). Thus, TS of the paired-pulse was applied at a time of increased GABAAR-mediated inhibition. Moreover, allosteric positive modulators of the GABAR enhanced the suppressive effect of SICI (Di Lazzaro et al. 2005, Di Lazzaro et al. 2007, Müller-Dahlhaus et al. 2008). Therefore, SICI is thought to represent short-lasting IPSPs mediated by GABAA receptors (Di Lazzaro et al. 2005, Di Lazzaro et al. 2007, Müller-Dahlhaus et al. 2008, Di Lazzaro et al. 2000, Ilic et al. 2002). In contrast to the hypothesis SICI might reduce primarily early TEPs, we found a suppression of the late TEPs, the N100 and the P180.

The N100 is the most pronounced and reproducible TEP component and is thought to reflect intracortical GABAB-mediated inhibition (Nikulin et al. 2003, Bender et al. 2005, Premoli et al. 2014b). An increased N100 potential implies augmented inhibition: whereas participants who actively attempt to resist an upcoming TMS-evoked movement showed an increased N100 (Bonnard et al. 2009), it was reduced by assisting a TMS-evoked movement (Nikulin et al. 2003), motor performance (Kicic et al. 2008) or motor response preparation (Bender et al. 2005). Recent studies revealed that the N100 amplitude correlates with the cortical silent period (CSP) (Farzan et al. 2013) and the slope of the N100 correlates with LICI (Rogasch et al. 2013a), both representing a measure of GABABergic neurotransmission (Siebner et al. 1998,

Premoli et al. 2014b). Pharmacological studies further fortify the assumption that the N100 potential reflects GABAB-mediated inhibition as it was selectively enhanced by baclofen, a specific GABABR agonist (Premoli et al. 2014a).

Therefore, the observed suppression of N100 by SICI would support the idea of a reduction in GABAB-mediated cortical inhibition. The assumed GABAAR-mediated control of GABABergic neurotransmission is in general agreement with previous studies. Experiments at cellular level showed that activation of GABAARs suppresses sIPSP generated by neocortical and hippocampal pyramidal cells by GABABRs (Lopantsev and Schwartzkroin 1999, Thomson and Destexhe 1999). It is supposed that the elevated concentration of intracellular chloride inhibits the potassium conductance on which the GABABR-mediated sIPSP is based (Lenz et al. 1997). Furthermore, GABAAR agonists lead to a decrease of the N100 amplitude (Premoli et al. 2014a) and resulted in a reduction of the cortical silent period (Inghilleri et al. 1996, Kimiskidis et al. 2006). Premoli et al. (2014b) showed that the suppressive effect of diazepam was strongest on LICI-evoked potentials in subjects with the strongest baseline LICI of corresponding potentials. In addition, a SICI-EEG experiment related the modulation of late TEPs to the activity of GABABRs (Ferreri et al. 2011). Studies of interhemispheric inhibition (IHI) support our findings as a GABAAR-mediated regulation of excitatory transcallosal fibers targeting contralateral GABABergic interneurons was revealed (Irlbacher et al. 2007, Palmer et al. 2013). Likewise, the topographical surface voltage maps of our study implicate that the corticocortical propagation of induced neural activity, especially to the contralateral hemisphere, is occluded by SICI at the N100.

As there is preexisting strong evidence that SICI is mediated by GABAAergic neurons and our study confirms several studies proposing that GABAAergic circuits control and are able to inhibit GABABergic inhibitory circuits, we assume that the suppressive effect of SICI on the N100 reflects a GABAAR-mediated inhibitory effect on GABABergic neurotransmission. However, our study could not reveal a specific TEP component as marker of GABAA mediated neurotransmission.

The P180 potential is suppressed by SICI ($p < 0.001$) and the P180 is also suppressed under the influence of diazepam at the single-pulse level ($p = 0.01$). Premoli et al. (2014a) found a trend towards a reduction of P180 in the single-pulse condition by diazepam. Both could indicate an underlying GABABergic mechanism also for P180 as GABABR-mediated activity does not stop at 100 ms after stimulation. In fact, the activity of presynaptic GABABRs peak around 100 ms post-stimulus (Davies et al. 1990, Lambert and Wilson 1994), while the sIPSP mediated by postsynaptic GABABR

peaked slightly later, approximately 150 to 200 ms after receptor activation (McCormick 1989, Davies et al. 1990, Deisz 1999). Due to its long latency and wide distribution, the P180 was already suggested to represent reverberant cortico-subcortical circuits (Ferreri et al. 2011).

