Pharmaco-TMS-EEG as a new tool to characterize human cortical excitability and connectivity

Dissertation

zur Erlangung des Grades eines

Doktors der Naturwissenschaften

der Mathematisch-Naturwissenschaftlichen Fakultät

und

der Medizinischen Fakultät

der Eberhard-Karls-Universität Tübingen

vorgelegt

von

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September-2018

Tag der mündlichen Prüfung:	15.11.2018
Dekan der Math.-Nat. Fakultät:	Prof. Dr. W. Rosenstiel
Dekan der Medizinischen Fakultät:	Prof. Dr. I. B. Autenrieth
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Ich erkläre, dass ich die zur Promotion eingereichte Arbeit mit dem Titel: ‘Pharmaco-TMS-EEG as a new tool to characterize human cortical excitability and connectivity’ selbständig verfasst, nur die angegebenen Quellen und Hilfsmittel benutzt und wörtlich oder inhaltlich übernommene Stellen als solche gekennzeichnet habe. Ich versichere an Eides statt, dass diese Angaben wahr sind und dass ich nichts verschwiegen habe. Mir ist bekannt, dass die falsche Abgabe einer Versicherung an Eides statt mit Freiheitsstrafe bis zu drei Jahren oder mit Geldstrafe bestraft wird.

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Summary

Excitation and inhibition in human cortex can be measured by transcranial magnetic stimulation (TMS) combined with electromyography (EMG) and electroencephalography (EEG) by way of specific markers of TMS-evoked muscle and brain responses. It has been shown that this capacity can be strongly enhanced by combining TMS-EMG/EEG with central nervous system (CNS) active drugs. Early studies have systematically investigated the role of a wide variety of CNS active drugs on motor evoked potentials (MEPs) and this knowledge is now partially applied to clinical settings. However, pharmacological alteration of TMS evoked EEG potentials (TEPs), which can provide direct information on cortical excitability and connectivity, has not been systematically elucidated yet. Here, we complement previous findings by using pharmaco-TMS-EEG/EMG approaches to explore the physiological signatures of TEPs. In Experiment 1, we studied the effects of the experimental compound S44819, a selective α5-GABAAR antagonist, on TEPs and MEPs in 18 healthy young adults in a phase I study. In experiment 2, we investigated the role of three anti-epileptic drugs (carbamazepine, brivaracetam and tiagabine) on TEPs and MEPs in 15 healthy male adults. 100 mg S44819 enhanced cortical excitability, as denoted by reduction of the amplitude of the N45 TEP component, as well as decrease of the motor threshold; carbamazepine decreased the amplitude of the P25 and P180 TEP components and increased motor threshold; brivaracetam decreased the N100 TEP amplitude and increased MEP threshold; tiagabine had no effect on TEPs and/or MEPs. Results of experiment 1 demonstrated for the first time effects of S44819 in the human cortex, that are relevant as S44819 showed potential to improve plasticity and learning in animal models of cerebral stroke. These findings led to further development of S44819 in a clinical phase II study to test its efficacy in enhancing recovery of function in stroke patients. Results of experiment 2 confirmed and extended previous findings that the P25 TEP component reflects axonal excitability of the corticospinal system, the N100 potential in the non-stimulated hemisphere propagated activity mediated by inhibition of presynaptic neurotransmitter release, and the P180 late activity dependent on voltage-gated sodium channels (VGSCs). We believe that these updated pharmacological characterization of TEPs will prove useful for the

understanding of normal and dysfunctional cortical excitability and inhibition of the human brain.

General Introduction

Transcranial magnetic stimulation (TMS) is a powerful non-invasive brain stimulation technique which is widely used in systems neurophysiology, cognitive and clinical neuroscience, and neuropsychiatry (Barker, Jalinous, & Freeston, 1985; Hallett, 2000; Walsh & Cowey, 2000). The rationale behind how TMS is able to induce changes in the electrical activity of the brain is based on Faraday's principle of induction of an electric current by a fluctuating magnetic field (Faraday, 1846). A simple TMS machine consists of a stimulating coil, a bank of energy-storing capacitors, and low resistance cables. When the TMS machine generates a single pulse, the stored energy in the capacitors is released and an intense time-varying current pulse runs through the coil, which in turns produces a brief intense magnetic field (Barker et al., 1985; Jalinous, 1991). This magnetic field induces an intracranial current without significant attenuation by the skull and/or dura. This can result in depolarization of cell membranes and initiation of action potentials, and thus change the neuronal firing pattern and organization which can in the short term excite or inhibit specific brain areas (Hallett, 2000; O'Shea & Walsh, 2007). Central motor pathways can easily be targeted by TMS and cause muscle responses on the contralateral side of the body (Muellbacher et al., 2002), while excitation of sensory systems is more challenging to attain. It is well-established that various stimulation settings (e.g., single pulse, paired-pulse and repetitive TMS) are able to produce dissimilar effects on cortical excitability and inhibition, either transiently or lastingly (RMMF Chen et al., 1997; Fitzgerald, Fountain, & Daskalakis, 2006; Pascual-Leone et al., 1998). The alteration of specific motor functions, as a consequence of TMS over primary motor cortex (M1), has traditionally been quantified by the consecutive or concurrent recording of neurophysiological responses through surface electromyography (EMG) from a muscle. The conventional readouts of single pulse TMS over M1 are resting motor threshold (RMT) and motor evoked potential (MEP) size, which are recorded by EMG. RMT is typically defined as the minimal stimulator intensity that is required to produce a small reliable MEP in a resting muscle (usually a hand muscle). It has been hypothesized that the RMT represents the excitability of a central core of neurons which are excited by TMS, and in response transmit action potentials (Hallett, 2000). The magnitude of an MEP is usually measured as either the average response to a series of pulses

applied at a steady stimulator intensity or as the growth in MEP size as a function of stimulus intensity (referred to as an MEP input-output curve) (E. M. Wassermann, 1998). It has been thought that the activity of neurons other than those in the hypothesized 'core region' contribute to the measured MEP amplitude, and these neurons have been shown to have a higher threshold for activation, probably due to their location (more remote from the center of stimulation) or nature (less excitable) (Hallett, 2000). However, the basic physiology behind measures of motor cortical excitability was less well understood until 1996, when Ziemann et al. laid the basis for pharmaco-TMS studies (Ziemann, Lonnecker, Steinhoff, & Paulus, 1996). The basic concept behind these studies is that CNS-active drugs, with distinct mechanisms of action, can be used to characterize TMS measures of motor cortical excitability since various drugs would produce unalike effects on MEP patterns. In pharmaco-TMS, a single dose of a drug of interest is given and changes in motor excitability, measured by TMS, are compared with a baseline and/or placebo condition. Many studies have systematically investigated the pharmacological characterization of MEPs. For instance, several studies showed that RMT can be influenced by drugs that affect voltage-gated ion channels, like carbamazepine and lamotrigine which acts as voltage-gated sodium channel (VGSC) blockers (for review see (Ziemann et al., 2015)). Whereas modulation of the major CNS neurotransmitter systems, like the gamma-aminobutyric acid (GABA) system, either had no effect, or inconsistent effects, on the motor threshold (for review see (Paulus et al., 2008)). Therefore, these results support the hypothesis that RMT represents axon membrane excitability. Results from pharmaco-TMS studies also suggest that MEP size reflects the transsynaptic excitation of corticospinal neurons, as some of neurotransmitters and neuromodulators (for example GABA, Glutamate, Dopamine, and Norepinephrine) have been shown to modulate MEP size and MEP input-output curve (mostly at the high-amplitude MEP range) (Ziemann et al., 2015) while voltage-gated ion channels had inconsistent or no effects on MEP size (for review see (Paulus et al., 2008)). Pharmaco-TMS studies also reported in several cases that the change in MEP size happens without changes in RMT, this also supports the initial hypothesis that the mechanisms underlying RMT and MEPs are different (E. Wassermann et al., 2008).

Pharmaco-TMS has not been limited to single-pulse stimulation protocols but has also been utilized with other protocols, such as paired-pulse techniques. In paired-pulse stimulation a sub-threshold conditioning stimulus (S1) followed by a supra-threshold test stimulus (S2) and the S1 induces a short-term modulation of the amplitude of the MEP produced by the S2. Several studies showed that the MEP amplitudes are inhibited at inter-stimulus intervals (ISIs) of 1–5 ms and are facilitated at ISIs of 7–20 ms and these phenomena are commonly referred to as short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF) (Kujirai et al., 1993; Sanger, Garg, & Chen, 2001; Ziemann, Corwell, & Cohen, 1998; Ziemann, Rothwell, & Ridding, 1996). It has been shown that positive modulators of GABAARs, such as benzodiazepines, increase SICI (for review see (Ziemann et al., 2015)), thus suggesting that intracortical inhibition may in principal be mediated by GABAARs. On the other hand, the physiological signature of ICF is less evident than SICI and one suggestion is that ICF might reflect excitability of an excitatory motor cortical pathway separate from the SICI circuitry (Ziemann et al., 2015; ULF Ziemann et al., 1996). It is worth mentioning that although pharmaco-TMS has contributed significantly to a better understanding of cortical excitability in healthy brains and in those with brain disorders (Robert Chen et al., 2008) a relevant limitation of this technique is that the TMS effects are measured indirectly from a muscle and not the brain, meaning that spinal and cortical mechanisms that may underlie the neural responses to TMS cannot be fully disentangled. Another limitation is that EMG measures are limited to motor cortex as only TMS of M1 produces muscle twitches (or in the other word MEPs) and so the impact of TMS on non-motor regions (i.e. prefrontal and occipital cortex) cannot be studied.

More recent work has sought to combine TMS with several other electrophysiological and neuroimaging techniques, such that neural processes outside the motor system can be directly examined. For example, investigation of the prefrontal and occipital cortex can be achieved by the concurrent recording of neurophysiological responses through scalp electroencephalography (EEG) following TMS pulses (Daskalakis et al., 2008; Farzan et al., 2009). EEG traces in response to TMS pulses reveal the temporal and spatial summation of the TMS-induced excitatory and inhibitory postsynaptic potentials (Kirschstein & Köhling, 2009). A series of studies have shown that TMS over cortex evokes a

sequence of positive and negative deflections within the first few 100 ms after stimulation onset. In particular, TMS over M1 has been shown to generate highly reproducible TMS-evoked potentials (TEPs) which are typically labeled according to their polarity and latency: P25, N45, P70, N100 and P180 (P: positive; N: negative) (Bonato, Miniussi, & Rossini, 2006; Casarotto et al., 2010). However, the neurophysiological mechanisms that underlie TEPs are not well-known. Pharmaco-TMS-EEG was thus recently developed to identify TMS-EEG markers of cortico-cortical excitability and effective connectivity in the healthy brain (Premoli, Castellanos, et al., 2014; Premoli, Rivolta, et al., 2014). This method has been utilized to characterize the underlying physiology of TMS-evoked brain responses by using CNS-active drugs with specific mechanisms of action, as well as to interrogate newly developed CNS-active drugs with respect to their modulation of specific TEP components. In these studies, changes in TEPs after application of a single dose of the study drug are compared to a baseline and/or placebo condition in a randomized, placebo-controlled, double-blind crossover design. Recent pharmaco-TMS-EEG studies have systematically demonstrated effects of several GABAergic drugs on TEPs. For instance, classical benzodiazepines (alprazolam and diazepam), which are positive modulators at the GABAA receptor, increase the N45 potential amplitude, and decrease the N100. Conversely, baclofen, which acts as a specific agonist at the GABAB receptor, raised the N100 potential amplitude and had no effect on the N45. Taken together, these results suggested that the N100 is negatively related to GABAA but positively to GABAB receptor-mediated neurotransmission (Premoli, Castellanos, et al., 2014; Premoli, Rivolta, et al., 2014). However, in order to gain a more comprehensive overview of how TEPs could be utilized as new markers of excitation and inhibition in the human brain, the effect of CNS active drugs with other modes of action on TEPs needed to be investigated. Such measures are useful in understanding the changes in brain physiology observed in physiological processes such as plasticity, and pathological processes in brain disorders. Several groups have already started preliminary clinical research in this direction; for example, TMS-EEG in epilepsy patients has shown that late motor cortex TEP components are abnormal in comparison to those in healthy controls (Del Felice, Fiaschi, Bongiovanni, Savazzi, & Manganotti, 2011; Julkunen et al., 2013; Shafi et al., 2015; Valentin et al., 2008).

Aims and scopes of this dissertation

In order to depict the pharmaco-physiological profile of TEPs and to explicate the mode of action of a newly industrialized CNS-active drug, we used the pharmaco-TMS-EEG/EMG approaches described in the introduction.

First, we tested the effects of a single oral dose of S44819, a novel competitive selective antagonist of α5-GABAAR, on cortical excitability in 18 male participants in a randomized, double-blinded, placebo-controlled, crossover phase I study (1st study, Appendix 1).

Second, we studied the pharmacological influences of three anti-epileptic drugs (AEDs) with well-defined modes of action including carbamazepine, a voltage-gated sodium channel (VGSC) blocker, brivaracetam, a ligand to the presynaptic vesicle protein VSA2, and tiagabine, a selective GABA reuptake inhibitor, on cortical excitability in a placebo-controlled crossover study in 15 male participants (2nd study, Appendix 2).

Summary of scientific findings

Study 1: 'Effects of the Selective alpha5-GABAAR Antagonist S44819 on Excitability in the Human Brain: A TMS-EMG and TMS-EEG Phase I Study'.

We showed that the 100 mg S44819 reached human cortex and significantly increased the corticocortical and corticospinal excitability as indexed by a decrease in motor threshold measured by TMS–EMG and a decrease of the amplitude of the N45 component of the TMS–EEG responses. Also, we indicated that the peak serum concentration of 100 mg S44819 correlated with the decrease in N45 amplitude.

Study 2: 'Effects of antiepileptic drugs on cortical excitability in humans. A TMS-EMG and TMS-EEG study'.

Using TMS-EEG/EMG and three anti-epileptic drugs with specific modes of action, we showed that carbamazepine increased both motor and MEP threshold, brivaracetam only increased MEP threshold and tiagabine didn't alter motor or MEP threshold. We then showed that carbamazepine suppressed the amplitudes of the P25 and P180 TEP components, with and without adjusting stimulus intensity by the change in motor threshold in the post-drug measurements, while brivaracetam decreased the N100 over the contralateral sensorimotor cortex. For tiagabine, no significant TEP modulation could be established. Drug-induced changes in spontaneous oscillatory spectral power in the resting-state EEG and pre-TMS periods revealed that tiagabine caused a strong broadband increase in spontaneous oscillatory power in all frequency bands (delta, theta, alpha, and beta) which was most pronounced in the delta and theta bands (7-8 fold).

Overall conclusion and outlook

The scope of this dissertation was first to deepen the understanding of the physiological processes underlying TEPs by combining TMS-EEG with CNS active drugs with specific modes of action (GABAergic system, voltage-gated ion channels etc.), and second to use TMS-EEG as a non-invasive method to characterize the mode of action of a newly developed CNS active drug (S44819). In this thesis, by employing TMS-EEG/EMG, we could show convincing evidence that a sufficient concentration of S44819 reached human cortex and subsequently increased corticospinal and cortical excitability. Noticeably, pharmacological reduction of α5-GABAAR-mediated inhibitory tone could be important in several neurological pathologies including stroke. For instance, ischemic stroke triggers a hypo-excitability in the peri-infarct motor cortex mainly due to over-activation of α5-GABAA receptors (Clarkson, Huang, MacIsaac, Mody, & Carmichael, 2010). Therefore, counteracting exaggerated α5-GABAAR-mediated inhibition could allow faster functional recovery after stroke (Clarkson et al., 2010; Hermann & Chopp, 2012).

Moreover, we extended and confirmed previous findings by identifying the TEP signatures of voltage-gated ion channels and GABAergic system activity in the human brain, by using three well-known AEDs. Although AEDs provide satisfactory control of seizures for most patients with epilepsy, a significant fraction of patients (around one-third) with newly diagnosed epilepsy do not achieve freedom from seizures with a modest dose of a single AED (Cascino, 2008; Kwan & Brodie, 2000). It should, however, be mentioned that the investigation of AED-induced modulation of cortical excitation in epileptic patients is complex due to the high number of specific syndromes and the remarkable diversity of molecular targets of AEDs. Hence, the pharmaco-TMS-EEG work presented in this dissertation may give new insights into the effects of AEDs on cortical functioning.

In the following paragraphs, the updated ‘big-picture’ of the neurophysiological underpinnings of primary motor cortex TEPs will be discussed.

P25: The early TEP components of motor cortex have been shown to be putative markers of excitation of the corticospinal system, since following M1 stimulation, motor cortical regions respond between 3-28 ms after the TMS pulse (Ilmoniemi et al., 1997; Komssi et al., 2002). Also, other studies showed that rTMS and tDCS

(methods that modulate cortical excitability) significantly alter only the early components of motor cortex (Esser et al., 2006; Veniero, Bortoletto, & Miniussi, 2012). Our results confirm this notion by demonstrating that carbamazepine (as a classic excitability-lowering drug) suppresses the P25 component at the site of stimulation (Darmani et al., under revision). Altogether these findings support the feasibility of measuring cortical excitability after pharmacological interventions and suggest that the P25 component is a marker of axonal excitability of the corticospinal system.

N45: We showed that after application of 100 mg S44819, only the N45 TEP component was suppressed, whereas other components remained unaffected (Darmani et al., 2016). These results complement previous findings which showed that benzodiazepines and zolpidem increase the amplitude of the N45 component (Premoli, Castellanos, et al., 2014; Premoli, Rivolta, et al., 2014) and therefore N45 is likely mediated by neurotransmission through GABAA receptors.

N100: Our TMS-EEG experiments in combination with brivaracetam updated previous findings (Premoli, Castellanos, et al., 2014; Premoli, Rivolta, et al., 2014) regarding the N100 component, and suggest that drugs with inhibition of presynaptic excitatory transmitter release (such as BRV) decrease the activity of the N100 in the non-stimulated hemisphere (Darmani et al., under revision). Although, the exact underlying physiology of N100 reduction in the non-stimulated hemisphere requires further investigations, but may indicate a reduction in overall signal propagation and long-range connectivity in the cortex.

P180: Previous results of a TMS-EEG study on the effects of AEDs on TEPs in healthy subjects showed a decrease in the P180 potential by lamotrigine (another classical VGSC blocker) (Premoli, Costantini, Rivolta, Biondi, & Richardson, 2017). The suppression of the P180 component under carbamazepine in our experiments, with and without adjusting stimulus intensity to the change in motor threshold, highlighted the sensitivity of the late TEPs to VGSC blockage (Darmani et al., under revision). Thus, P180 might reflect VGSC activity.

It should be mentioned that we used a neuronavigation system to make sure that TMS positioning over each subject's head is consistent across sessions. This is methodologically crucial especially if one wants to replicate/extend the present findings to cortical areas outside of the motor strip (Casarotto et al., 2010; Lioumis, Kicic, Savolainen, Makela, & Kahkonen, 2009).

Although pharmaco-TMS-EEG is potentially a very useful tool to investigate the intrinsic and functional properties of brain systems non-invasively, several limiting and confounding factors should be considered when using this technique, in order to broaden its applications and increase the reliability of results across studies. In the following, some of these issues will be discussed briefly:

The use of TMS-EEG has been mainly limited to the cerebral cortex as the EEG signals cannot reflect deeper brain activity emerging from subcortical structures. Therefore, in order to reliably localize the cortical areas that play a part in the pharmacological modulation of TEPs, cortical source modeling of TEPs would be needed in future studies. One other possibility to overcome this limitation is to combine TMS-EEG with other techniques such as fMRI to investigate causal interactions between cortical as well as subcortical areas at a better spatial resolution.

Recent studies, including ours, show that drug effects on TEPs may depend on the stimulation intensity, since drugs may modulate motor and MEP thresholds (Darmani et al., under revision; Premoli et al., 2017). Therefore, it would be important to consider these threshold changes and adjust stimulation intensities in individual subjects in post-drug measurements accordingly.

It has also been shown that instantaneous brain state may have an impact on the reliability of TEP measurements (Bortoletto, Veniero, Thut, & Miniussi, 2015; Keil et al., 2013; Rosanova et al., 2009). In addition, since some drugs influence spontaneous brain oscillations dramatically, the reliable detection of TEPs in post-drug measurements may consequently be impeded as a result of drug-induced altered brain states.

A final issue are the methodological problems in dealing with TMS related artifacts. Studies have shown that TMS at effective stimulus intensities may cause significant unwanted auditory and somatosensory pathway co-activation due to a loud click and a tapping sensation as a result of the TMS pulse. These auditory-evoked potentials (AEP) and sensory-evoked potentials (SEP) are superimposed over the true TEPs and difficult to disentangle from direct transcranial cortical activation (Conde et al., 2018; Conde et al., 2012; Rogasch et al., 2014). Some solutions to these problems have been suggested by (Farzan et al., 2016; Herring, Thut, Jensen, & Bergmann, 2015) and could potentially be used in future studies to reduce the effects of AEPs and SEPs. However, a more

secure solution is realistic sham stimulation to account for the contamination of drug effects on TEPs by drug-induced changes in AEPs and SEPs.

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Appended papers/manuscripts

Appendix 1: Darmani, G., Zipser, C.M., Böhmer, G.M., Deschet, K., Müller-Dahlhaus, F., Belardinelli, P., Schwab, M., Ziemann, U., (2016) Effects of the Selective alpha5-GABAAR Antagonist S44819 on Excitability in the Human Brain: A TMS-EMG and TMS-EEG Phase I Study. Journal of Neuroscience 36, 12312-12320.

Appendix 2: Darmani, G., Bergmann, T.O., Zipser, C.M., Baur, D, Müller-Dahlhaus, F., Ziemann, U., (2018) Effects of antiepileptic drugs on cortical excitability in humans. A TMS-EMG and TMS-EEG study. Under revision in Human Brain Mapping (September 2018)

Statement of contributions

Study 1: Darmani, G., Zipser, C.M., Böhmer, G.M., Deschet, K., Müller-Dahlhaus, F., Belardinelli, P., Schwab, M., Ziemann, U., (2016) Effects of the Selective alpha5-GABAAR Antagonist S44819 on Excitability in the Human Brain: A TMS-EMG and TMS-EEG Phase I Study. Journal of Neuroscience 36, 12312-12320.

G.D., and C.M.Z. performed research; **G.D.**, and P.B., analyzed data; G.M.B., K.D., F.M.-D., M.S., and U.Z. designed research; **G.D.**, and U.Z. wrote the paper.

Study 2: Darmani, G., Bergmann, T.O., Zipser, C.M., Baur, D, Müller-Dahlhaus, F., Ziemann, U., (2018) Effects of antiepileptic drugs on cortical excitability in humans. A TMS-EMG and TMS-EEG study. Under revision in Human Brain Mapping (July 2018)

G.D., D.B., and C.M.Z. performed research; **G.D.**, and T.O.B. analyzed data; F.M.D, and U.Z. designed research; **G.D**., T.O.B., and U.Z., wrote the paper.

Acknowledgments

I am very thankful to my thesis supervisor Prof. Dr. Ulf Ziemann for giving me the opportunity to work in his lab and for supporting me during my Ph.D. Under Prof. Ziemann's supervision, I have learned to be disciplined, a critical thinker and goal oriented which added to my skills and boosted my commitments toward research. I am very grateful to Dr. Til Ole Bergmann for his valuable help, scientific discussions and guidance throughout my Ph.D. He has taught me, both consciously and unconsciously, how good physiological experiments are done. Big thanks to Christine Riegraf for her kindness and help with all of the administration stuff. I thank Florian Müller-Dahlhaus, Carl Zipser, Julia Kiraly and Hanna Faber for their great friendship as well as their good advice and collaboration. Many thanks to my advisory board members, Prof. Dr. Jan Born and Dr. Marc Himmelbach, for their useful advice and comments during my advisory board meetings. I am also very thankful to Graduate Training Center (GTC) and the GTC staff, Prof. Horst Herbert, Dr. Katja Thieltges, Dr. Petya Georgieva and Sandra Fischer, for their continuous support of the master and doctoral students and their kindness and patience.

Lastly, I would like to thank my family. All of my academic achievements would never have been real without having a great parent who supported me in every step. I still remember that my father made himself busy to stay awake with me all night through the exam periods during my time in Iran. My mother has been filling my life abroad with her supportive calls and love. And most of all I want to thank a very special person, my husband, Hamid for his continued and unfailing support, encouragement, and patience throughout all these years in Tübingen. He was always around at times when I thought that it was impossible to continue and helped me to keep things in perspective. Thank you, Hamid.

Appendix 1:

Darmani, G., Zipser, C.M., Böhmer, G.M., Deschet, K., Müller-Dahlhaus, F., Belardinelli, P., Schwab, M., Ziemann, U., (2016) Effects of the Selective alpha5-GABAAR Antagonist S44819 on Excitability in the Human Brain: A TMS-EMG and TMS-EEG Phase I Study. Journal of Neuroscience 36, 12312-12320.

Systems/Circuits

Effects of the Selective α5-GABAAR Antagonist S44819 on Excitability in the Human Brain: A TMS–EMG and TMS–EEG Phase I Study

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Alpha-5 gamma-aminobutyric acid type A receptors (α5-GABAARs) are located extrasynaptically, regulate neuronal excitability through tonic inhibition, and are fundamentally important for processes such as plasticity and learning. For example, pharmacological blockade of α5-GABAAR in mice with ischemic stroke improved recovery of function by normalizing exaggerated perilesional α5-GABAAR-dependent tonic inhibition. S44819 is a novel competitive selective antagonist of the α5-GABAAR at the GABA-binding site. Pharmacological modulation of α5-GABAAR-mediated tonic inhibition has never been investigated in the human brain. Here, we used transcranial magnetic stimulation (TMS) to test the effects of a single oral dose of 50 and 100 mg of S44819 on electromyographic (EMG) and electroencephalographic (EEG) measures of cortical excitability in 18 healthy young adults in a randomized, double-blinded, placebo-controlled, crossover phase I study. A dose of 100 mg, but not 50 mg, of S44819 decreased active motor threshold, the intensity needed to produce a motor evoked potential of 0.5 mV, and the amplitude of the N45, a GABAAergic component of the TMS-evoked EEG response. The peak serum concentration of 100 mg S44819 correlated directly with the decrease in N45 amplitude. Short-interval intracortical inhibition, a TMS–EMG measure of synaptic GABAAergic inhibition, and other components of the TMS-evoked EEG response remained unaffected. These findings provide first time evidence that the specific α5-GABAAR antagonist S44819 reached human cortex to impose an increase in cortical excitability. These data warrant further development of S44819 in a human clinical trial to test its efficacy in enhancing recovery of function after ischemic stroke.

Key words: α5-GABAAR; excitability; human cortex; motor evoked potential; TMS–EEG; tonic inhibition

Significance Statement

The extrasynaptic α-5 gamma-aminobutyric acid type A receptor (α5-GABAAR) regulates neuronal excitability through tonic inhibition in the mammalian brain. Tonic inhibition is important for many fundamental processes such as plasticity and learning. Pharmacological modulation of α5-GABAAR-mediated tonic inhibition has never been investigated in the human brain. This study demonstrates that S44819, a selective α5-GABAAR antagonist, increases cortical excitability in healthy human subjects, as indicated by specific markers of transcranial magnetic stimulation-induced muscle and brain responses measured by electromyography and electroencephalography. Our findings imply that tonic inhibition in human cortex can be modified effectively and that this modification can be quantified with noninvasive brain stimulation methods. The actions of S44819 may be suitable to improve plasticity and learning.

Introduction

The α5-subunit containing gamma-aminobutyric acid type A receptors (α5-GABAARs) predominate in the hippocampus, but are also expressed in the neocortex (Quirk et al., 1996; Möhler et al., 2002). They are located extrasynaptically at the base of the spines and on the adjacent shafts of pyramidal cell dendrites and are therefore in a privileged position to modulate excitatory input to pyramidal cells through tonic inhibition (Brünig et al., 2002;

Received May 26, 2016; revised Sept. 18, 2016; accepted Oct. 17, 2016.

Author contributions: G.M.B., K.D., F.M.-D., M.S., and U.Z. designed research; G.D., C.M.Z., and G.M.B. performed research; G.D., F.M.-D., P.B., and U.Z. analyzed data; G.D., P.B., and U.Z. wrote the paper.

This work was supported by the Institut de Recherches Internationales Servier. M.S. was supported by Robert Bosch Stiftung, Stuttgart, Germany. We thank Drs. Moerike, Gleiter, and Igel for local study support.

Farrant and Nusser, 2005; Möhler, 2006). Accordingly, increasing tonic inhibition shifts the input–output relationship of single cells to the right; that is, the probability of action potential generation to a given excitatory input decreases (Mitchell and Silver, 2003). Animal models demonstrated that specific pharmacological blockade, point mutations, or null mutants of α5-GABAARs enhance learning processes (Crestani et al., 2002; Maubach, 2003; Martin et al., 2010), whereas activation of α5-GABAARs reduces synaptic plasticity (Martin et al., 2010).

Acute ischemic stroke in mice and rats causes hypoexcitability in the peri-infarct cortex through increased tonic inhibition by overexpression and overactivation of α5-GABAARs (Clarkson et al., 2010; Schmidt et al., 2012). Pharmacological blockade or genetic lowering of the expression of α5-GABAARs enhances functional recovery after stroke in mice (Clarkson et al., 2010). Although increased tonic inhibition may be neuroprotective in the acute phase after ischemic stroke, counteracting excessive α5-GABAAR-mediated tonic inhibition in the subacute phase of stroke may allow a greater and/or more rapid recovery in stroke patients (Carmichael, 2012).

S44819 is a novel potent, competitive,and selective antagonist at the GABA-binding site of the α5-GABAAR tested *in vitro* (Etherington et al., 2016). It is as of yet unclear to what extent a single oral dose of S44819 is capable of reducing inhibition mediated by α5-GABAARs and thus increasing excitability in the human brain. Here, transcranial magnetic stimulation (TMS) was used in young healthy adults to obtain electromyographic (EMG) and electroencephalographic (EEG) markers of cortical excitability. Motor threshold, intensity needed to elicit a motor evoked potential (MEP) of a given amplitude, and short-interval intracortical inhibition (SICI) were obtained as classical TMS–EMG markers. Motor threshold represents axon membrane excitability, while SICI reflects synaptic GABAAergic inhibition of corticospinal neurons (for review (Ziemann et al., 2015)). The N45 component of the TMS-evoked EEG potential (TEP) involves GABAAergic activity, as benzodiazepines, i.e., allosteric positive modulators at GABAARs increase this potential (Premoli et al., 2014a; Premoli et al., 2014b). We hypothesized that S44819 would decrease the motor threshold and the intensity needed to elicit an MEP of a given amplitude, signifying a leftward shift in the input-output relationship of corticospinal neurons to TMS excitation, but would not affect SICI, a paired-pulse TMS measure of synaptic (phasic) rather than extrasynaptic (tonic) inhibition. Furthermore, we expected that S44819 would decrease the N45 amplitude given its previously established GABAAergic nature.

Materials and Methods

Participants

Eighteen healthy male volunteers (mean age ± SD: 27.5 ± 6.0 years; range 21–43) participated in this study after having provided written informed consent. All subjects were strongly right-handed according to the Edinburgh handedness inventory (mean laterality index of handedness ± SD: 87 ± 9; range 75–100; Oldfield, 1971), free of medication and any drug abuse (including alcohol and nicotine), and without any history of neurological or psychiatric diseases. All subjects underwent the Transcranial Magnetic Stimulation Adult Safety Screen (TASS; Keel et al., 2001), followed by a physical examination and a diagnostic EEG to rule out any contraindications against the TMS procedures of this study. At screening, participants were included if the resting motor threshold (RMT) was ≤50% of maximum stimulator output (MSO) and the stimulus intensity (SI) needed to elicit MEPs of, on average, 0.5 mV in peak-to-peak amplitude was ≤70% MSO. Blood alcohol test, drug screening, and urinary cotinine test were performed on the day before each experiment and participants had to have negative results in all tests to be allowed to take part in the study. Experimental procedures conformed to the Declaration of Helsinki and the Federal Institute for Drugs and Medical Devices (Bundesinstitut für Arzneimittel und Medizinprodukte) and the local ethics committee of the Medical Faculty of Eberhard-Karls-University Tübingen approved the study (EudraCT #2014-004681-13).

Experimental design

To evaluate whether the antagonistic action of S44819 on α5-GABAARs detected in preclinical studies *in vitro* is relevant for the modulation of the human primary motor cortex (M1) excitability, a randomized, double-blinded, placebo-controlled, crossover study investigated the effects of a single oral dose of 50 and 100 mg of S44819 on TMS–EMG and TMS–EEG measures of corticospinal and cortical excitability. Participants were assigned to one of the six possible sequences of treatment (three subjects were allocated in each of the six sequences of treatment), which all included three successive treatment periods during which placebo, 50 mg S44819 or 100 mg S44819 was administered. One week separated each of the treatment periods to exclude the possibility of carryover effects between treatment periods according to a S44819 serum half-life of ~7 h in human (Institut de Recherches Internationales Servier, 2014). Drug dosages were chosen based on extrapolation of pharmacokinetic (PK) results in animal models and doses presenting satisfactory clinical safety in a first human phase I study of S44819 (Institut de Recherches Internationales Servier, 2016). To ensure controlled conditions for food and fluids for all participants, they were admitted to our phase I unit on the evening of the day before each treatment period. TMS measurements were performed on next morning starting either at 8:00 A.M. or 10:00 A.M. (always at the same time for a given participant). TMS sessions always followed the same sequence and timing of investigations (Fig. 1): baseline TMS–EMG and TMS–EEG measurements; first PK blood sampling, oral study drug intake; waiting period of 150 min; second PK blood sampling (at +2 h after study drug intake); TMS–EEG and TMS–EMG postdrug measurements; and further PK blood samplings (at +4 h, +6 h, +8 h after study drug intake). The timing of TMS postdrug measurements was based on the PK data from a first human phase I study estimating the maximum systemic levels of S44819 between ~2.5 and 4 h after oral intake (Institut de Recherches Internationales Servier, 2014).

Data recording

TMS. Participants were asked to sit on a comfortable reclining chair and stay awake with eyes open. Monophasic TMS pulses were applied over the hand area of the dominant (left) M1 using two Magstim 200^2 magnetic stimulators connected to a figure-eight coil (outer diameter of each wing, 70 mm) through a BiStim Module (Magstim). The coil was placed tangentially on the scalp with the handle pointing backwards and 45° away from the midline. This way, the direction of the TMS-induced current in the brain was from lateral–posterior to medial–anterior, leading to largely transsynaptic excitation of corticospinal cells through horizontal corticocortical connections (Di Lazzaro et al., 2008). The hand representation of the left M1 was determined and marked with a pen on the scalp as the coil position, where TMS at a marginally suprathreshold stimulus intensity consistently resulted in largest MEPs in the right first dorsal interosseus (FDI) muscle. MEPs were recorded by using surface EMG with Ag-AgCl cup electrodes in a belly–tendon arrangement. EMG data were recorded by spike2 software (Cambridge Electronic Design), the raw signal was amplified (Digitimer D360 8-channel amplifier), band-pass filtered (20 Hz to 2 kHz), and digitized at an A/D rate of 5 kHz (CED Micro 1401; Cambridge Electronic Design). Single-pulse TMS was used to determine RMT, active motor threshold (AMT), and stimulus

U.Z. received personal fees from Biogen Idec GmbH, Bayer Vital GmbH, Bristol Myers Squibb GmbH, CorTec GmbH, Medtronic GmbH and grants from Biogen Idec GmbH and Janssen Pharmaceuticals NV outside of the submitted work. The remaining authors declare no competing financial interests.

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DOI:10.1523/JNEUROSCI.1689-16.2016

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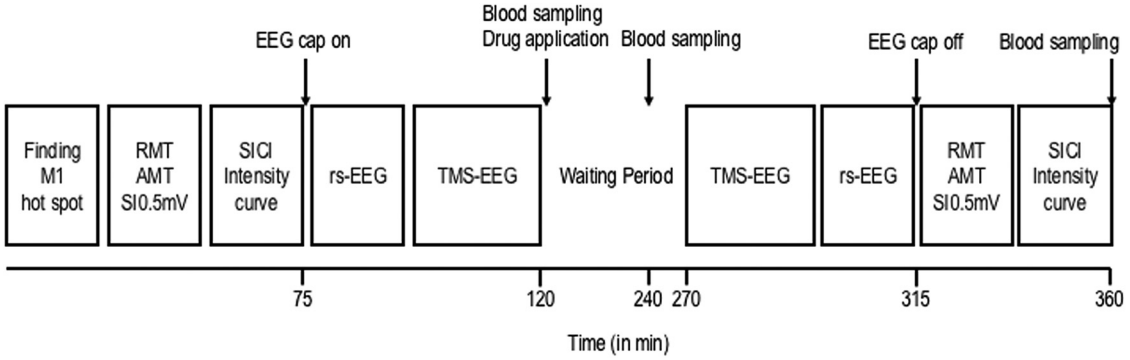

Figure 1. Timeline of experiments. TMS–EMG and TMS–EEG measures were obtained immediately before and 150 min after study drug intake (100 mg S44819, 50 mg S44819, or placebo).

intensity needed to elicit a motor evoked potential of 0.5 mV amplitude (SI0.5mV). RMT was defined as the lowest stimulus intensity eliciting a MEP of ≥50 μV in peak-to-peak amplitude in at least five of 10 successive trials (Groppa et al., 2012). AMT was determined in the slightly voluntarily contracting FDI muscle (~10% of maximum voluntary contraction monitored by audiovisual feedback of the EMG signal) as the lowest stimulus intensity resulting in a MEP of >100 μV in peak-to-peak amplitude in at least five of 10 successive trials. SI0.5mV was determined as the stimulus intensity required for MEPs of, on average, 0.5 mV in peak-to peak amplitude in the voluntarily relaxed FDI. SICI was tested by paired-pulse TMS. The SICI paradigm involved pairing of a conditioning stimulus (CS) followed by a test stimulus (TS) at a short interstimulus interval of 2.0 ms to avoid possible contamination by short-interval intracortical facilitation (Peurala et al., 2008). A SICI intensity curve was obtained with CS intensities ranging from 50% AMT to 120% AMT in steps of 10% AMT (i.e., 8 different CS intensities) and TS intensity of SI0.5mV. TS intensity was adjusted to maintain a test MEP amplitude of, on average, 0.5 mV in the postdrug SICI measurements. CS/TS and TS alone conditions were repeated 10 times each in randomized order in a block of 90 trials. The intertrial interval varied randomly between 4 and 8 s to limit anticipation of the next trial. SICI datasets from 5/18 subjects had to be discarded from analysis because of incomplete voluntary relaxation of the FDI or too small test MEP amplitudes (<200 μV). Both factors can lead to a nonspecific (drug-unrelated) reduction of SICI (Ridding et al., 1995; Sanger et al., 2001).