Interestingly, in our study the N45 potential was not significantly modulated by SICI. In previous TMS-EMG experiments, SICI demonstrated different affinities towards various subtypes of the GABAAR. Diazepam and lorazepam, but not zolpidem, a positive modulator mainly at the α1-GABAAR, increased SICI (Di Lazzaro et al. 2006). This lead to the hypothesis that SICI is mediated by the α2- or α3-subunit-containing subtype of the GABAAR (Di Lazzaro et al. 2006). In addition, in single-pulse TMS trials zolpidem increased the N45 only. Thus, the N45 is thought to represent activity of α1-subunit-bearing GABAARs (Premoli et al. 2014a). Our result is in line with previous studies as SICI did not modulate the TEP component attributed to α1-subunit-bearing GABAAR activity. This is of importance, because certain subtypes of inhibitory interneurons target different GABAAR subtypes (Nusser et al. 1996, Fritschy et al. 1998, Nyiri et al. 2001, Klausberger et al. 2002).

4.2.2 Drug effects on SICI

If our hypothesis that SICI represents GABAA-mediated intracortical inhibition is true, diazepam should enhance and baclofen might reduce the suppressive effect of SICI on early TEPs. Indeed, SICI was modulated in opposite directions by diazepam and baclofen respectively, however at the N100. Whereas diazepam reduced SICI of the N100 expressed over frontal sites of the non-stimulated hemisphere ($p = 0.03$), baclofen showed an increase in the frontal area ($p = 0.007$).

While GABAARs mediate a Cl^- influx via postsynaptic chloride channels (Bormann 1988), presynaptic GABABRs suppress neurotransmitter release by inhibition of voltage-dependent calcium channels (Mintz and Bean 1993) and postsynaptic GABABRs activate inward rectifying K^+ channels (Luscher et al. 1997). GABAB-mediated autoreceptors inhibit the release of GABA, whereas heteroreceptors inhibit the release of glutamate and other neurotransmitters (Kaupmann et al. 1998). Diazepam potentiates the action of GABA by increasing the opening frequency of GABAARs containing a γ-subunit (Bormann 1988, Macdonald and Olsen 1994, Haefely 1984). Baclofen is a selective agonist for GABABRs and might act on pre- and postsynaptic sites, although its exact mechanism of action is unknown (Misgeld et al. 1995). Pre- and postsynaptic GABABR effects were shown to have distinct pharmacological properties (Dutar and Nicoll 1988). In addition, at high baclofen

concentrations the presynaptic effect on neurotransmitter release dominated, whereas at lower concentrations postsynaptic effects were more marked (Hirata et al. 1992). Furthermore, studies observed a reduction of the GABAA Cl^-- IPSP by baclofen (Scholfield 1983, Blaxter and Carlen 1985, Inoue et al. 1985, Deisz and Prince 1989) due to hyperpolarization of inhibitory neurons at low concentrations and acting directly on transmitter release at higher concentrations (Hirata et al. 1992, Lambert and Wilson 1993).

In our study, the SICI of N100, which was the only potential modulated by both of the GABAergic agonists, could be related to the activity of presynaptic (peaks around 100 ms) and postsynaptic (peaks around 150 to 200 ms) GABABRs starting around 50 ms and lasting up to a few hundred milliseconds (Tamás et al. 2003, McDonnell et al. 2006, Davies et al. 1990, McCormick 1989). We assume also regarding the baseline results, that this experiment could provide information about the interaction between GABAAergic and GABABergic neurotransmission. It illustrates the enhancement of GABAAergic or GABABergic neurotransmission in presence of GABAA-mediated inhibitory effects of SICI on sIPSP mediated by GABABRs.