EEG recordings. To evaluate TEPs, EEG was recorded parallel with the EMG recordings. EEG signals were acquired through TMS-compatible EEG equipment (BrainAmp DC; Brain Products) using a 64-channel EEG cap (BrainCap-Fast'n Easy; Brain Products). FCz and AFz served as the active reference and ground electrodes, respectively. To monitor eye movement artifacts and blinks, two more electrodes were placed outside of the outer canthus and over the right eye. Electrode impedances were maintained at <5kΩ throughout the experiment. EEG signals were recorded via BrainVision Recorder software version 1.20 (BrainProducts) with a resolution of 0.5 μV/bit, a low-pass filter of 1 kHz, and a sampling rate of 5 kHz. During the TMS–EEG recordings, white noise was applied through ear phones to mask the TMS click and to avoid TMS-evoked auditory potentials (Massimini et al., 2005; Casarotto et al., 2010). A total of 130 TMS pulses were applied at baseline and 150 min after drug intake over the FDI hotspot of the left M1 at 100% RMT as determined at baseline; that is, no adjustment of stimulus intensity was made in the postdrug TMS–EEG measurements. Because there were no drug effects on RMT (cf. Fig. 3), the observed drug effects on TEPs (see below) cannot be accounted for by changes motor threshold. MEPs were monitored visually during TMS–EEG recording. No or only miniature MEPs were elicited. This ensured that the somatosensory afferent signals caused by muscle twitches were absent and therefore did not contaminate the TEPs. The intertrial interval varied randomly between 4 and 8 s to limit anticipation of the next trial.

Data analyses

TMS–EMG analysis. EMG data were analyzed via Spike2 software (Cambridge Electronic Design) and MATLAB (R2015a, RRID:SCR_000903; The MathWorks). Peak-to-peak MEP amplitudes were calculated for each trial and averaged per each condition. The SICI intensity curve was obtained by calculating the ratio of mean conditioned MEP (eight different CS intensities: 50%, 60%, 70%, 80%, 90%, 100%, 110%, and 120% AMT) over mean test MEP (SI0.5mV). For assessment of possible drug-induced changes in RMT, AMT, SI0.5mV, and test-MEP, two-way repeated measures ANOVA (rmANOVA) was used, with the main within-subject effects of time (two levels: baseline and postdrug) and drug condition (three levels: placebo, 50 mg S44819, and 100 mg S44819). For SICI, a three-way rmANOVA with the main within-subject effects of time, drug condition, and CS intensity (8 levels: 50–120% AMT) was run. Order effects were assessed by substituting the main effect of drug condition by period (three levels: period 1, period 2, and period 3). In case of significant interactions between time and drug condition (or period), *post hoc* tests were applied to compare effects between the single drug conditions. Differences were considered significant whenever $p < 0.05$.

TMS–EEG analysis. EEG data were processed offline using BrainVision Analyzer software (version 2.0, RRID:SCR_002356; BrainProducts) and the Fieldtrip open source toolbox (www.ru.nl/fcdonders/fieldtrip/, RRID:SCR_004849) running in MATLAB (R2015a; The MathWorks). The EEG raw data were re-referenced to linked mastoid electrodes and downsampled to 1 kHz. All trials were inspected visually to remove artifact-contaminated trials caused by movements, blinks, or TMS-related muscle artifacts. Artifact-free trials were segmented from −500 ms to 500 ms relative to the TMS pulse and then, to remove the electromagnetic TMS artifact, a linear interpolation was applied from −10 ms to 10 ms around each TMS pulse (Thut et al., 2011; Premoli et al., 2014b). Next, epochs were baseline corrected by subtracting the mean amplitude of the channel signal during an interval between −500 ms and −100 ms before the TMS pulse. A digital band-pass filter was then applied (2–80 Hz). Further, a notch filter with a stop band centered at 50 Hz was applied for noise-line correction. Independent component analysis was then used to remove components reflecting TMS-induced muscle activity and TMS artifacts (within the first 50 ms after the TMS pulse) from TEPs based on each participant's data (Rogasch and Fitzgerald, 2013; Rogasch et al., 2014). Averages at each recording channel were calculated across the retained trials (mean ± SEM, 98 ± 4, range 74–118), and finally grand averaged TEPs were computed by averaging per condition (2 levels of time, 3 drug conditions) across all participants. A 45 Hz low-pass filter was applied to smooth the TEP components with latency <200 ms. Five TEP components were considered (P25, N45, P70, N100, and P180) due to their consistent reproducibility upon M1 stimulation as reported in several other studies (Bonato et al., 2006; Lioumis et al., 2009; Ferreri et al., 2011; Premoli et al., 2014b). For their quantitative analysis, five time windows of interest (TOIs) were defined, based on the grand average TEP components: P25 (15–35 ms), N45 (38–60 ms), P70 (63–82 ms), N100 (85–119 ms), and P180 (156–230 ms; Fig. 2; Premoli et al., 2014a; Premoli et al., 2014b). These TOIs were adjusted individually to take into account interindividual variability of TEP peak latencies and peak amplitudes of the TEP components were determined for each participant,

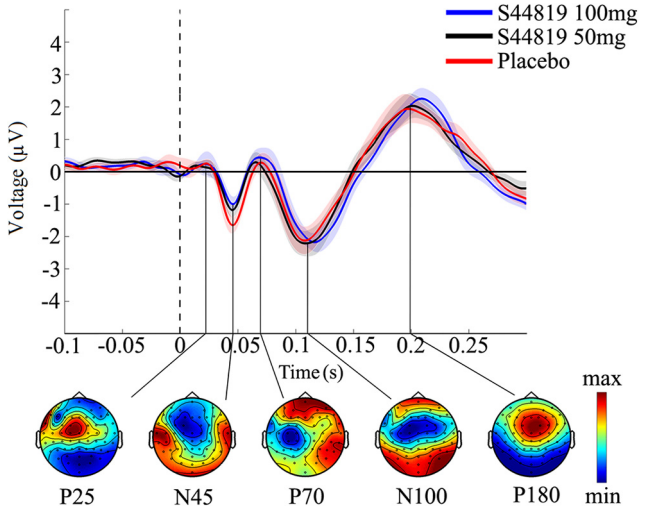

Figure 2. Grand average TEPs before drug intake. TEPs were averaged over all channels and artifact-free trials at baseline in the three different drug conditions (100 mg S44819, 50 mg S44819, and placebo) and labeled based on their polarity (P: positive; N: negative) and approximate latency relative to the time of applying TMS (time 0, vertical dashed line) over the left M1 (P25, N45, P70, N100, and P180). Topographical distributions of surface voltages illustrated in the bottom were grand averaged over the three drug conditions in nonoverlapping TOIs after TMS (P25: 15–35 ms; N45: 38 – 60 ms; P70: 63– 82 ms; N100: 85–119 ms; P180: 156 –230 ms). Color coding of each map was calibrated according to the maximum positivity (red) and negativity (blue) of the separate grand-averaged TEPs.

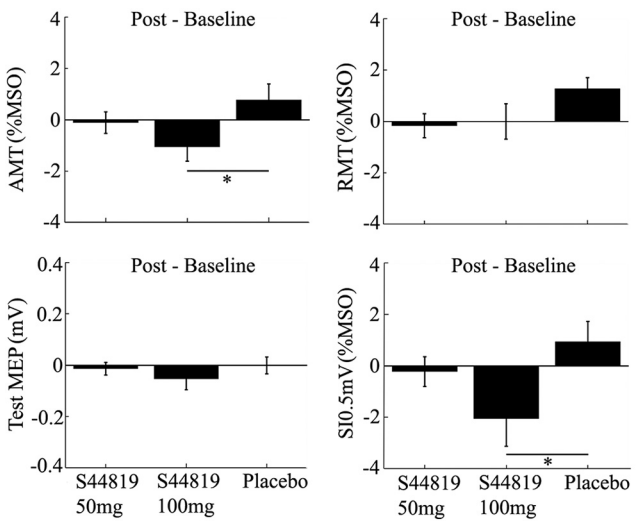

Figure 3. Mean changes (±1 SEM) in RMT, AMT, SI0.5mV, and test-MEP (postdrug − baseline) in the three drug conditions (50 mg S44819, 100 mg S44819, and placebo). The 100 mg S44819 dose decreased AMT and SI0.5mV compared with placebo (*$p < 0.05$).

Table 1. TMS–EMG measures at baseline in the different drug conditions

Measure	100 mg S44819	50 mg S44819	Placebo
RMT (%MSO)	46.00 ± 6.61	45.44 ± 4.88	45.22 ± 5.46
AMT (%MSO)	39.56 ± 5.27	38.94 ± 3.23	38.94 ± 4.29
SI0.5mV (%MSO)	59.50 ± 10.23	58.11 ± 8.37	56.67 ± 9.03
Test-MEP (mV)	0.37 ± 0.08	0.37 ± 0.11	0.34 ± 0.08

time point, and drug condition. To correct for multiple comparisons (i.e., electrodes, time points within TOIs), a cluster-based permutation analysis (Maris and Oostenveld, 2007) was conducted as implemented in FieldTrip (http://fieldtrip.fcdonders.nl/; Litvak et al., 2007; Premoli et al., 2014a; Premoli et al., 2014b). A paired *t* test was applied to compare the postdrug versus baseline data within the same drug condition or postdrug data between different drug conditions for each electrode at each data point within the five different TOIs. *t*-values exceeding an *a priori* threshold of $p < 0.05$ were clustered based on adjacent data points and neighboring electrodes. Cluster-level statistics were calculated by taking the sum of the *t*-values within every cluster. The statistical comparisons were done with respect to the maximum values of summed *t*-values. By means of a permutation test (i.e., randomizing data across postdrug and baseline conditions and rerunning the statistical test 1000 times), a reference distribution of the maximum of summed cluster *t*-values was obtained for evaluating the statistics of the actual data. Clusters in the original dataset were considered to be significant at an α level of 5% if <5% of the permutations used to construct the reference distribution yielded a maximum cluster-level statistic larger than the cluster-level value observed in the actual data.

The data from three participants had to be discarded from analysis due to large artifacts in at least one recording session. Therefore, the presented TEP data and analyses are based on 15 participants.

Analysis of resting-state EEG data postdrug versus predrug. To investigate drug-induced changes of spontaneous oscillations, 3 min periods of eyes-closed resting-state EEG (rs-EEG) data recorded postdrug versus predrug (cf. Fig. 1) were analyzed. Data were preprocessed consistently with the TEP analysis (see above) and then divided into nonoverlapping 2 s time windows. The power spectra of the rs-EEG signal postdrug and

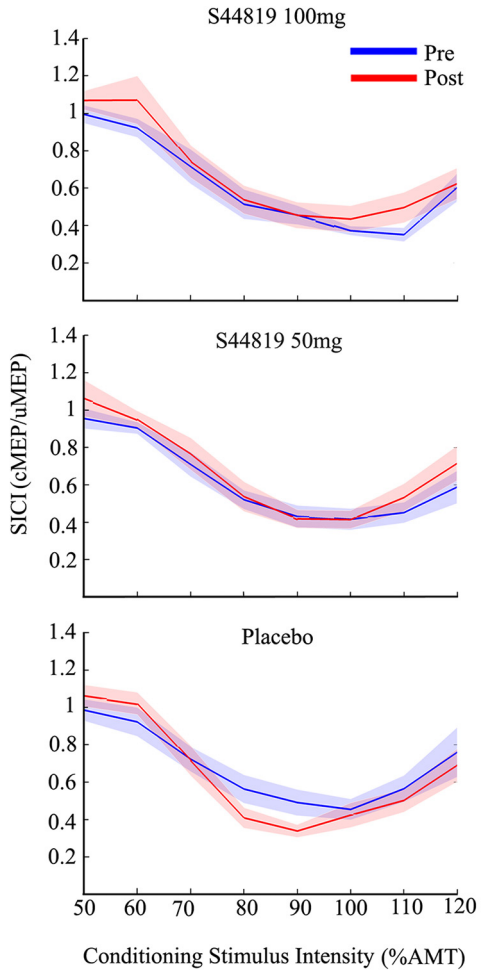

Figure 4. Mean SICI intensity curves (shadings: ±1 SEM) plotted against eight different conditioning stimulus intensities before (blue) and after (red) intake of S44819 (100 mg; top), S44819 (50 mg; middle), and placebo (bottom).

predrug were analyzed in the theta-frequency (4–7 Hz), alpha-frequency (8–12 Hz), and beta-frequency (13–30 Hz) bands by means of Hanning taper with frequency-dependent window length (frequency steps: 1 Hz in the range 4–50 Hz). Then, the same cluster statistical test described previously for TEPs was applied to the topoplots of the rs-EEG power in the three bands (postdrug vs predrug; Maris and Oostenveld, 2007).

Results

S44819 and all study procedures were generally well tolerated. Serious adverse events did not occur.

PK

The mean (±SD) peak serum concentrations were 9.09 ± 1.94 ng/ml (range 6.0–12.6 ng/ml) for 50 mg S44819 and 16.47 ± 4.70 ng/ml (range 10.0–28.9 ng/ml) for 100 mg S44819. A model-based PK approach was used to assess individual and mean PK parameters. Mean time to peak concentration (Fig. 1) was 198 min for 50 mg S44819 and 184 min for 100 mg S44819. These times correspond to 318 min and 314 min, respectively, on the time axis in Figure 1. Therefore, the time to peak concentration was reached, on average, at approximately the end of the postdrug TMS–EEG recordings and immediately before the postdrug TMS–EMG recordings.

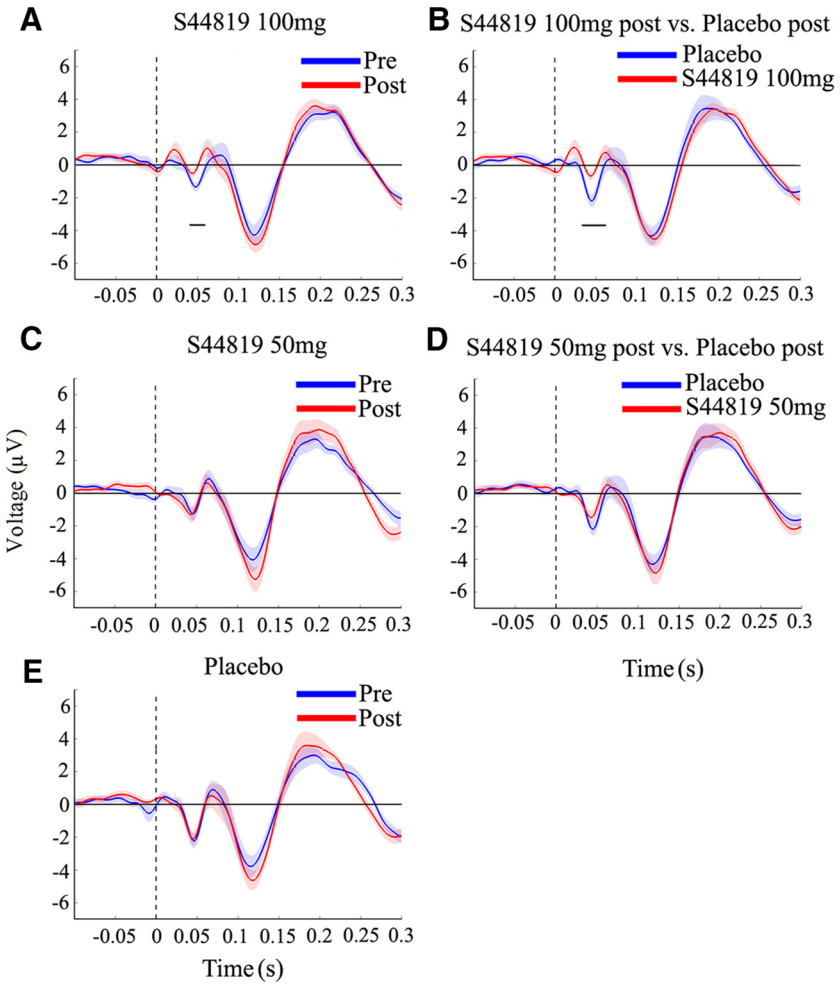

Figure 5. Grand-averaged TEPs (shadings: ±1 SEM) elicited by TMS of left M1 before (blue) versus after (red) intake of S44819 (100 mg; ***A***), S44819 (50 mg; ***C***), and placebo (***E***) or after drug intake comparing S44819 (100 mg; red) versus placebo (blue; ***B***) and S44819 (50 mg; red) versus placebo (blue; bottom; ***D***). The 100 mg S44819 dose decreased specifically the N45 TEP component compared with the baseline measurement (***A***) and compared with postdrug placebo (***B***), whereas there were no changes in other drug conditions and/or TEP components. Horizontal black bars underneath the N45 denote the significant periods of drug-induced changes. Data are grand averages of those channels that showed a significant difference in the N45 TEP component in the 100 mg S44819 postdrug versus baseline (***A***) or between the postdrug 100 mg S44819 and placebo conditions (***B***). These channels are indicated in Figure 6, *A* and *B*, respectively.

Drug effects on RMT, AMT, SI0.5mV, and unconditioned test-MEP amplitude

RMT, AMT, SI0.5mV, and unconditioned test-MEP amplitude in the SICI measurements were not different at baseline between drug conditions (Table 1, all $p > 0.05$). This ensured that baseline differences could not account for any of the drug effects (see below). Drug effects on RMT, AMT, SI0.5mV, and unconditioned test-MEP amplitude (postdrug − baseline) are summarized in Figure 3. Two-way rmANOVA with the main effects of time (two levels: baseline and postdrug) and drug condition (three levels: placebo, 50 mg S44819, and 100 mg S44819) did not reveal any significant main effects or an interaction of time and drug condition for RMT and test-MEP. In contrast, AMT and SI0.5mV showed a significant interaction between time and drug condition (AMT: $F_{(2,34)} = 3.361$, $p = 0.047$; SI0.5mV: $F_{(2,34)} = 3.526$, $p = 0.041$). *Post hoc* testing revealed that these effects were explained by a significant decrease in AMT and SI0.5mV in the 100 mg S44819 condition compared with the placebo condition (AMT: $p = 0.004$; SI0.5mV: $p = 0.022$; Fig. 3). None of the other *post hoc* comparisons between drug conditions was significant. Two-way rmANOVAs with the main effects of time (two levels: baseline and post drug) and period (period 1, period 2, and period 3) did not reveal any significant main effect or interaction of time and period for RMT, AMT, SI0.5mV, or unconditioned test-MEP, signifying that there were no order effects.

Drug effects on SICI

The three-way rmANOVA with main effects of time (two levels: baseline and postdrug), CS intensity (eight levels: 50% to 120% AMT), and drug condition (three levels: placebo, 50 mg S44819, and 100 mg S44819) did not reveal any significant interaction between time and drug conditions ($F_{(2,24)} = 1.538$, $p = 0.235$), or time, drug condition, and CS intensity ($F_{(5.48,65.73)} = 0.698$, $p = 0.640$; Fig. 4). Similarly, the three-way rmANOVA with the main effects of time, CS intensity, and period (three levels: period 1, period 2, and period 3) did not show significant interactions of time with period, or time, period, and CS intensity, excluding an order effect.

Drug effects on TEPs

Figure 2 illustrates the grand average TEPs of 15 participants at baseline in each of the three drug conditions (placebo, 50 mg S44819, and 100 mg S44819). Five typical TEP components were identified (P25, N45, P70, N100, and P180) and are shown with their topographical surface voltages. TEPs were highly reproducible and there were no differences at baseline between the three drug conditions.

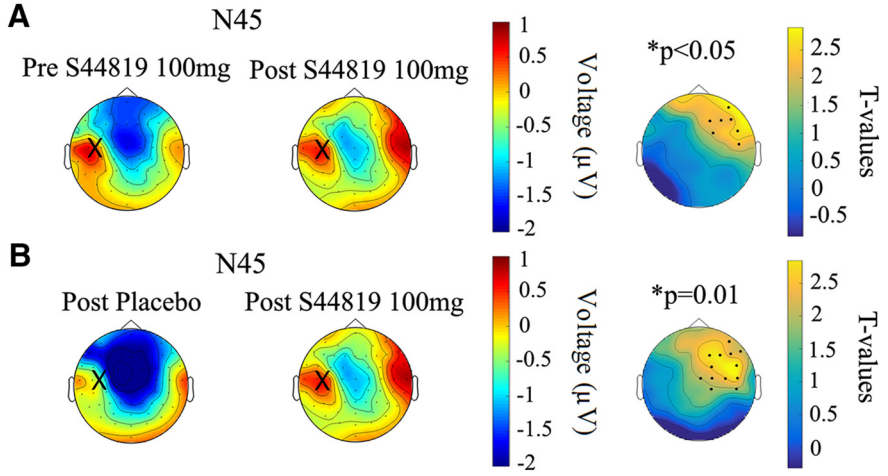

Figure 6. Topographical surface voltage plots of the N45 TEP component. ***A***, Topoplots before (left column) and after (middle column) intake of 100 mg S44819 and *t*-statistic map (right column) of the postdrug versus baseline differences (postdrug − baseline 100 mg S44819). ***B***, Topoplots of the N45 TEP component after intake of placebo (right column) and S44819 (100 mg; middle column), and *t*-statistic map (right column) of the postdrug 100 mg S44819 versus post placebo differences (postdrug 100 mg S44819 − post placebo). Large crosses on the left hemispheres indicate the site of TMS over left M1. Yellow color on the *t*-statistic maps represents a decrease of N45 negativity. Black dots on the *t*-statistics maps represent the channels showing a significant difference. Note that these channels are located in the nonstimulated right hemisphere in the region where the N45 is predominantly expressed.

The 100 mg S44819 dose suppressed the N45 over the period of 43–61 ms after stimulus (postdrug: -0.52 ± 0.27 μV; baseline: -1.34 ± 0.27 μV; $p < 0.05$; Fig. 5*A*). There was also a significant reduction in the N45 in the postdrug 100 mg S44819 condition versus postdrug placebo condition over the period 33–55 ms after stimulus (postdrug 100 mg S44819: -0.64 ± 0.27 μV; postdrug placebo: -2.17 ± 0.38 μV; $p = 0.01$; Fig. 5*B*). In contrast, there were no significant differences in postdrug versus baseline in the placebo (Fig. 5*E*) or 50 mg S44819 conditions (Fig. 5*C*) or the postdrug 50 mg S44819 versus postdrug placebo conditions (Fig. 5*D*) in any of the TOIs (all $p > 0.05$).

Figure 6 shows the topological distribution of changes in EEG surface voltage in the N45 component caused by the study drug (Fig. 6*A*: postdrug vs baseline 100 mg S44819; Fig. 6*B*: postdrug 100 mg S44819 vs postdrug placebo). Cluster-based analyses revealed that this significant depression of N45 amplitude occurred largely in the nonstimulated right frontal and central cortex (postdrug vs baseline 100 mg S44819: $p < 0.05$, significant channels: FC2, FC6, F2, F4, F6, C6, and AF8; postdrug 100 mg S44819 vs postdrug placebo: $p = 0.01$, significant channels: FC2, FC6, F2, F4, F6, F8, Cz, C2, C4, C6, CP2, CP6, and AF8); that is, in a region where the N45 is typically expressed (cf. Fig. 2 and left column of Fig. 6). Finally, the individual N45 data are displayed in Figure 7 to demonstrate the consistency of the suppressive effect by 100 mg S44819 across subjects. In summary, results suggest a highly specific effect of S44819; that is, a selective depression of the N45 TEP component at the dose of 100 mg S44819.

Linear regression analyses were performed to determine whether the depression in N45 amplitude (postdrug − baseline 100 mg S44819) correlated with the TMS–EMG changes observed for AMT and SI0.5mV. None of these correlations showed significance (all $p > 0.05$).

Finally, peak 100 mg S44819 serum concentration correlated with the change in N45 amplitude (postdrug − baseline 100 mg S44819; Fig. 8, Pearson correlation coefficient $r = 0.066$, $p = 0.015$), but not with the changes in AMT or SI0.5mV (all $p > 0.05$).

Drug effects on rs-EEG power

The cluster-based analysis did not reveal any significant effect of any of the drugs on rs-EEG power in any of the frequency bands (theta-, alpha-, and beta-frequency bands; all $p > 0.05$).

Discussion

Significant effects of the specific α5-GABAAR antagonist S44819 on excitability of the human cortex could be demonstrated by TMS–EMG and TMS–EEG assessments. Main findings were decreases in AMT and SI0.5mV and a reduction specifically of the N45 TEP component of the TMS-induced EEG response, whereas SICI and other TEP components remained unaffected. In the following paragraphs, these findings will be discussed and interpreted.

Dose effects

All significant effects of S44819 on TMS–EMG and TMS–EEG measures of cortical excitability were observed only with the 100 mg dose, but not with the 50 mg dose. The presence of significant effects strongly suggests that a single oral dose of 100 mg S44819 reaches the human brain to a relevant extent. All observed effects are consistent with the notion that 100 mg S44819 increased cortical excitability via reduction of tonic inhibition. Importantly, the decrease in N45 amplitude correlated directly with the peak serum concentration of 100 mg S44819, suggesting that the modulation of N45 can be used as a direct marker of S44819 action, most likely on α5-GABAARs, in the human brain.

SICI

S44819 did not change SICI. Several factors likely account for this nil finding. SICI has been established as a marker for GABAAR-mediated inhibition (for review, see Ziemann et al., 2015). Benzodiazepines, allosteric positive modulators of GABAARs containing α1-, α2-, α3-, or α5-subunits, consistently increased SICI (Ziemann et al., 1996a; Di Lazzaro et al., 2000; Ilić et al., 2002; Di Lazzaro et al., 2005; Di Lazzaro et al., 2006; Di Lazzaro et al., 2007; Müller-Dahlhaus et al., 2008; Teo et al., 2009). There have been two important specifications to these findings. First, zolpidem, a benzodiazepine-like hypnotic with largely specific positive modulation of the α1-GABAAR, had no effect on SICI (Di Lazzaro et al., 2007; Teo et al., 2009). This suggested that SICI represents inhibition mediated by GABAARs other than the α1-GABAAR (i.e., the α2-, α3-, and/or α5-subtypes of the GABAAR). Second, the benzodiazepine antagonist flumazenil did not change SICI (Jung et al., 2004). This provided evidence that there is normally no significant endogenous activity at the benzodiazepine GABAAR-binding site in the human M1.

Furthermore, cortical polarization by anodal or cathodal transcranial direct current stimulation (tDCS) did not affect SICI (Nitsche et al., 2005). This is a very important nil finding because pharmacological blockade of the α5-GABAAR would be expected to shift the resting membrane potential toward depolarization (similar to anodal tDCS; and see below on the effects of S44819 on AMT and SI0.5mV). In contrast to the synaptic α1-, α2-, and α3-GABAARs, the α5-GABAAR is localized extrasyn-

aptically (Brünig et al., 2002; Farrant and Nusser, 2005) and is involved in tonic inhibition that decreases pyramidal neuron excitability (Mitchell and Silver, 2003; Caraiscos et al., 2004). Because neither intervention (tDCS or S44819) affected SICI, the most straightforward conclusion is that SICI measures specifically synaptic, but not extrasynaptic, GABAAergic inhibition in human M1. This conclusion is in full accordance with the currently accepted notion that the subthreshold CS of the SICI paired-pulse protocol excites low-threshold inhibitory interneurons that synapse via GABAARs onto pyramidal neurons that are then less excitable to the succeeding suprathreshold test stimulus (Ilić et al., 2002; Di Lazzaro and Ziemann, 2013).

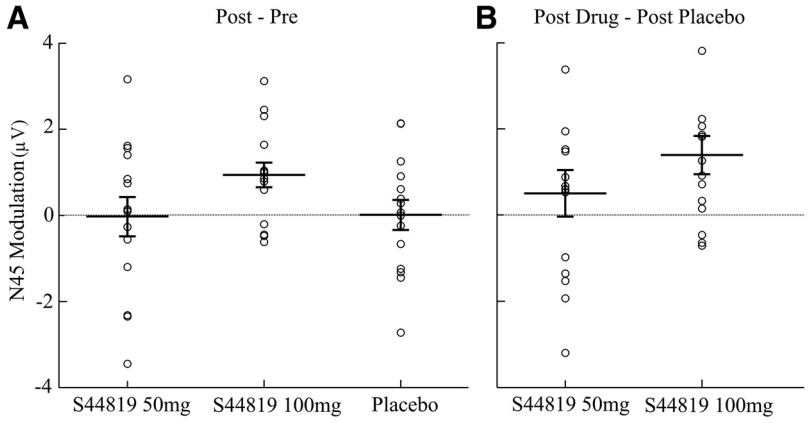

Figure 7. Drug-induced modulation of the N45 TEP component (single-subject data). Scatter plots of individual amplitude modulations (***A***: postdrug − baseline; ***B***: postdrug − postplacebo) of the N45 for all conditions are illustrated. In all conditions, data were extracted from EEG channels showing a significant difference for postdrug 100 mg S44819 − baseline S44819 (100 mg; ***A***), or postdrug 100 mg S44819 − postplacebo (***B***). Error bars indicate the group mean ±1 SEM.

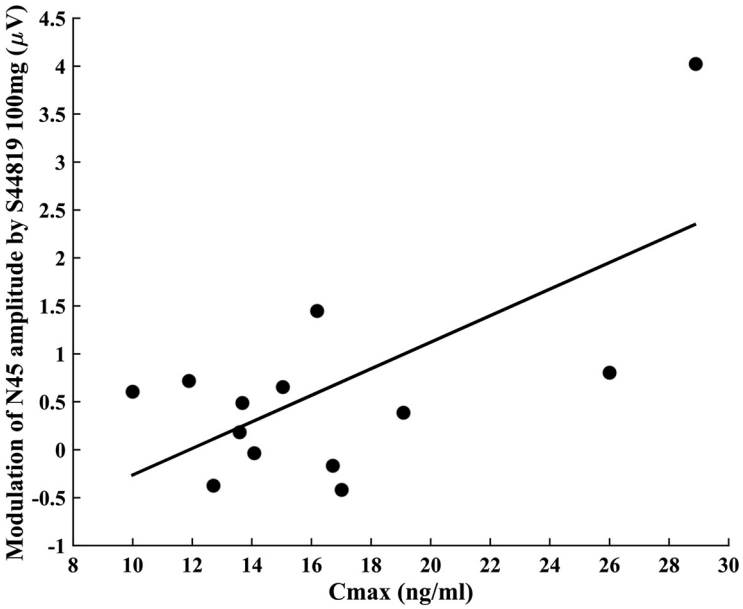

Figure 8. Correlation between the peak 100 mg S44819 serum concentration and the change in N45 amplitude (postdrug 100 mg S44819 − baseline). Pearson correlation coefficient $r = 0.66$, $p = 0.015$. The data from only 13/15 subjects were subjected to this correlation analysis because two subjects had no identifiable N45 potential.

Single pulse TMS–EMG measures (RMT, AMT, SI0.5mV, test MEP)

The 100 mg S44819 dose reduced AMT and SI0.5mV. This suggests that blockade of extrasynaptic α5-GABAARs increases the excitability of corticospinal neurons to single-pulse TMS. This is plausible because the blockade of extrasynaptic α5-GABAARs by S44819 will result in a less hyperpolarized (i.e., more depolarized) state of pyramidal neurons in M1. Basic experiments showed that an increase of tonic inhibition shifts the input–output relationship of single cells to the right; that is, the probability of action potential generation to a given excitatory input is decreased (Mitchell and Silver, 2003). At the systems level, depolarization, probably of the somatic region of corticospinal cells, by anodal tDCS over M1 increases MEP amplitude (Nitsche and Paulus, 2000, 2001; Nitsche et al., 2005). However, RMT and AMT remain unchanged after anodal tDCS (Nitsche et al., 2005). In addition, an increase of ambient GABA in the extracellular space by vigabatrin, an irreversible inhibitor of the GABA transaminase, or by tiagabine, a GABA-transporter-blocking GABA reuptake inhibitor, had no effect on motor thresholds or MEP amplitude (Ziemann et al., 1996b; Werhahn et al., 1999). However, the change of GABA concentration at extrasynaptic sites by these drugs may be too small to drive changes in excitability of corticospinal neurons to single-pulse TMS. Consistent with the current findings, some, but not all, of the previous pharmacological TMS–EMG studies that tested the effects of benzodiazepines reported an increase in motor threshold (Ilić et al., 2002) or a decrease in MEP amplitude (Di Lazzaro et al., 2000; Boroojerdi et al., 2001). These effects may be explained by the positive modulation of benzodiazepines at the extrasynaptic α5-GABAAR. S44819 did not affect RMT, but there was a nonsignificant trend for 100 mg S44819 to reduce RMT compared with placebo ($p = 0.074$, cf. Fig. 3). There were no significant drug effects on the unconditioned test-MEP amplitude because test stimulus intensity was adjusted, whenever necessary, to maintain a test MEP amplitude of, on average 0.5 mV, in the postdrug paired-pulse SICI measurements.

TMS–EEG measures of TEPs

The 100 mg S44819 dose reduced the N45 amplitude and the amount of N45 amplitude reduction was correlated directly with the peak serum concentration of 100 mg S44819. The effect was expressed in the frontal and central region of the nonstimulated right hemisphere, where the N45 potential is predominantly localized (Komssi et al., 2004; Bonato et al., 2006; Litvak et al., 2007; Rogasch et al., 2013; Premoli et al., 2014a; Premoli et al., 2014b). In addition, the effect was specific because none of the other TEP amplitudes (P25, P70, N100, or P180) was altered by S44819. Furthermore, the absence of drug effects on rs-EEG power suggests that the observed reduction of N45 amplitude by 100 mg S44819 cannot be explained by concomitant alterations in rs-EEG power. The reduction of the N45 amplitude by S44819 is

opposite to the significant increase observed in our previous studies by single doses of the benzodiazepines alprazolam and diazepam and the hypnotic zolpidem, a largely selective positive modulator at the α1-GABAAR (Premoli et al., 2014a; Premoli et al., 2014b). Because alprazolam, diazepam, and zolpidem exhibit a common receptor affinity profile targeting the α1-subunit of the GABAAR, these data strongly suggested that activation of α1-subunit-containing GABAARs contributes to the generation of the N45 potential. The current findings extend this notion by providing evidence that the α5-GABAAR also contributes to the generation of the N45 potential.

The lack of an effect of S44819 on the N100 TEP component may be explained by the fact that activation of GABABRs, but not GABAARs, contributes to its generation at the site of its predominant expression in the stimulated M1, as indicated by the significant increase of the N100 amplitude by baclofen, a selective GABABR agonist (Premoli et al., 2014b). Furthermore, we had observed a decrease of the N100 amplitude in our previous studies by alprazolam and diazepam, but not zolpidem (Premoli et al., 2014b). The current findings suggest that the transcallosal and/or corticothalamo-cortical interactions, which are probably responsible for the modulation of the N100 amplitude in the nonstimulated hemisphere by diazepam and alprazolam, depend on activation of α2- and/or α3-GABAARs, but not α1- (zolpidem) or α5-GABAARs (S44819).

Two limitations of this study should be noted: behavioral data have not been obtained because this study was designed to test primarily the effects of S44819 on cortical and corticospinal excitability as measured with TMS–EEG and TMS–EMG. However, investigation of behavioral measures, in particular on memory and learning processes that are expected to be influenced by S44819, and the relation of the currently obtained electrophysiological measures with behavior would be of interest in future studies. Furthermore, implementation of navigated TMS would be advantageous to optimize test–retest reliability in future pharmaco-TMS–EEG studies (Casarotto et al., 2010).

Conclusions

The present data provide evidence that the selective α5-GABAAR antagonist S44819 reached human cortex at a sufficient concentration to impose an increase in corticospinal and cortical excitability, as indexed by a decrease in motor threshold measured by single-pulse TMS–EMG and a decrease of the amplitude of the N45 component of the TMS–EEG response. These data warrant the further development of S44819 in a human clinical trial to test its efficacy in enhancing recovery of function after ischemic stroke, in which tonic inhibition mediated by α5-GABAARs is abnormally increased in the peri-infarct zone.

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Appendix 2:

Darmani, G., Bergmann, T.O., Zipser, C.M., Baur, D, Müller-Dahlhaus, F., Ziemann, U., (2018) Effects of antiepileptic drugs on cortical excitability in humans. A TMS-EMG and TMS-EEG study. Under revision in Human Brain Mapping (September 2018)

Effects of antiepileptic drugs on cortical excitability in humans. A TMS-EMG and TMS-EEG study

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Abstract

Brain responses to transcranial magnetic stimulation (TMS) recorded by electroencephalography (EEG) are emergent non-invasive markers of neuronal excitability and effective connectivity in humans. However, the underlying physiology of these TMS-evoked EEG potentials (TEPs) is still heavily underexplored, impeding a broad application of TEPs to study pathology in neuropsychiatric disorders. Here we tested the effects of a single oral dose of three antiepileptic drugs with specific modes of action (carbamazepine, a voltage-gated sodium channel (VGSC) blocker; brivaracetam, a ligand to the presynaptic vesicle protein VSA2; tiagabine, a gamma-aminobutyric acid (GABA) re-uptake inhibitor) on TEP amplitudes in 15 healthy adults in a double-blinded randomized placebo-controlled crossover design. We found that carbamazepine decreased the P25 and P180 TEP components, and brivaracetam the N100 amplitude in the non-stimulated hemisphere, while tiagabine had no effect. Findings corroborate the view that the P25 represents axonal excitability of the corticospinal system, the N100 in the non-stimulated hemisphere propagated activity suppressed by inhibition of presynaptic neurotransmitter release, and the P180 late activity particularly sensitive to VGSC blockade. Pharmaco-physiological characterization of TEPs will facilitate utilization of TMS-EEG in neuropsychiatric disorders with altered excitability and/or network connectivity.