Regarding the EEG curve of SICI, there are obviously two peaks around 100ms and 150 ms modulated differently after drug-intake. We suppose that these two peaks are related to inhibitory pre- and postsynaptic GABABergic activity respectively with a similar time course reported in previous studies (Tamás et al. 2003, McDonnell et al. 2006, Davies et al. 1990, McCormick 1989). This would imply that diazepam reduces the inhibitory effect of SICI on postsynaptic GABABRs and thus results in an increased activity of postsynaptic GABABRs. Postsynaptic GABABergic neurotransmission is thought to be responsible for the suppression of late I-waves and thus the reduction of MEPs in response to TS (Chen et al. 2008, Di Lazzaro et al. 2008). Baclofen leads to an enhancement of presynaptic GABABergic inhibition in the presence of SICI and may lead to a non-significant reduction of postsynaptic GABABR activity most likely via autoinhibitory processes (Deisz 1999, Davies et al. 1990).

Further investigation needs to be done to strengthen this line of argumentation. In particular, paired-pulse studies are required recording brain responses applying different stimulus intensities, interstimulus intervals and drug doses. Future source modelling analyzes will be more accurate in determining possible origins of inhibitory potentials.

According to our hypothesis, diazepam is thought to potentiate the GABAA-mediated suppressive effects of SICI. Since at baseline conditions no early TEPs were

modulated but the SICI of the N100, enhanced activity of GABAARs may have led to higher suppression of the GABABR-mediated N100, thus to an increased SICI of the N100. In contrast, the SICI of N100 was reduced by diazepam.

However, this discrepancy suggests that the results have to be integrated into a more complex neuronal framework. The effects of GABAA- and GABAB-mediated cortical inhibition might not be restricted only to early and late TEPs, they rather may replace each other and the weakening of one may strengthen the other (Misgeld et al. 1995). In recent studies, it has been confirmed that GABAR activation can also take place in a less spatially and temporally restricted manner. GABA from the synaptic cleft can activate receptors on presynaptic terminals or synapses nearby on the same or close-by neurons. Additionally, low concentration of GABA in the extracellular space can lead to a persistent activation of GABAARs, named tonic activation (Farrant and Nusser 2005).

Several studies confirmed that GABAA- and GABAB-mediated inhibition tightly controls each other. The concept that GABARs may interact is not surprising as metabotropic GABABRs are known to reduce presynaptic release of GABA (Deisz and Prince 1989, Kobayashi et al. 2012). Shen et al. (2017) suggested that the feed-forward crosstalk between GABARs depends on the subunit composition of the GABAAR. The metabotropic GABABR can suppress the activity of α-subunit, but not *ρ*-containing GABAARs in human amygdala. Additionally, baclofen was shown to enhance tonic GABAAR current in thalamus and dentate gyrus where the GABAAR δ-subunit is present (Connelly et al. 2013, Tao et al. 2013), whereas opposite effects occurred in amygdala that lacks δ-subunits (Wisden et al. 1992). Therefore GABABR activity can promote both excitation and inhibition (Shen et al. 2017, Garaycochea and Slaughter 2016). Furthermore, chloride influx via GABAA IPSPs was found to reduce GABABR-mediated IPSPs in hippocampal pyramidal cells (Lopantsev and Schwartzkroin 2001). Based on their kinetic properties, the interaction between metabotropic and ionotropic receptors is thought to suppress repeated, rapid GABAergic inhibition (Shen et al. 2017)

4.2.3 Comparison of EEG responses between SICI and LICI

Comparing the SICI and the LICI paradigm at the level of TEPs, cluster-based permutation analysis showed no significant effects of all TEP components (all $p < 0.05$) apart from the P70 with a higher amplitude in the EEG signal of LICI ($p = 0.02$).

At first sight, this negative result seems to be surprising as previous TMS-EMG measurements have revealed that different cell populations mediate SICI and LICI.

Variation in strength of the test stimulus had different effects and no correlation in the extent of SICI and LICI (Sanger et al. 2001). Higher stimulus intensities might recruit neurons less excitable or spatially further away from the center of activation (Sanger et al. 2001).

However, the largely identical EEG pattern of SICI and LICI does not necessarily imply the same cortical mechanisms, but implies that TMS-EEG and TMS-EMG measurements are probably measuring separate phenomena. Hitherto, it remained unclear whether inhibition recorded by EEG reflects similar mechanisms to MEP inhibition. MEPs reflect corticospinal excitability elicited by indirect-induced descending action potentials originating in pyramidal neurons via presynaptic IPSPs and EPSPs (Rossini et al. 1994). The motor output is affected by cortical, subcortical and spinal mechanisms (Kiers et al. 1993), though the exact sequence of central pathways is not well understood (Ziemann and Rothwell 2000). On the contrary, TEPs asses directly cortical responses representing the summation of temporally and spatially distributed electrical currents in the superficial cortical layers produced by IPSPs and EPSPs without influence of spinal inhibitory mechanisms (Nikulin et al. 2003, Bender et al. 2005, Ilmoniemi and Kicic 2010).

Previous studies showed a dissociation of MEP and N100 effects highlighting different origins of the evoked signals (Day et al. 1989, Di Lazzaro et al. 2004). Especially, the N100 occurs even at subthreshold intensities and no correlation between the N100 peak amplitude and the MEP size was found (Paus et al. 2001, Nikulin et al. 2003, Komssi et al. 2004, Komssi et al. 2007).

At present, the neurophysiology of the P70 is not known in detail, but pre-existing evidence suggests a contribution of somatosensory feedback caused by muscle twitches of suprathreshold TMS pulses (Petrichella et al. 2017). A stronger P70 in the LICI paradigm may be explained by the use of 100% RMT (Premoli et al. 2014b) as CS in contrast to the SICI protocol with 70%RMT.

Additionally, Ferreri et al. (2011) compared the EEG waveforms of SICI (ISI = 3ms) and ICF (ISI = 11ms) which are thought to represent separate intracortical phenomena (Ziemann et al. 1996c). Similarly to our study, their comparison resulted in nearly identical potential patterns induced by differing TMS intensities and paradigms suggesting that the similar sequence of neuronal events is independently triggered for each peak and the same cortical and subcortical circuits are recruited illustrating functional connectivity (Lee et al. 2003b). Furthermore, these experiments provide information about the large-scale connected networks regulated via different interneural

circuit activation we were able to modulate by the paired-pulse paradigm and GABAergic drugs. Finally, TMS-evoked activity should be considered as a balance of inhibitory and excitatory circuit net activities (Ferreri et al. 2011).

We conclude that the results of TMS-EMG studies measuring corticospinal activity are limited in their value explaining cortical mechanisms. Our data strongly suggest that modulation of paired-pulse TMS-induced EEG potentials cannot be deduced directly from paired-pulse TMS-induced MEP amplitudes. This is not surprising as only the N15-P30 complex is significantly related to the MEP amplitude (Cash et al. 2017, Maki and Ilmoniemi 2010). In the same manner, this study does not necessarily imply that the EEG modulations by SICI and LICI are based on equal intracortical mechanisms. In order to define a satisfactory framework of functional connectivity experimental paradigms need to be tested by electrophysiological, neuroimaging or functional imaging techniques in combination. In future, source localization will allow detecting the origin of inhibitory activity more precisely.

4.3 Outlook

Our study further contributed to the understanding of the mechanisms of cortical inhibition. Although SICI has been linked to GABAAergic neurotransmission (Kujirai et al. 1993), we could not reveal a specific TEP component as marker of GABAAergic neurotransmission. However, we demonstrated a tight interaction between GABAergic receptor subtypes controlling the balance between inhibitory and excitatory activity at the level of human cortex. Future TMS-EEG paired-pulse studies varying the interstimulus interval, the stimulus intensities and the drug doses with concordant electrophysiological or functional imaging techniques will be able to clarify this relationship more precisely. An increased number of subjects and number of TMS stimuli will reduce the variability of the study results. Recent methodological improvements as neuronavigation or an optimal control for sensory feedback after TMS-evoked muscle contraction, will minimize the contamination of physiological brain activity.