Keywords

Brivaracetam; carbamazepine; electroencephalography; excitability; human cortex; tiagabine; TMS-evoked EEG response; transcranial magnetic stimulation

Introduction

Many neuropsychiatric disorders are caused by or associated with abnormal neuronal excitability and/or network connectivity. However, it is still difficult to measure these abnormalities non-invasively. Transcranial magnetic stimulation (TMS) evoked electroencephalographic (EEG) potentials (TEPs) provide a relatively novel technique to test excitability and connectivity of the human brain (Chung, et al., 2015; Ilmoniemi and Kicic, 2010; Ilmoniemi, et al., 1997; Rogasch and Fitzgerald, 2013; Ziemann, 2011). However, the physiological mechanisms underlying TEPs remain heavily underexplored impeding their clinical application, even though several studies have used them to study a variety of patients, for instance with epilepsy (Julkunen, et al., 2013; Kimiskidis, et al., 2017; Shafi, et al., 2015; Ter Braack, et al., 2016; Valentin, et al., 2008), traumatic brain injury (Bashir, et al., 2012), multiple sclerosis (Zipser, et al., 2018), stroke (Pellicciari, et al., 2018), Alzheimer's disease (Ferreri, et al., 2016), or depression (Sun, et al., 2016).

When TMS is applied to the primary motor cortex (M1) then a typical sequence of TEPs can be recorded that are named according to their relative polarity and latency (in ms): P25, N45, P70, N100, and P180 (Bonato, et al., 2006; Lioumis, et al., 2009). One way to characterize the physiology of these TEPs is to test their changes in healthy subjects under challenge with central nervous system active drugs, which have specific modes action (Ziemann, et al., 2015b). Neurotransmission through the gamma-butyric acid type A (GABAA) receptor contributes to the N45 potential because positive modulators at the GABAA receptor, such as benzodiazepines and zolpidem, and the SV2A ligand levetiracetam increased the N45 potential amplitude (Premoli, et al., 2017a; Premoli, et al., 2014a; Premoli, et al., 2017b), whereas S44819, a specific antagonist of the alpha-5 subunit bearing subtype of the GABAA receptor decreased it (Darmani, et al., 2016). Moreover, neurotransmission through the GABAB receptor contributes to the N100 potential because baclofen, a specific agonist at the GABAB receptor, increased the N100 potential amplitude at the site of stimulation (Premoli, et al., 2014a). Positive modulators at the GABAA receptor resulted in a decrease of the N100 potential in frontal areas of the non-stimulated hemisphere, suggesting that propagation of neuronal activity into areas remote from the stimulation site is under the control of neurotransmission

through the GABAA receptor (Ferrarelli, et al., 2010; Premoli, et al., 2014a). Finally, late TEP components, such as the P180 may be controlled by axonal excitability, as the voltage-gated sodium channel (VGSC) blocker lamotrigine resulted in a depression of the P180 amplitude (Premoli, et al., 2017b).

Here we extend previous findings by studying the effects of carbamazepine (CBZ), brivaracetam (BRV) and tiagabine (TGB) on cortical excitability and inhibition in healthy human subjects testing both TMS evoked electromyographic (EMG) and TMS-EEG responses. Drugs were chosen because of their common use as antiepileptic drugs and well-defined specific modes of action: CBZ is a VGSC blocker (Macdonald, 1995), BRV decreases neuronal excitability primarily through selective binding to the presynaptic vesicle protein SV2A (Klein, et al., 2018; Klitgaard, et al., 2016), and TGB is a selective GABA reuptake inhibitor (Suzdak and Jansen, 1995). Subjects received a single oral dose of CBZ, BRV, or TGB in a double-blinded, randomized, placebo-controlled crossover design. The study was exploratory but, given the previous pharmaco-TMS-EEG data, we expected suppression of the P180 under CBZ and, possibly, an increase of N45 under BRV, although the detailed modes of action of BRV and levetiracetam are different, with levetiracetam but not BRV showing relevant inhibitory action on glutamatergic neurotransmission through AMPA and NMDA receptors (Lee, et al., 2009; Niespodziany, et al., 2017).
We consider this work important to elucidate the physiological underpinnings of TEPs, potentially to use them in the future as biomarkers to inform on specific abnormalities in excitability and/or connectivity of human cortex.

Materials and Methods

Participants

Fifteen right-handed healthy male volunteers (mean age = 28 years, SD = 2.6 years, age range: 22 – 33 years) were recruited to participate in the study. Right-handedness was confirmed using the Edinburgh handedness inventory (laterality score ≥ 75%, (Oldfield, 1971)). All participants gave written informed consent prior to study enrolment. Participants underwent a physical and neurological examination followed by a structured clinical interview to exclude subjects with conditions that would predispose them to potential adverse effects related to TMS, MRI, or any of the study drugs (Rossini, et al., 2015). The general exclusion criteria included 1) drug or alcohol abuse, 2) any history of neurological or psychiatric diseases, 3) a history of cardiac, hematopoietic, liver and/or kidney disease, 4) current use of CNS active drugs, 5) a family history of epilepsy, and 6) contraindications to the study medications (CBZ, BRV, and TGB). To screen for atrioventricular block, a 12-channel ECG was performed at the inclusion visit. The experiments were conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of the Medical Faculty of Eberhard-Karls-University Tübingen (protocol 026/2016BO1).

Experimental design

The study followed a double-blinded, placebo-controlled crossover design, measuring motor-evoked potentials (MEPs) and TMS-evoked EEG potentials (TEPs) in response to single- and paired-pulse TMS of the left M1 hand area before (pre) and after (post) administration of either of the three study drugs or placebo. Subjects participated in four experimental sessions in pseudo-randomized order, balanced across subjects, and separated by at least one week to avoid carry-over effects from the previous session. Study drugs were: 1) CBZ, a VGSC blocker (Macdonald, 1995), 2) BRV, a specific ligand to the presynaptic vesicle protein SV2A with high affinity and selectivity (Klein, et al., 2018; Klitgaard, et al., 2016), and 3) TGB, a selective GABA re-uptake inhibitor (Suzdak and Jansen, 1995). The timeline of an experimental session is illustrated in **Figure 1**. Resting motor threshold (RMT), the TMS intensity to elicit MEPs of 1mV peak-to-peak amplitude (SI1mV), short-interval intracortical inhibition (SICI) intensity curves, MEP input-output curves, resting state EEG

(rs-EEG), and TMS-evoked EEG potentials (TEPs) were measured prior to study drug intake (pre-drug measurements) and again following a defined waiting period after study drug intake (post-drug measurements).

Drug dosages were chosen based on effective standard daily doses in the (chronic) treatment of epilepsy patients and according to previous TMS-EMG reports (Sommer, et al., 2012; Werhahn, et al., 1999b; Ziemann, et al., 1996a; Ziemann, et al., 2015b). Single dosages of either CBZ 600 mg (Carbamazepine AbZ®, AbZ-Pharma GmbH), BRV 100 mg (Briviact®, UCB Pharma SA), TGB 15 mg (Gabitril®, Cephalon UK Ltd.) or placebo (P-Tabletten Lichtenstein, Winthrop) were administered (**Table 1**). A common waiting period of 150 min was chosen based upon the drugs individual t_{max}, and upon TMS studies that previously demonstrated an effect on cortical excitability and/or GABAergic activity after this waiting period (Werhahn, et al., 1999a; Ziemann, et al., 1996a). Placebo tablets had roughly the same size as CBZ, BRV and TGB, and subjects were asked to close their eyes before tablet intake in order to prevent recognition of the tablet by color.

Table 1: Pharmacokinetics for the study drugs and placebo.

Drug	Dosage	Administration form	Median t_{max} (range)	Median $t_{1/2}$ (range)
CBZ	600 mg	tablet	4.5 (4.0-5.0)	45.0 (25.0-65.0)
BRV	100 mg	tablet	1.0 (0.25- 3.0)	9.0 (N.R.)
TGB	15 mg	tablet	0.75	8.0 (7.0-9-0)
PBO	N.A.	tablet	N.A.	N.A.

CBZ, carbamazepine; BRV, brivaracetam; TGB, tiagabine; PBO, placebo; Median t_{max} indicates time to peak plasma concentrations in hours; Median $t_{1/2}$ indicates median biological half-life (in hours) of the substances as given in the full prescribing information of each medication respectively (resources: US food and drug administration, FDA); N.R., not reported; N.A., not applicable.

Prior to the first session, a high-resolution T1-weighted anatomical magnetic resonance (MR) image (voxel size = 1 × 1 × 1 mm; FoV read = 250, FoV phase = 93.8%, TR = 2300 ms, TE = 4.18 ms, FA = 9.0°) was obtained from each subject using a 3T MRI scanner (Magnetom Prismafit, syngo MR D13D; Siemens) to allow neuronavigation of the TMS coil. In each experimental session, pre-drug TMS measurements were conducted either at 8:00 am or

10:30 am (always at the same time for a given participant) after participants had a light caffeine-free breakfast. One hour after drug intake a cereal bar (without chocolate) was ingested. During the waiting period subjects were located in the waiting area of the lab, where they could be monitored constantly (in order to prevent sleeping and to monitor possible adverse effects). At 1:00 pm or 3:30 pm, respectively, post-drug TMS measurements were started. The total experimental session time was 6:35 h ± 20 min (mean ± SD). In order to control for blood pressure changes during the experimental sessions, blood pressure was measured at the beginning of each session, immediately prior to drug intake, one and two hours post drug intake, and at the end of the session. All 15 subjects participated in 4 experimental sessions, however, only a subset of 12 subjects was able to complete the experimental session after taking TGB. Three subjects experienced adverse effects in the TGB session including dizziness, nausea, vomiting, somnolence, coordination problems, concentration difficulties, confusion and nervousness. In these subjects, the post drug measurements could not be obtained.

Experimental procedures and data recording

Transcranial magnetic stimulation (TMS). Subjects were seated in a comfortable reclining armchair, and watched a fixation point directly in front of them. To reduce head movement, a vacuum pillow was placed around the neck. Monophasic TMS pulses were delivered through a 90-mm figure-of-eight coil using a Magstim 200 stimulator (Magstim Co., Whitland, UK), in case of single-pulse TMS, or two Magstim 200 stimulators connected by a BiStim module, in case of paired-pulse TMS. To ensure reproducibility of the stimulation site across sessions, we used a frameless stereotactic neuronavigation system (Localite GmbH, Sankt Augustin, Germany) to digitize EEG electrode positions and to navigate the TMS coil based on each subject's anatomical MRI scan. To have a comparable EEG cap positioning across sessions, EEG electrode positions were digitalized at the beginning of the first session individually and cap position was kept identical in the following sessions with the help of the stored electrode positions (this is an important step to obtain comparable TEP results for the different sessions). The TMS coil was placed tangentially to the scalp and perpendicular to the central sulcus to optimally excite corticospinal motor neurons in M1 with an induced current direction in the brain from

posterior to anterior (Di Lazzaro, et al., 2008; Mills, et al., 1992). Then the coil position was optimized individually to evoke the largest and most consistent responses (MEPs) in the relaxed abductor pollicis brevis (APB) muscle of the right hand at a marginally suprathreshold stimulus intensity. The coil was maintained at the desired position with the help of a holding arm (Magic Arm, Manfrotto, Cassola, Italy) and coil position was monitored in real-time by the neuronavigation system to ensure targeting consistency.

Resting motor threshold (RMT) was determined as the lowest stimulation intensity (in percent maximum stimulator output [MSO]) evoking MEPs of at least 50 μV peak-to-peak amplitude in at least 5 out of 10 successive trials (Groppa, et al., 2012). SI1mV was determined as the stimulus intensity required to evoke average MEPs of 1mV peak-to-peak amplitude in the relaxed APB muscle. Short interval intracortical inhibition (SICI) was tested by applying paired-pulse TMS and investigating the effect of a first subthreshold (conditioning) stimulus on a second suprathreshold (test) stimulus (Kujirai, et al., 1993). The test stimulus (TS) was delivered at a short interstimulus interval (ISI) of 2.0 ms after the conditioning stimulus (CS); 2.0 ms was selected since maximum SICI typically occurs at this ISI and short-interval intracortical facilitation does not compromise the SICI effect at this particular interval (Peurala, et al., 2008a; Roshan, et al., 2003; Ziemann, et al., 1998). SICI was obtained at six different CS intensities (50%, 60%, 70%, 80%, 90%, and 100% of RMT) with TS intensity set to SI1mV. The amplitude of CS responses was expressed as percentage of the amplitude of the TS responses. Eight trials for each CS intensity condition and sixteen trials for the TS alone condition were delivered in randomized order, i.e., a total of 64 trials. For post-drug measurements, TS intensity was adjusted to ensure that MEPs in response to TS alone still showed a 1mV peak-to-peak amplitude on average, despite a potential drug- or time-related change in corticospinal excitability. This was important to avoid non-specific alteration of SICI related to changes in test MEP amplitude (Sanger, et al., 2001). Subjects were provided with audio-visual feedback of APB muscle activity to assist in maintaining complete muscle relaxation, avoiding any drug-unrelated reduction of SICI by pre-innervation (Ridding, et al., 1995). MEP input-output curves were also acquired in the resting APB muscle. Seven stimulus intensities (90%, 100%, 110%, 120%,

130%, 140%, and 150% of RMT) were tested in randomized order, and eight trials were obtained per stimulus intensity, resulting in a total of 56 single-pulse stimuli. For post-drug MEP input-output curves, stimulus intensity was not adjusted in case of RMT change, i.e., the same absolute stimulus intensities were used for pre- and post-drug measurements. TMS-evoked EEG potentials (TEPs) were measured in response to 200 single TMS pulses, delivered at an intensity of 100% RMT (as determined at baseline), pre- and post-drug intake with a jittered 4-6 s inter-trial interval to reduced anticipation of the next trial. Here, post-drug TEP measurements were repeated with an adjusted stimulus intensity if RMT had changed by more than 2% MSO (**Figure 1**). Auditory white noise masking via in-ear headphones was used to attenuate auditory co-stimulation by the TMS click and prevent contamination of TMS-evoked EEG responses with auditory evoked potentials (Casarotto, et al., 2010; Massimini, et al., 2005).

EMG recordings. MEPs were recorded from the APB muscle using EMG adhesive hydrogel electrodes (Kendall, Covidien) in a bipolar belly-tendon montage and a ground electrode placed on the right wrist. EMG data were recorded (20 Hz – 2 kHz bandpass filter, 50 Hz notch filter, 5 kHz sampling rate) by Spike2 software (Cambridge Electronic Design) and stored for offline analysis.

EEG recordings. Subjects were instructed to fixate a cross, minimize eye blinks, and keep their face and hand muscles relaxed during data recording. EEG was recorded using a 64-channel EEG cap with TMS-compatible sintered Ag/AgCl electrodes (Multitrodes, BrainCap-Fast'n Easy; Brain Products). FCz and AFz served as recording reference and ground electrode, respectively. To minimize TMS-related artifacts during TEP measurements, EEG signals were recorded in DC mode with an anti-aliasing low-pass filter of 1000 Hz, and digitized with a resolution of 0.1 μV/bit at a sampling rate of 5 kHz using BrainVision Recorder software (version 1.20; BrainProducts). Additional electrodes for horizontal and vertical electrooculography were placed at the outer canthus and below of the right eye to monitor eye movement artifacts. The position of each EEG electrode relative to the head was recorded and stored

using the neuronavigation system to allow precise repositioning of the cap for subsequent sessions of the same subject. Electrode impedances were regularly checked and kept below 5 kΩ throughout the experiment. In each experimental session, 5 min of both, eyes-closed and eyes-open resting-state EEG were acquired additional to TEP recordings before and after drug intake to investigate drug-induced changes in spontaneous oscillatory brain activity.

Data analyses

TMS–EMG analysis. EMG data were analyzed blind to experimental conditions using customized MATLAB scripts (R2015a, MathWorks). Data were imported from Spike2 to MATLAB and MEP peak-to-peak amplitudes were determined per trial and averaged across trials per time point (pre, post drug) and drug condition. For assessment of drug-induced changes in RMT and SI1mV, normalized (post/pre) data were computed separately for all drug conditions, and two-sided one-sample t-tests against 1 for each drug condition and two-sided paired t-tests for comparing drug conditions with placebo were conducted. MEP input-output curves were obtained by calculating conditional averages (7 stimulation intensity levels: 90% - 150% RMT in steps of 10% RMT) of the peak-to-peak MEP amplitudes. SICI intensity curves were calculated as the ratio of conditional averages of conditioned MEP (6 CS intensities: 50% - 100% RMT in steps of 10% RMT) over the average test MEP (TS intensity: SI1mV). For MEP input-output and SICI intensity curves, three-way rmANOVAs were conducted separately for each study drug relative to placebo, with the factors TIME (2 levels: pre-drug, post-drug), DRUG (2 levels: drug, placebo), and INTENSITY (7 levels for MEP input-output curves; 6 levels for SICI intensity curves). In addition, a two-way rmANOVA with the factors TIME and INTENSITY was conducted within each drug condition. Post-hoc paired t-tests were applied in case of significant main effects. Significance threshold was set to $p < 0.05$.

TMS-EEG analysis. EEG data were analyzed blind to experimental conditions using MATLAB and the Fieldtrip open source toolbox (www.ru.nl/fcdonders/fieldtrip; (Oostenveld, et al., 2011)), and in accordance with established artifact removal pipelines (Herring, et al., 2015; Rogasch, et al.,

2017). Raw data were initially segmented into longer epochs from 1.5 s before to 1.5 s after the TMS pulse to avoid filter artifacts before later reducing segments to the actual epoch of interest (i.e., from -100 ms to 300 ms after the TMS pulse). Long epochs were baseline corrected by subtracting the average of the interval from -220 ms to -20 ms. The initial TMS pulse artifact (amplifier ringing and step artifact) and subsequent cranial muscle artifacts (resulting from co-stimulation of peripheral motor nerves) were cut and data was cubically interpolated between -2 ms to 12 ms. Then, trials and channels were inspected visually using the summary function of Fieldtrip to remove artifact-contaminated trials. On average, 37± 23 (mean ± SD) trials were removed per block of 200 trials. Subsequently, a 2 Hz 4^{th} order zero-phase (two-pass) Butterworth high-pass filter were applied to suppress low-frequency fluctuations, followed by demeaning and downsampling EEG data to 1 kHz. Note that high-pass filtered and down-sampled data was used for independent component analysis training only (see below). Independent component analysis (FastICA) was applied to the down-sampled data to capture the residual muscle and exponential decay artifacts as well as sharp edges caused by interpolation of the pulse artifact. Artifact components were identified based on the topography and temporal pattern of the TMS-locked responses and in agreement with published procedures (Herring, et al., 2015; Rogasch, et al., 2017). After identifying artifact contaminated components (on average 3 ± 2 (mean ± SD) per subject), the same unmixing matrix was applied to the interpolated EEG signal before high-pass filtering and down-sampling had been applied, and bad components were removed from those data before back-projection into channel space. This procedure prevented filter and down-sample artifacts to contaminate our data. Then, a second round of high-pass filtering (2 Hz), demeaning, downsampling (1 kHz), and ICA was applied to identify and remove other TMS-unrelated artifacts such as eye blinks, eye movements, line noise, and tonic muscle artifacts according to standard criteria (Chaumon, et al., 2015). Again, the unmixing matrix was applied to the signal cleaned by the first ICA before the second round of high-pass filtering and downsampling had been applied, and the selected artefactual components were removed before back-projecting that signal into channel space. Finally, time-locked averages (TEPs) of the remaining artifact-free trials (163 ± 23 (mean ± SD) per participant and

measurement) were computed per measurement time point and drug condition for each channel, and eventually a 2 Hz high-pass filter and a 45 Hz low-pass filter were applied followed by baseline correction from -200 to 0 ms and re-referencing of EEG data to the common average of all EEG channels.

Five TEP components: P25 (time window of interest [TOI]: 15–35 ms), N45 (36–50 ms), P70 (51–85 ms), N100 (86–150 ms), and P180 (151–280 ms) were studied due to their high reproducibility for M1 stimulation, and in accordance with the literature (Bonato, et al., 2006; Darmani, et al., 2016; Lioumis, et al., 2009; Premoli, et al., 2014a; Premoli, et al., 2014b). The specific TOIs were chosen around the respective peaks based on grand-average TEPs. Two-tailed paired t-tests were applied to compare pre- and post-drug TEP peak amplitudes within each drug condition and to test for the interaction between TIME and DRUG, i.e. drug(post-pre)–placebo(post-pre) for each electrode and at each time point, separately for the five TOIs. Non-parametric, cluster-based permutation statistics (Maris and Oostenveld, 2007) were used to control for multiple comparisons across channels and time points within TOIs. Clusters were defined as adjacent time point-channel pairs for which the t-statistic exceeded a threshold of $p < 0.05$. Cluster-level statistics were calculated based on the sum of t-values within each cluster. Monte Carlo p-values were computed based on 10000 random permutations and a value of $p < 0.05$ was used as the cluster-statistical significance threshold for all tests.

Since there were strong drug-induced increases in spontaneous oscillatory power in the post-TGB measurement, which contaminated the TEPs, we used General Eigenvalue Decomposition (GEiD) for extracting and removing the spontaneous oscillatory components from the concatenated pre- and post-drug measurements of the TGB session only (Cohen, 2017). Given that delta and theta frequency bands demonstrated the largest increase, we used the respective peak frequencies for each subject (as determined from the individual Fast Fourier Transform (FFT); see below) to individualize the targeted frequency band for optimal results. Separate GEIDs were calculated for each frequency band. To identify truly endogenous oscillations, time-frequency representation, inter-trial coherence, and power spectra of the components were considered. However, to ensure that spontaneous oscillatory components were removed to the maximal possible degree, we were very conservative with

respect to the components kept, which may have resulted in slight overcorrection and removal of actual TEP components.

Pre-TMS EEG analysis. To further investigate the above mentioned drug-induced changes of spontaneous oscillations, a FFT was performed for the pre-TMS time periods. Data preprocessed for the TEP analysis between -1030 ms and -30 ms before the TMS pulse were analyzed using a Hanning-tapered FFT for frequency bins from of 1 to 45 Hz in steps of 1 Hz, averaged across trials, separately for each channel, measurement time point and drug condition, and eventually as grand-average across all participants. The same cluster statistical test outlined for the TEP analysis was used to test post-drug vs. pre-drug for each drug condition, as well as the interactions between TIME and DRUG, i.e. drug(post-pre)–placebo(post-pre), separately for each frequency band: i.e., delta (1–3 Hz), theta (4–7 Hz), alpha (8–12 Hz), and beta (13–30 Hz).

Resting-state EEG analysis. In addition to the pre-TMS analyses, we also quantified drug-induced changes in spontaneous oscillations for the 5 min eyes-open resting-state EEG. For initial ICA pre-processing, rs-EEG data were segmented into 4 s epochs, and concatenated across all drug conditions and measurements per subject. Data were 2 Hz high-pass filtered, demeaned, and down-sampled (1 kHz), and ICA was conducted to identify eye blinks, eye movements, or muscle/movement artifacts. Similar to the TEP analysis described above, the unmixing matrix was then applied to the original data before high-pass filtering and down-sampling. The artefactual components were removed before back-projection into channel space. Data were visually inspected, and trials contaminated by residual artifact were removed manually. The cleaned rs-EEG data were then re-referenced to the average of all EEG channels. Power spectra were determined via a Hanning-tapered FFT for frequency bins from 1 to 45 Hz in steps of 0.25 Hz, and spectra were averaged across segments and EEG channels. Cluster-based permutation tests were performed as described above separately for delta, theta, alpha and beta frequency bands.

Results

CBZ and BRV had tolerability profiles comparable to placebo. TGB, however, was associated with considerable side effects (see Methods section), causing 3 out of 15 participants to abort the respective session, leading to a reduced sample size of N = 12 for the analyses of this condition.

Drug effects on RMT and SI1mV

Drug effects on RMT and SI1mV (post-drug/pre-drug) are summarized in **Figure 2** and **Table 2**. CBZ increased RMT and SI1mV, both with respect to pre-drug baseline (RMT: $t_{14} = 3.57$, $p = 0.003$; SI1mV: $t_{14} = 4.34$ $p < 0.001$) and compared to placebo-related changes (RMT: $t_{14} = 3.38$, $p = 0.004$; SI1mV: $t_{14} = 1.9$, $p = 0.08$, non-significant). Under BRV, there was a non-significant ($p < 0.1$) RMT increase relative to both pre-drug baseline ($t_{14} = 1.86$, $p = 0.08$) and compared to placebo-related changes ($t_{14} = 1.84$, $p = 0.08$), as well as a significant increase in SI1mV relative to pre-drug baseline ($t_{14} = 2.35$, $p = 0.03$). No significant effects were found for TGB (RMT: all $p > 0.7$; SI1mV: all $p > 0.3$) or placebo (all $p > 0.5$).

Table 2. Mean ± SD for RMT and SI1mV before (pre) and after (post) drug intake for all drug conditions.

	CBZ		BRV		TGB		PBO	
	pre-drug	post-drug	pre-drug	post-drug	pre-drug	post-drug	pre-drug	post-drug
RMT (%MSO)	42±7.2	45±7.9	41.8±7.2	42.5±6.8	43.4±6.7	43.3±6.9	42.4±6.8	42.4±7
SI1mV (%MSO)	56±11.1	60.3±11.6	55.4±9.6	58±11.4	57.1±10.8	58.9±12.3	55.7±11.4	56.2±11.3

CBZ, carbamazepine; BRV, brivaracetam; TGB, tiagabine; PBO, placebo.

Drug effects on MEP input-output curve

Drug effects on MEP input-output curves are illustrated in **Figure 3**. A 2x2x7 three-way rmANOVA with factors TIME (pre vs. post), DRUG (drug vs. placebo), and INTENSITY (90, 100, 110, 120, 130, 140, 150 % RMT) revealed a main effect of DRUG for CBZ ($F_{1,392} = 5.47$, $p = 0.02$), and a TIME x DRUG interaction for BRV ($F_{1,392} = 4.45$, $p = 0.03$). A follow-up within-drug 2x7 two-way rmANOVA for BRV with the factors TIME and INTENSITY did reveal a non-significant ($p < 0.1$) main effect of TIME only ($F_{1,392} = 3.68$, $p = 0.056$),

suggesting a general BRV-induced decrease in corticospinal excitability, irrespective of stimulation intensity. No significant change of MEP input-output curves was found for TGB or placebo. Trivially, all main effects of INTENSITY were highly significant ($p < 0.0001$).

Drug effects on SICI intensity curve

SICI intensity curves per drug condition (TS intensity adjusted for SI1mV changes, CS intensity non-adjusted, see Methods) are provided in **Figure 4**. A 2x2x6 three-way rmANOVA with factors TIME, DRUG, and CS-INTENSITY (50, 60, 70, 80, 90, 100 % RMT) revealed a significant interaction between TIME and DRUG ($F_{1,336} = 5.24$, $p = 0.02$) for CBZ. A follow-up within-drug 2x6 two-way rmANOVA for CBZ with the factors TIME and CS-INTENSITY for the CBZ session, revealed main effects of TIME ($F_{1,168} = 8.95$, $p = 0.003$) but no interaction effect ($p > 0.3$), indicating a general CBZ-induced decrease in SICI, irrespective of CS-INTENSITY. For BRV, the three-way rmANOVA revealed a main effect of DRUG only ($F_{1,336} = 3.9$, $p = 0.04$) but no interaction effect ($p > 0.3$), and the respective two-way rmANOVA for the BRV session showed no significant main effect of DRUG or interaction ($p > 0.3$). No significant change of SICI intensity curves was found for TGB or placebo. As expected, CS-INTENSITY effects were highly significant in all drug conditions ($p < 0.0001$).

Drug effects on TEPs

Cluster-based permutation analysis was used to test for differences between pre-drug TEPs across drug conditions, drug-induced TEP changes within each drug condition (i.e., post-drug – pre-drug; indicated as *vs. baseline* in the following), and drug-induced TEP changes relative to placebo (i.e., the interaction contrast drug(post-pre)-placebo(post-pre); indicated as *vs. placebo* in the following). Because CBZ increased RMT in some subjects, TEP analyses were conducted for the measurements with adjusted and non-adjusted stimulation intensity (see Methods). Note that in the following *increases* and *decreases* of TEP components always refer to a modulation of their amplitude in absolute values (e.g., a decreased N100 means a negative potential of reduced amplitude). Also note that the same TEP component can have opposite signs (direction of deflection) in different channels as a result of the

dipole orientation of the underlying neuronal source and the common average referencing (e.g., the P25 being positive over the stimulated sensorimotor cortex, but negative at contralateral posterior sites, with a reduction in absolute amplitude of that negativity at contralateral posterior sites would still being considered a decrease of the very same potential).

Figure 5 shows the grand average TEPs at pre-drug baseline for all drug conditions. In line with the literature, five classic TEP components (P25, N45, P70, N100, and P180) were identified and selected for further analysis. Importantly, pre-drug TEPs did not differ between drug conditions, demonstrating reliability of TMS-evoked EEG responses in our neuronavigated multi-session TEP measurements. Moreover, there was no significant modulation of the TEP over time in the placebo condition, besides a non-significantly ($p < 0.1$) increased P25 amplitude over the stimulated left sensorimotor cortex ($p = 0.06$), possibly reflecting a general effect of time, that is taken into account by analysis of the interaction contrasts (drug(post-pre)-placebo(post-pre)).

CBZ modulated both early and late TEP components as depicted in **Figure 6** (**Supplementary Table S1** provides the individual channels for each of the significant clusters mentioned below) and in **Figure 8A**. Notably, those changes were independent of the general decrease in corticospinal excitability (as reflected by an increase in RMT and SI1mV in the TMS-EMG measurements), as the following results were obtained with the adjusted stimulation intensity. CBZ suppressed the P25 potential over the stimulated left sensorimotor cortex (vs. placebo: $p = 0.003$; vs. baseline: $p = 0.08$) and the parieto-occipital cortex in the non-stimulated right hemisphere (vs. placebo: $p = 0.0001$; vs. baseline: $p = 0.001$). CBZ also caused a reduction of the P180 potential (vs. placebo: $p = 0.03$; vs. baseline: $p = 0.0005$) and a non-significant ($p < 0.1$) attenuation of the N100 potential (vs. placebo: $p = 0.09$; vs. baseline: $p = 0.09$), both over the non-stimulated right sensorimotor cortex. The N100 potential was also decreased in amplitude over the occipito-parietal cortex (vs. placebo: $p = 0.058$; vs. baseline: $p = 0.03$). Notably, without readjustment of the stimulation intensity, results remained largely identical for the P25 and P180 potential (see **Supplementary Table S1**); merely the non-significant ($p < 0.1$) N100 did not

survive, and instead the N45 potential was increased over the stimulated left sensorimotor cortex (vs. placebo: $p = 0.03$; vs. baseline: $p = 0.09$) and over the non-stimulated right sensorimotor cortex (vs. placebo: $p = 0.0006$; vs. baseline: $p = 0.10$) (as shown in **Supplementary Figure S1**).

BRV only modulated late TEP components as depicted in **Figure 7** and **Supplementary Figure S2** (**Supplementary Table S2** provides the individual channels for each of the significant clusters mentioned below) and in **Figure 8B**. BRV decreased the N100 potential (vs. placebo: $p = 0.03$; vs. baseline: $p = 0.03$) and caused a non-significant ($p < 0.1$) attenuation of the P180 potential (vs. baseline: $p = 0.08$), with drug-induced changes of both TEP components topographically being located over the non-stimulated right sensorimotor cortex.

For TGB, in the reduced sample of N = 12 subjects, and despite all attempts to remove the increased spontaneous oscillatory activity corrupting TEP averages (see Methods, and below), no significant modulation of any of the TEP components could be observed (all $p \geq 0.2$).

Drug effects on spontaneous oscillations

Drug-induced changes in spontaneous oscillatory spectral power in the pre-TMS time periods are shown in **Figure 9** and **Supplementary Figure S3**. CBZ caused an increase in the power of spontaneous oscillations that was significant only for a circumscribed central cluster in the beta band during pre-TMS EEG periods (vs. placebo: $p = 0.007$; vs. baseline: $p = 0.0009$), but extended to more widespread global increases in the theta (vs. baseline: $p = 0.004$) and alpha (vs. placebo: $p = 0.004$; vs. baseline: $p = 0.01$) bands in addition to the beta band (vs. placebo: $p = 0.04$; vs. baseline: $p = 0.01$) during eyes open resting-state EEG recordings.

TGB caused an even stronger broadband and spatially widespread boost of oscillatory spectral power, including delta (vs. placebo: $p = 0.0009$; vs. baseline: $p = 0.0009$), theta (vs. placebo: $p = 0.0009$; vs. baseline: $p = 0.0009$), alpha (vs. placebo: $p = 0.0009$; vs. baseline: $p = 0.01$), and beta (vs. placebo: $p = 0.003$; vs. baseline: $p = 0.0009$) bands during both pre-TMS time periods

and eyes open resting-state EEG. Oscillatory power changes were most pronounced (i.e. 7-8 fold) in the delta and theta bands (see **Figure 9** and **Supplementary Figure S3**).

No significant modulation of spontaneous resting EEG spectral power was observed for BRV or placebo in any of the four frequency bands (all $p > 0.2$).

Discussion

The novel findings of this work relate to the drug induced changes in TMS-evoked EEG potentials (TEPs). In the context of previous studies that investigated the effects of drugs with other specific modes of action this allows to further characterize the pharmaco-physiology of TEPs, which are considered important non-invasively measured signatures of excitability and connectivity of the human brain (Chung, et al., 2015; Rogasch and Fitzgerald, 2013). In the following the main reported drug effects on TMS-EMG and TMS-EEG measures will be discussed.

TMS-EMG results

*RMT and SI1mV***.** CBZ increased RMT and SI1mV. This confirms previous TMS-EMG findings that consistently demonstrated that CBZ increases motor threshold (for review see (Ziemann, et al., 2015a)). Motor threshold depends on membrane excitability, and blockade of VGSCs decreases the axonal excitability of cortico-cortical and corticospinal neurons to single-pulse TMS. VGSCs are crucial in regulating axon excitability and their blockade will result in a more hyperpolarized (i.e., less excitable) state of cortico-cortical axons in M1 (Hodgkin and Huxley, 1952), and since these axons have synaptic contacts with cortico-spinal neurons, VGSC blocking drugs such as CBZ increase motor threshold (Ziemann, et al., 2015b).

BRV increased the SI1mv and there was also a non-significant ($p < 0.1$) increase in RMT, however, the increases in SI1mv and RMT after BRV were less pronounced than after CBZ, which was expected since BRV has primarily neurotransmitter-modifying properties. There is some inconsistency in the reports after levetiracetam intake (also a ligand to the presynaptic vesicle protein SV2A), with some studies reporting an increase in motor threshold (Premoli, et al., 2017b; Solinas, et al., 2008), while others reported no change

(Heidegger, et al., 2010). The slight increase in motor threshold after BRV intake reported here may relate to a secondary mode of action of BRV in blocking VGSCs (Niespodziany, et al., 2015).

TGB didn't alter RMT or SI1mv, in line with previous results (Werhahn, et al., 1999a).

*MEP input-output curve***.** CBZ didn't change the MEP amplitudes. MEP input-output curve is a measure of trans-synaptic excitation of corticospinal neurons regulated by glutamatergic, GABAergic and neuromodulating neurotransmitters, but no effects of ion channels on MEP amplitudes have been established (Ziemann, et al., 2015b). Note that significant changes in motor threshold may occur without changes in MEP amplitudes, which supports the idea that the mechanism underlying motor threshold and MEP amplitudes are different, and this may explain the lack of a significant effect of CBZ on MEP input-output curve despite its effects on motor threshold. Also note that the assessment of changes in RMT and MEP curve, was not only based on two independent measurements, and determined by two very different procedures, but also relied on different statistical tests (paired t-tests vs. three-way rm-ANOVA), which may partially explain this apparent discrepancy. In fact, if conducting post-hoc comparisons per intensity condition (which is not justified given the lack of interaction in the three way ANOVA) the single significant post-pre difference would have been at 100% RMT, but no other intensity. Others have described a rightward shift of the MEP input-output curve reflecting the increase in motor threshold after VGSC blocker intake (Boroojerdi, et al., 2001).

BRV produced a non-significant ($p < 0.1$) decrease in the MEP input-output curve. This effect may be explained by its specific binding to the presynaptic vesicle protein SV2A. This protein is expressed on excitatory and inhibitory neurons throughout the central nervous system (Klein, et al., 2018), but BRV may exert its antiepileptic effects predominantly through depression of excitatory neurotransmission (Yang, et al., 2015).The current BRV findings are in agreement with previous TMS-EMG studies that reported a depression of MEP input-output curves under levetiracetam (Reis, et al., 2004; Sohn, et al., 2001).

TGB didn't modulate the MEP input-output curve, in accord with previous findings (Werhahn, et al., 1999a).

SICI intensity curve. SICI reflects synaptic GABAAergic inhibition of corticospinal neurons, and VGSC blockers had no effect on SICI in previous studies (Ziemann, et al., 2015b). The observed decrease in SICI after CBZ in the present study (cf. **Figure 4**) is most likely simply a rightward shift of the SICI input-output curve caused by the increase in RMT, for which CS intensity was not adjusted for and, therefore, a non-specific finding. This is supported by the observation that motor and SICI thresholds are closely related to each other (Ziemann, et al., 1996b).