Additionally, we found that cortical inhibitory mechanisms are explained insufficiently by TMS-EMG studies testing corticospinal activity, and thus also non-cortical processes might contribute to its generation, although SICI is thought to originate primarily at the cortical level. Thus we summarize, that paired-pulse TMS-induced EEG potentials cannot be deduced directly from MEP amplitudes. We further confirmed that paired-pulse effects on TEPs are modulated largely similarly irrespective of the interstimulus interval. To address the extent of contribution of subcortical sources to SICI, future

experiments applying source localization analyses are needed to evaluate the origin of inhibitory potentials more accurately.

Our findings provide the basis for the interpretation of paired-pulse TMS-EEG measurements in patients with impaired GABAergic neurotransmission as epilepsy or schizophrenia (Hasan et al. 2012, Kimiskidis et al. 2014, Lewis et al. 2005) and will facilitate the understanding of the pathophysiology and treatment of various neurological and psychiatric disorders as amyotrophic lateral sclerosis (Shibuya et al. 2017), Alzheimer disease and frontotemporal dementia (Benussi et al. 2017) or stroke patients (McDonnell and Stinear 2017).

5 Conclusion

Cortical inhibitory processes can be measured by paired-pulse transcranial magnetic stimulation (TMS). Until recently, short-interval intracortical inhibition (SICI) and long-interval intracortical inhibition (LICI) were quantified as motor evoked potential (MEP) inhibition in the hand muscle. Pharmacological experiments consider them as a measure of GABAA and GABAB receptor-mediated neurotransmission, respectively.

The effect of SICI on TMS-evoked EEG potentials (TEPs) and its pharmacological properties using combined TMS and electroencephalography (TMS-EEG) have not been systematically studied. Thus, the present study aims to investigate the physiological underpinnings of TEPs modulated by SICI, to examine its pharmacological characteristics and to compare SICI with a separate paired-pulse paradigm LICI.

Sixteen healthy male subjects participated in three sessions of the pseudo-randomized, placebo-controlled, double-blinded crossover study. Paired-pulse TMS was tested over the left motor cortex and the evoked brain responses were recorded by a high-density 64-channel EEG. Neuroactive drugs acting as positive modulator at GABAA- (diazepam, 20mg) or as specific agonist at GABAB (baclofen, 50mg) receptors were applied, probing the pharmacological characteristics of SICI.

We analyzed the effects of a conditioning stimulus (CS) applied 2 ms prior to a test stimulus (TS) on TEPs. Here, SICI was calculated as the difference between paired-pulse TEPs (corrected for late EEG responses evoked by the conditioning pulse) and single-pulse TEPs. Cluster-based permutation analysis showed that SICI before drug-intake significantly suppressed late TEPs (N100 and P180, both $p < 0.001$). Whereas diazepam reduced SICI of the N100 over the non-stimulated hemisphere ($p = 0.03$), baclofen increased SICI of the N100 over frontal sites of both hemispheres ($p = 0.007$). The comparison of the effects of two paired-pulse paradigms with differing interstimulus intervals (ISI), SICI (ISI = 2 ms) and LICI (ISI = 100 ms), showed a largely similar modulation on TEPs irrespective of the interstimulus interval.

These findings demonstrate for the first time that cortical inhibitory mechanisms are explained insufficiently by TMS-EMG studies testing corticospinal activity; TEPs of SICI and LICI cannot be deduced directly from MEP amplitudes. Furthermore, we demonstrated a tight interaction between GABAergic receptor subtypes controlling the balance between inhibitory and excitatory activity at the level of human cortex. The modulation by paired-pulse paradigms increases our knowledge of TMS-EEG measurements identifying functional abnormalities with altered inhibitory mechanisms.

5.1 *Zusammenfassung*

Intrakortikale Hemmung kann mittels transkranieller magnetischer Stimulation (TMS) durch Anwendung von Doppelpulsprotokollen untersucht werden. Intrakortikale Hemmung bei kurzen Interstimulusintervallen (engl. short-interval intracortical inhibition, SICI) und intrakortikale Hemmung bei langen Interstimulusintervallen (engl. long-interval intracortical inhibition, LICI) wurden bisher üblicherweise als motorisch evozierte Potentiale (MEP) durch Oberflächenelektorden über intrinsische Handmuskeln abgeleitet. Studien belegen die unterschiedlichen pharmakologischen Profile der GABAA-Rezeptor vermittelten SICI und der GABAB-vermittelten LICI.