BRV had no effect on SICI. This is in accord with the literature where levetiracetam also did not alter SICI (Reis, et al., 2004; Sohn, et al., 2001; Solinas, et al., 2008), and with the mode of action of BRV and levetiracetam without any modulating activity directly at the GABAA receptor (Klein, et al., 2018; Lyseng-Williamson, 2011).

In contrast to previous work (Werhahn, et al., 1999a), we did not observe a depression of SICI after TGB intake. Those authors also demonstrated a prolongation of the cortical silent period (CSP) duration and an increase in long-interval intracortical inhibition, both putative measures of GABAB receptor mediated cortical inhibition (McDonnell, et al., 2006; Ziemann, et al., 2015b), and interpreted the TGB-induced reduction in SICI through enhanced GABAB receptor-mediated presynaptic autoinhibition (Werhahn, et al., 1999b). We have not tested here TMS-EMG measures of GABAB receptor-mediated inhibition, and there are other methodological differences between the two studies, for example the ISI of 3 ms (Werhahn, et al., 1999b) vs. 2 ms (our study) for SICI testing that may have contributed to the disparate findings (Peurala, et al., 2008b).

TMS-EEG results

Carbamazepine. After application of repetitive TMS (rTMS) or transcranial direct current stimulation (tDCS), which modulate synaptic strength and cortical excitability as indicated by TMS-EMG measures (Ziemann, et al., 2008), only early components of TEPs changed significantly (Esser, et al., 2006; Pellicciari, et al., 2013; Veniero, et al., 2012). Also, Ilmoniemi et al. showed that after stimulating M1, motor cortical areas responded within the first 28 ms (Ilmoniemi, et al., 1997) and these results suggest that the amplitude of early TEPs (<

30ms) might be a putative marker of excitation of the corticospinal system. Moreover, the amplitude of the N15-P30 complex correlated directly with MEP amplitude (Mäki and Ilmoniemi, 2010). In patients with progressive myoclonus epilepsy, the P25 waveform was increased as a sign for corticospinal and cortico-cortical hyperexcitability (Julkunen, et al., 2013). Therefore, P25 suppression by CBZ, predominantly at the site of stimulation, most likely reflects reduction of corticospinal excitability, a finding that remained significant even with stimulation intensity adjusted to RMT change (**Figure 5**). This corroborates the previously established notion that TMS-EEG measures may be more sensitive than TMS-EMG measures in detecting change in cortical excitability after intervention (Ferreri and Rossini, 2013). The N15-P30 complex has been reported to be strongly affected by TMS coil orientation (Bonato, et al., 2006). However, we were able to exclude this possible confound by careful application of neuronavigated TMS.

A decrease of N100 was observed after administration of diazepam and alprazolam, while baclofen increased N100, suggesting that the N100 is negatively related to GABAA but positively to GABAB receptor mediated neurotransmission (Premoli, et al., 2014a; Premoli, et al., 2014b). The non-significant ($p < 0.1$) reduction of N100 amplitude after CBZ should be considered with caution because it took place only when compared to baseline but not when compared to placebo, and not with unadjusted stimulus intensities (**Figure 6**, **Supplementary Figure S1**). Therefore, we consider this a nil finding, that is in accordance with a lacking effect on the N100 by lamotrigine, another VGSC blocker (Premoli, et al., 2017b).

A decrease in P180 amplitude was observed after lamotrigine intake, both with and without adjusting stimulus intensity to compensate for RMT change (Premoli, et al., 2017a; Premoli, et al., 2017b). Our results complement these findings, as CBZ reduced P180 amplitude both with and without adjusting stimulation intensity. These results suggest that P180 is reactive to excitability-lowering drugs, e.g., classic VGSC blockers (CBZ and lamotrigine), while GABAergic drugs had no effect (Premoli, et al., 2014a).

Part of the P180 component is likely caused by auditory evoked activity induced by the click of the stimulating coil (Conde, et al., 2018; Rogasch, et al., 2014). Therefore, it is possible that a reduction of the cortical auditory evoked potential

after CBZ has contributed to the observed P180 reduction. This is, however unlikely, given the findings in the literature that have only shown changes in latency but not amplitude of cortical auditory evoked potentials after CBZ (Japaridze, et al., 1993) but significant amplitude depression after diazepam (Noldy, et al., 1990). This double dissociation with the reported TMS-EEG P180 data strongly suggests that the reported depression by VGSC blockers is caused by their effects on direct TMS-evoked brain responses rather than brain activity related to the auditory input.

*Brivaracetam***.** The N100 is thought to be a marker of GABABergic inhibition due to the enhancing effect of baclofen, a specific GABAB receptor agonist, on N100 amplitude at the site of stimulation (Premoli, et al., 2014a). On the other hand, benzodiazepines (Premoli, et al., 2014a) and levetiracetam (Premoli, et al., 2017a) resulted in N100 amplitude depression in the non-stimulated hemisphere. The present results of BRV, showing N100 amplitude depression in M1 area of the non-stimulated hemisphere (**Figure 7**) are in full agreement with those previous findings. The underlying physiology of this effect, particularly on propagated neuronal activity remote from the site of stimulation remains unclear, but may indicate a suppression of long-range cortico-cortical effective connectivity and signal propagation under the influence of drugs with positive modulation at the GABAA receptor (Ferrarelli, et al., 2010; Sarasso, et al., 2015) and drugs with inhibition of presynaptic excitatory transmitter release (levetiracetam, BRV).

Tiagabine. TGB didn't modulate any of the TEP components. One possible reason might be that the TGB dose was not sufficient to cause any effects. However, this can be largely excluded as one previous study found significant effects on TMS-EMG measures with the same dose (15 mg) (Werhahn, et al., 1999b). Furthermore, we found that TGB strongly increased pre-TMS EEG power in all frequency bands (**Figure 9** and **Supplementary Figure S3**), confirming findings of two resting-state magnetoencephalographic studies (Muthukumaraswamy and Liley, 2018; Nutt, et al., 2015) and, therefore, indicating a significant effect of TGB on brain activity in the present experiments.

Nutt and colleagues also had investigated gaboxadol, a positive modulator specifically at the extrasynaptic alpha-4 delta unit bearing subtype of the GABAA receptor, and zolpidem, a positive modulator with strong positive modulating activity at the synaptic alpha-1 unit bearing subtype of the GABA receptor. Gaboxadol but not zolpidem resulted in a similar enhancement of resting-state activity as TGB, and they concluded that the effects by TGB may therefore be largely related to its action on tonic inhibition mediated by extrasynaptic GABAA receptors (Nutt, et al., 2015). However, this is unlikely to be the reason for the nil findings of TGB on TEP amplitudes in the present study as previous studies demonstrated significant effects of ethanol, another positive modulator at the extrasynaptic alpha-4 delta unit bearing subtype of the GABAA receptor (Kahkonen, et al., 2001; Kahkonen, et al., 2003).

The reason for the nil findings may be explained by the specific mode of action of TGB, which increases the concentration of GABA in the synaptic cleft by inhibition of the GABA transporter 1, but without having significant affinity for any neurotransmitter receptor binding sites in the central nervous system (Suzdak and Jansen, 1995). Importantly, in rat hippocampal slice preparations, tiagabine had no effect on the amplitude of low- and high-intensity single-pulse evoked inhibitory post-synaptic potentials (Jackson, et al., 1999), in contrast to positive modulators at GABAA receptors, such as benzodiazepines (Thomson, et al., 2000). For this reason, TGB may have failed to modulate single-pulse TMS evoked neuronal activity in the human brain as measured by TEP amplitudes.

In conclusion, the present study used three different drugs with specific modes of action to further elucidate the pharmaco-physiological characteristics of TMS-evoked EEG potentials (TEPs), emergent non-invasive markers of excitability and effective connectivity of the human brain. We found that carbamazepine, a VGSC blocker depressed the P25 and P180 potentials, while brivaracetam that decreases neuronal excitability through binding to the presynaptic protein SV2A decreased the N100 potential in the non-stimulated hemisphere, and tiagabine, a GABA re-uptake inhibitor without direct modulating action on receptors in the central nervous system had no effect. Together with data from previous pharmaco-TMS-EEG studies the present findings corroborate the view that the

P25 represents axonal excitability of the corticospinal system, the N100 in the non-stimulated hemisphere propagated activity suppressed by positive modulation of GABAA receptors, and the P180 late activity particularly sensitive to VGSC blockade.

Limitations

Pharmaco-TMS-EEG is still a pioneering approach with many challenges, but we did our best to prevent potential confounds by running a double-blinded, randomized, placebo-controlled crossover design and state-of-the-art neuronavigated TMS-EEG procedures and analyses. Nonetheless, there are a few caveats to consider: While we controlled for multiple comparisons regarding the number of EEG channels and time points, the assessment of multiple different drugs inevitably comes at the risk of false positive findings, no matter whether within a single-study or across multiple studies and research groups. The only real solution to this issue is replication across labs, which we hereby explicitly encourage. In general, TEPs can be inherently confounded by auditory and somatosensory co-stimulation (Conde, et al., 2018; Gordon, et al., 2018; Herring, et al., 2015). While auditory noise masking can reduce its impact, and the post-pre and drug-placebo comparison remove most of these confounds, it is possible that a pharmacological modulation of residual auditory and somatosensory evoked potentials adds to the drug-induced changes in truly transcranial evoked brain responses. This potential confound has to be carefully assessed for each individual case, in particular for components that appear remotely to the stimulation site, such as the P180 as discussed in detail in the TMS-EEG section above. In general, remote TEP components are more difficult to interpret than those at the stimulation site, as they may have multiple origins. While they can indeed reflect multisensory co-activations, they may also result from actual signal propagation (also transcallosal) within the stimulated network (Ilmoniemi, et al., 1997; Massimini, et al., 2005), or from projections to the remote surface from dipoles located at deeper sources (Litvak, et al., 2007). In the future, realistic sham conditions and source localized TEP analyses may help to disentangle these contributions. However, TEP analyses in channel space are well established and, importantly, allow comparability with previously published studies.

Conflicts of interest. U.Z. reports grants from Bristol Myers Squibb, Janssen Pharmaceuticals NV, Servier, and Biogen Idec, and personal fees from Pfizer GmbH, Bayer Vital GmbH, CorTec GmbH, and Medtronic GmbH, all outside the submitted work.

Acknowledgements. This work has been supported by a grant from the German Research Foundation to U.Z. (DFG ZI 542/9-1).

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Figures

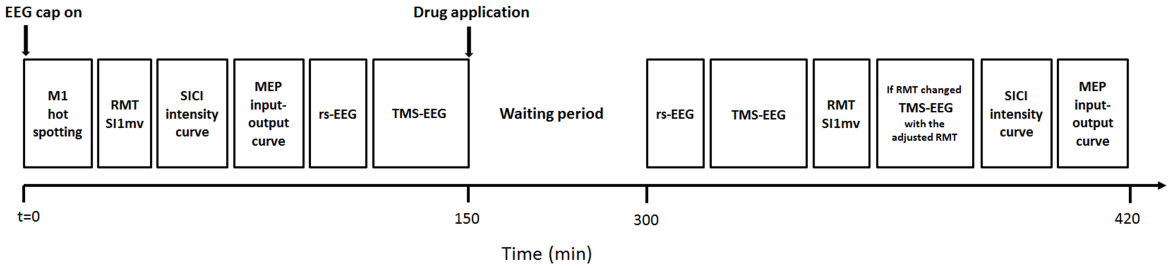


Figure 1: Time line of an experimental session.

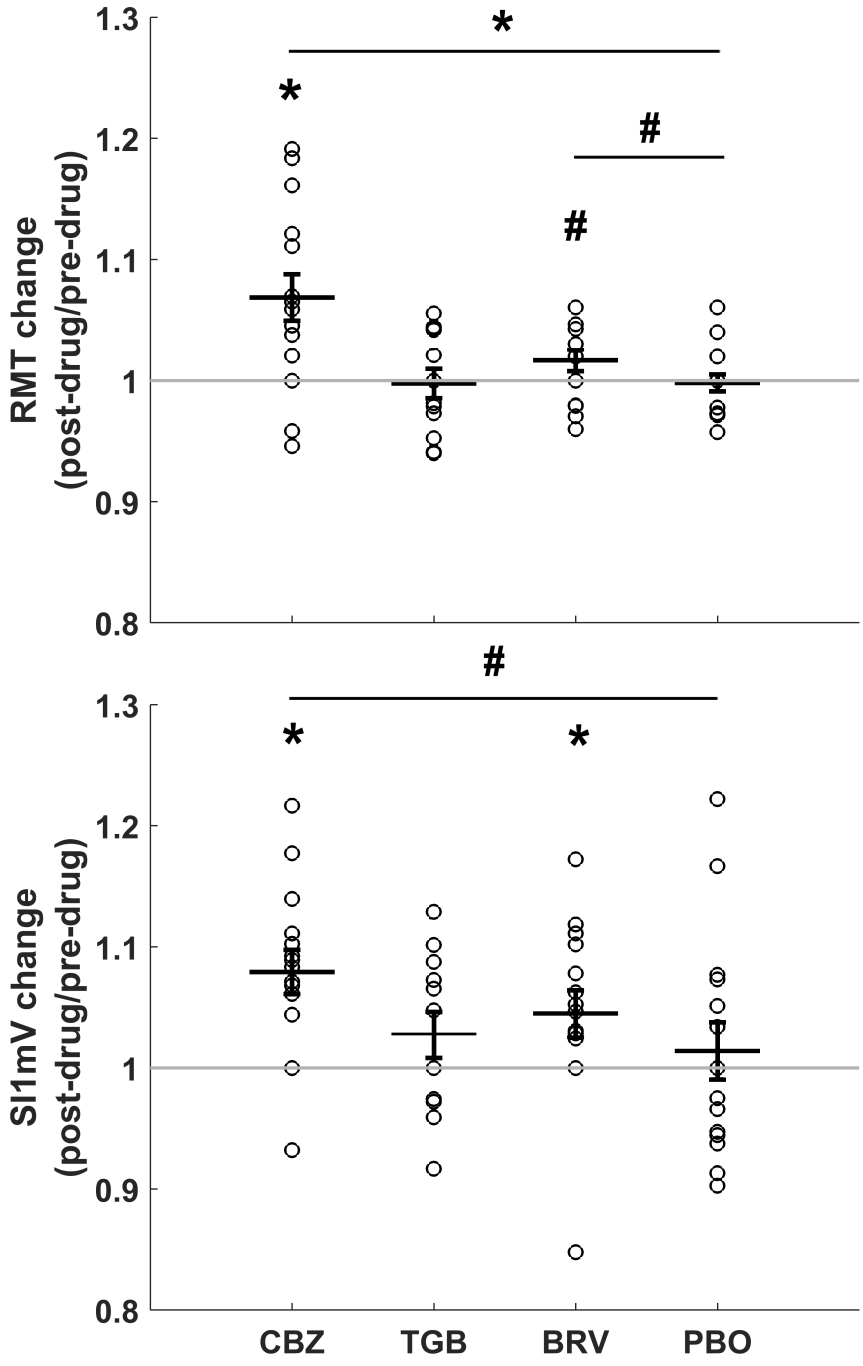


Figure 2. Individual and mean changes (± SEM) in RMT and SI1mV (post-drug/pre-drug) in all drug conditions (CBZ, carbamazepine; TGB, tiagabine; BRV, brivaracetam; and PBO, placebo). CBZ increased RMT compared to baseline and placebo ($p < 0.05$) and both CBZ and BRV increased S1mV compared to baseline ($p < 0.05$). Asterisks indicate significant comparisons with $p < 0.05$, hashtags indicate non-significant comparisons with $p < 0.1$.

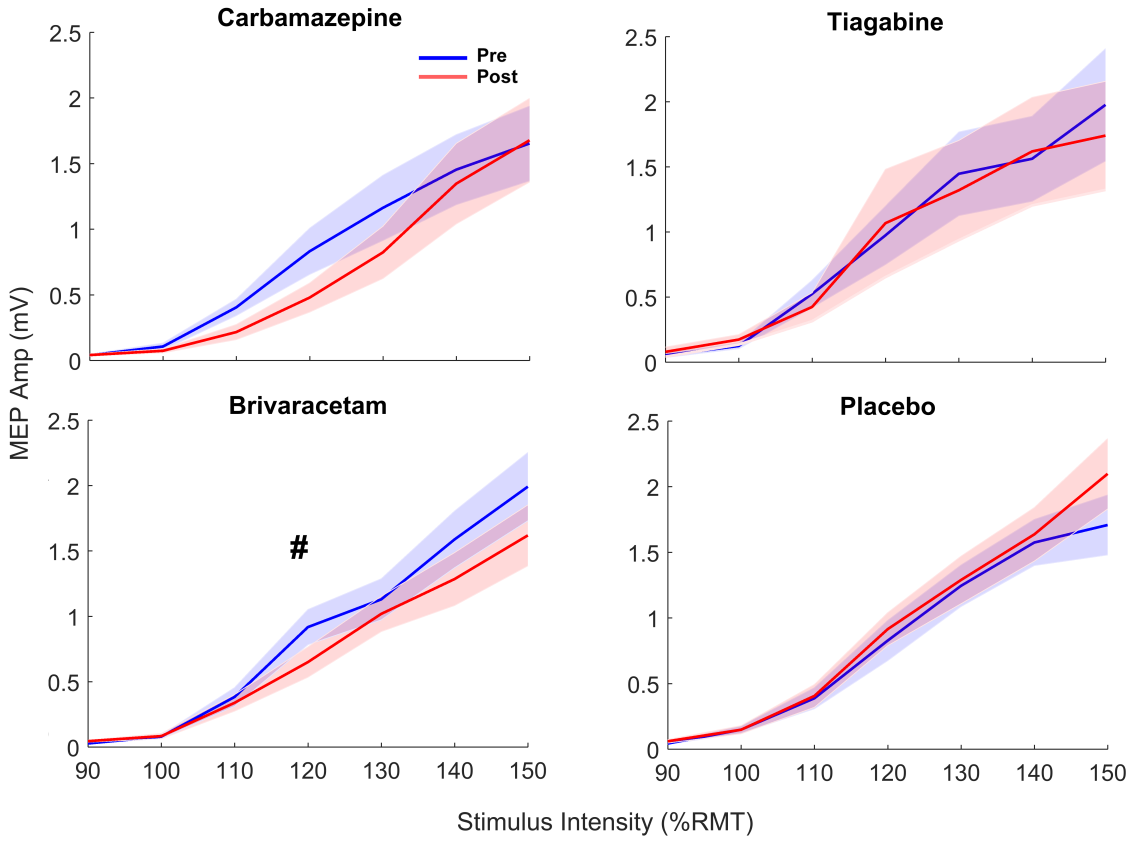


Figure 3. Mean MEP input-output curves (shadings: ± 1 SEM) plotted against seven different stimulus intensities before (black) and after (gray) intake of the four drug conditions: carbamazepine (top left), tiagabine (top right), brivaracetam (bottom left) and placebo (bottom right). The hashtag indicates a non-significant ($p < 0.1$) drug-related change of the MEP curve. RMT, resting motor threshold, as determined before drug intake.