Die vorliegende Arbeit zielt auf die Untersuchung der neurophysiologischen Wirkung von SICI auf TMS-induzierte EEG-Potentiale (engl. TMS-evoked EEG potentials, TEPs) und dessen pharmakologischen Eigenschaften sowie auf einen Vergleich von SICI mit einem weiteren Doppelpulsprotokoll, LICI.

Sechzehn gesunde, männliche Probanden nahmen an drei Sitzungen der pseudo-randomisierten, placebokontrollierten und doppelblinden Crossover-Studie teil. Die Aufzeichnung der fokalen Doppelpuls-TMS über dem linken motorischen Kortex erfolgte durch ein räumlich hochauflösendes 64-Kanal EEG. Die Applikation neuroaktiver Medikamente wie dem allosterischen Modulator des GABAA-Rezeptors (Diazepam, 20 mg) und dem spezifischen Agonisten des GABAB-Rezeptors (Baclofen, 50 mg) ermöglichte die Erhebung der pharmakologischen Charakteristika von SICI.

Wir analysierten den Effekt auf die kortikale Erregbarkeit durch einen konditionierenden Stimulus (CS) und einem nach 2 ms folgenden Teststimulus (TS). SICI berechnete sich als Differenz zwischen den Doppelpuls TEPs - bereinigt für spät evozierte EEG Signale durch den CS - und den Einzelpuls TEPs. Ein clusterbasiertes Permutationsverfahren zeigte vor Medikamenteneinnahme eine signifikante Hemmung später TEPs (N100 und P180, beide $p < 0.001$) durch SICI. Diazepam führte zu einer Reduktion von SICI des N100 über der nicht-stimulierten Hemisphäre ($p = 0.03$), wohingegen Baclofen das SICI des N100 im frontalen Bereich beider Hemisphären verstärkte. Eine größtenteils identische Modulation der TEPs ergab sich im Vergleich zweier Doppelpulsprotokolle mit verschiedenen Interstimulusintervallen (ISI), SICI (ISI=2 ms) und LICI (ISI=100 ms).

Diese Resultate zeigen erstmals, dass intrakortikale Hemmung durch TMS-EMG Messungen nur unvollständig dargestellt wird. Folglich können die TEPs von SICI und LICI nicht ohne Weiteres von deren MEP Amplituden abgeleitet werden. Zudem konnte eine enge Interaktion GABAerger Rezeptorsubtypen festgestellt werden.

Doppelpuls-TMS-EEG stellt eine Grundlage zur Interpretation von funktionellen Abnormitäten neurologischer oder psychiatrischer Erkrankungen mit beeinträchtigter GABAerger Neurotransmission dar.

6 Literature

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7 Erklärungen zum Eigenanteil

Erklärung zum Eigenanteil der Dissertationsschrift

Die Arbeit wurde im Fachbereich Medizin in der Eberhard Karls Universität zu Tübingen unter 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 Frau Dr. Isabella Premoli.

Die Versuche wurden nach Einarbeitung durch Labormitglieder, namentlich Frau Dr. Isabella Premoli und mit Unterstützung durch Herrn Dr. Carl Zipser, von mir eigenständig durchgeführt.

Die zum Vergleich herangezogenen Daten (Unterpunkt 3.5, LICI) wurden freundlicherweise aus einer Publikation mit Einverständnis von Frau Dr. Isabella Premoli zur Verfügung gestellt.

Die statistische Auswertung erfolgte nach Anleitung durch Frau Dr. Isabella Premoli und Herrn Dr. Paolo Belardinelli durch mich.

Bereits veröffentlichte Ausschnitte des Methodenteils wurden in kursiver Schrift kenntlich gemacht. Diese wurden von mir selbstständig verfasst.

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

Tübingen, den 30.07.2018

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Julia Király

8 Veröffentlichungen

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

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