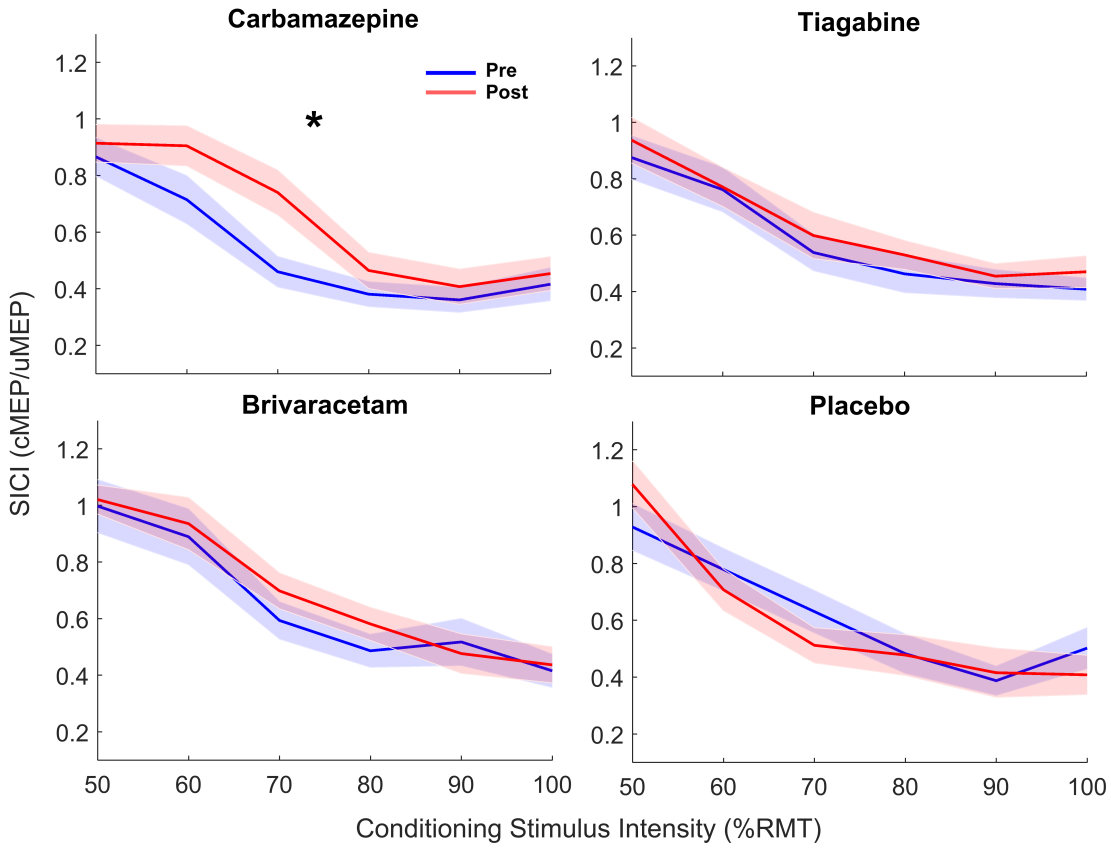


Figure 4. Mean SICI intensity curves (shadings: ± 1 SEM) plotted as a function of the six conditioning stimulus (CS) intensities before (black) and after (gray) intake of carbamazepine (top left), tiagabine (top right), brivaracetam (bottom left), and placebo (bottom right). The asterisk indicates a significant ($p < 0.05$) drug-related change of the SICI intensity curve. RMT, resting motor threshold; cMEP, conditioned motor evoked potential amplitude; uMEP, unconditioned motor evoked potential amplitude.

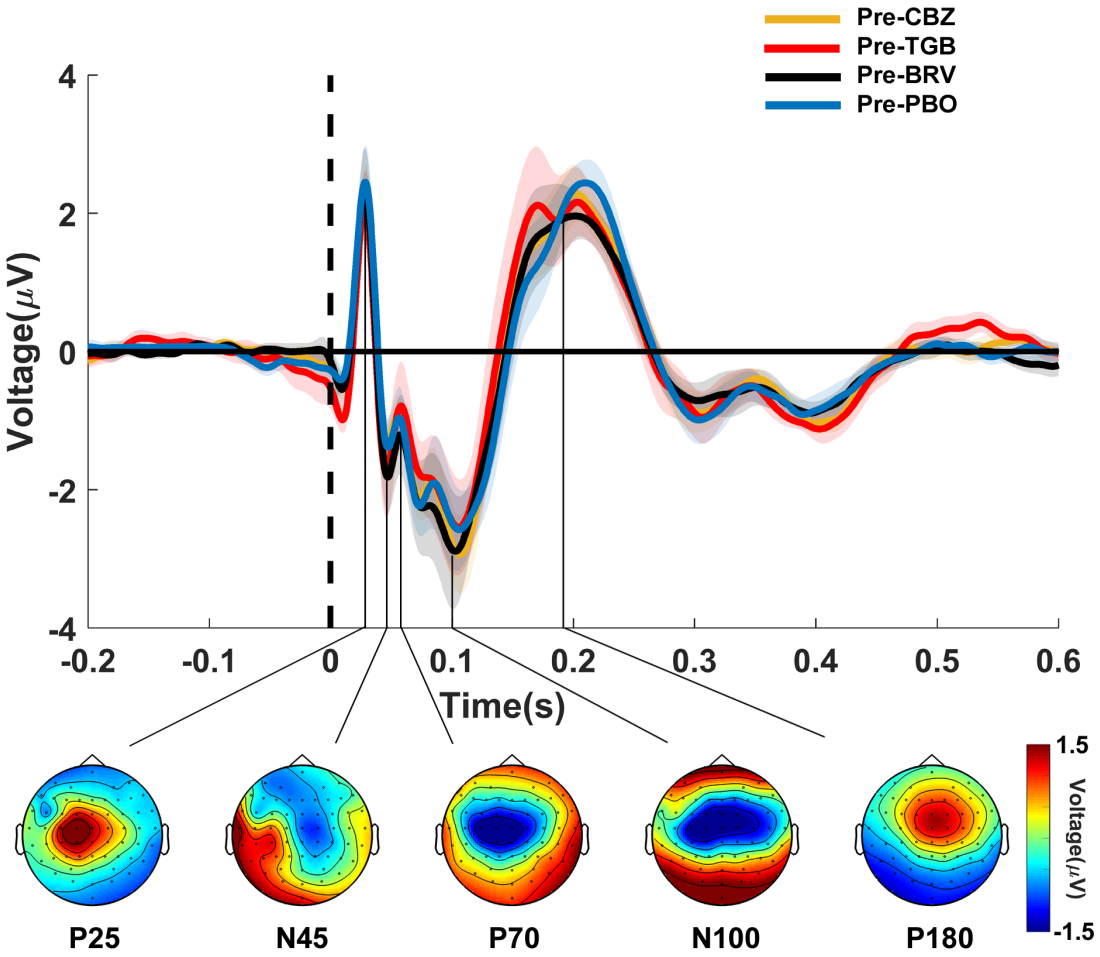


Figure 5. TEPs before drug intake. TEPs (shadings: ± 1 SEM) were plotted for channel Cz at baseline (pre-drug) in the four different drug conditions (CBZ, carbamazepine; TGB, tiagabine; BRV, brivaracetam; and PBO, placebo) and labeled based on their approximate latency (P25, N45, P70, N100, and P180) relative to the time of the TMS pulse over the left M1 (time 0, vertical dashed line). Topographical distributions of surface voltages illustrated in the bottom were grand averaged over the four drug conditions in non-overlapping TOIs after TMS (P25: 15–35 ms; N45: 36–50 ms; P70: 51– 85 ms; N100: 86–150 ms; P180: 151 –280 ms). Note that voltage topographies may not reveal accurate locations for low amplitude components (e.g., N45, P70) that peak during the flanks of high amplitude components (e.g., N100).

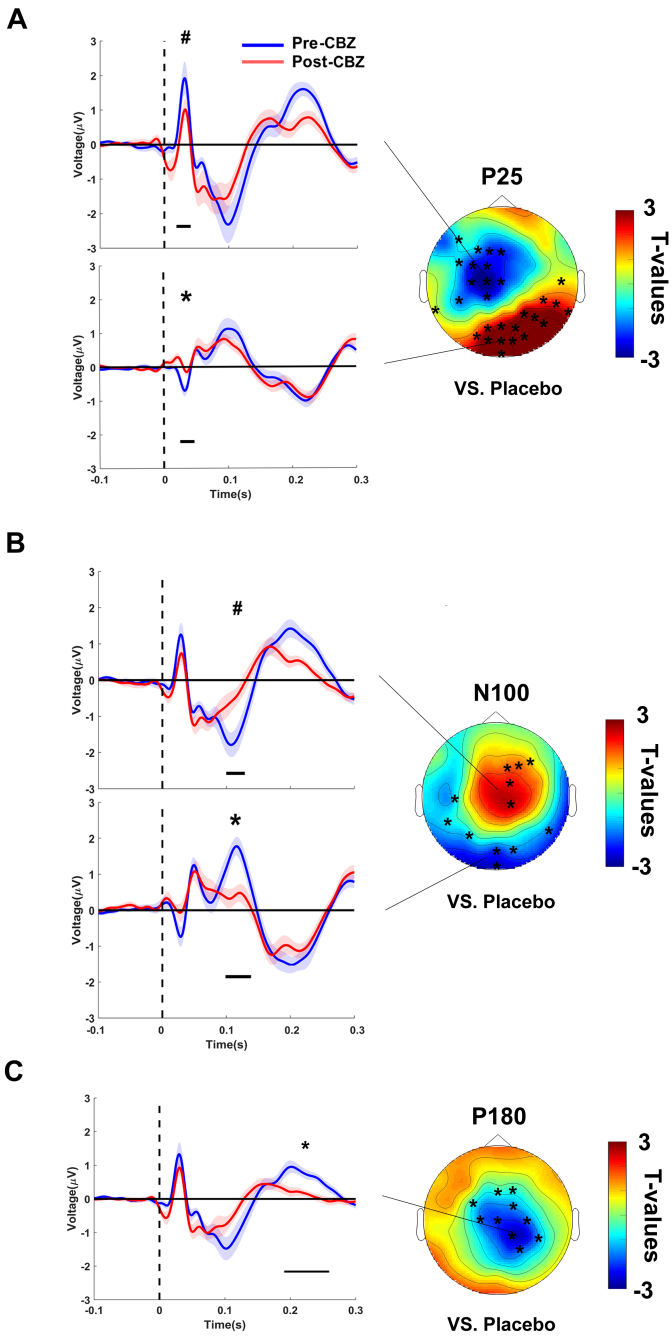


Figure 6. Carbamazepine-induced changes of TEPs. CBZ decreased the (A) P25, (B) N100 and (C) P180 TEP components. TEPs (shadings: ± 1 SEM) plotted for grand averages of those channels constituting a cluster of significant differences versus baseline (post-CBZ – pre-CBZ), separately for pre-drug (blue) and post-drug (red) measures. Horizontal black bars underneath the TEPs denote the significant clusters in time (*$p < 0.05$, $^{\#}p < 0.1$). T-statistic maps of the TEP amplitude plotted versus placebo (CBZ(post-pre) vs. placebo(post-pre)). Channels constituting significant clusters of changes in this interaction are indicated by asterisks in the t-statistic maps.

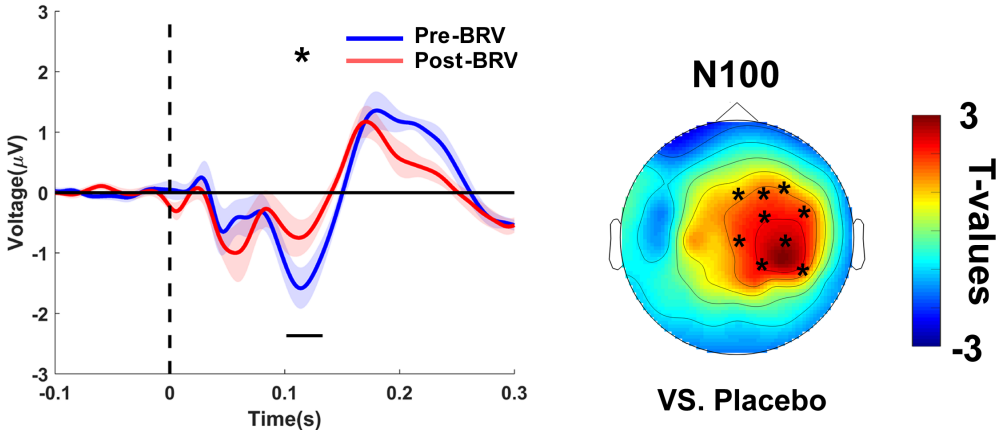


Figure 7. Brivaracetam-induced changes of TEPs. BRV decreased the N100 potential in the non-stimulated right hemisphere. TEPs (shadings: ± 1 SEM) plotted for grand averages of those channels that showed a significant difference versus baseline (post-BRV – pre-BRV), separately for pre-drug (blue) and post-drug (red) measures. Horizontal black bars underneath the TEPs denote significant clusters in time (*$p < 0.05$). T-statistic maps of the TEP amplitude plotted versus placebo (BRV(post-pre) vs. placebo(post-pre)). Channels constituting significant clusters of changes in this interaction are indicated by asterisks in the t-statistic maps.

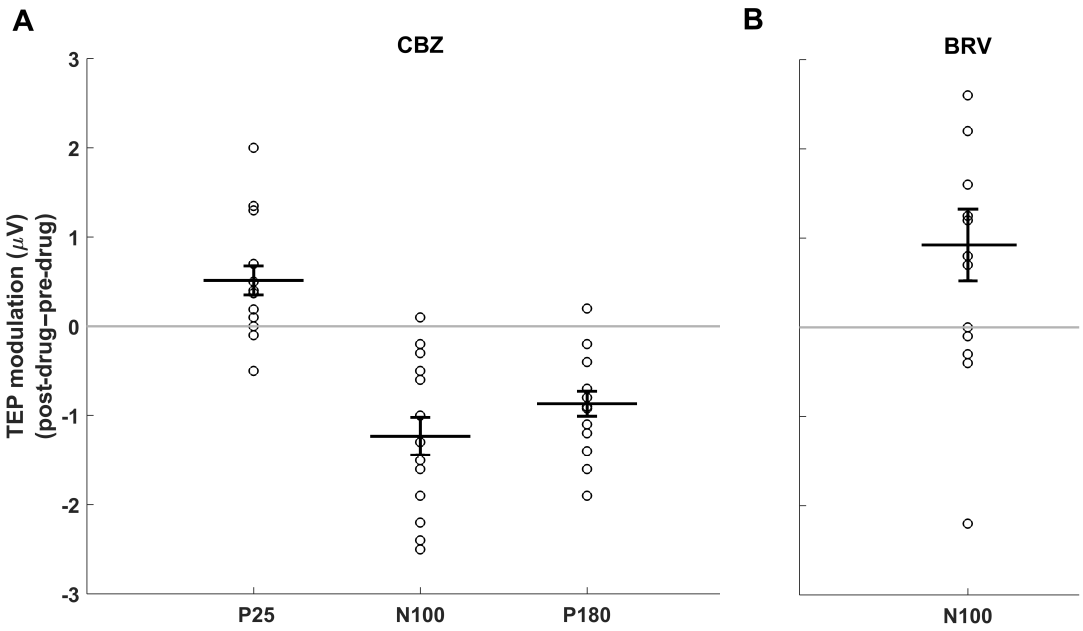


Figure 8. Individual and mean changes (± SEM) of drug-induced changes in TEP component amplitudes, averaged across channels within each significant ($p < 0.05$) cluster that was detected for (A) CBZ (cf. Figure 6) and BRV (cf. Figure 7).

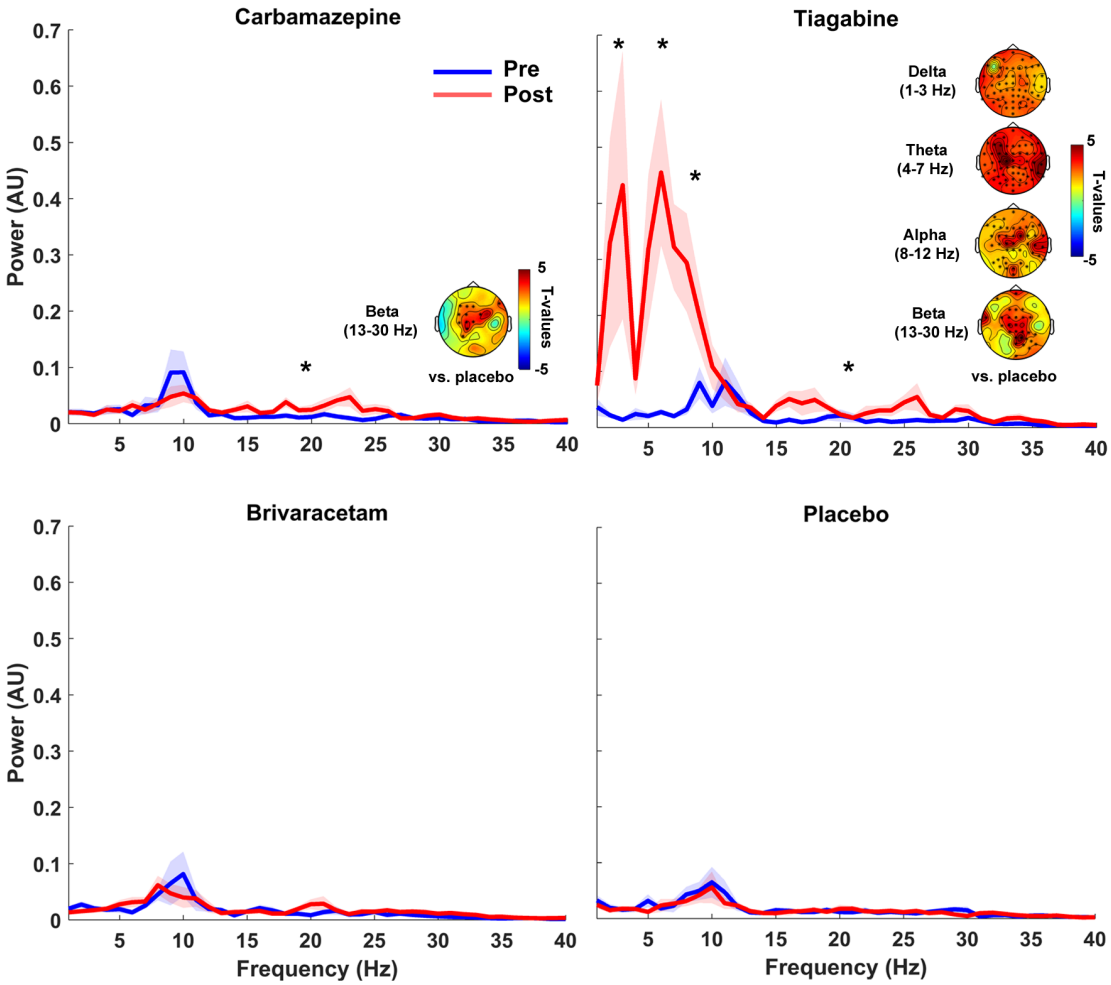


Figure 9. Drug-induced changes in spontaneous oscillatory power. Grand average power spectra (shadings: ± 1 SEM) are plotted for the pre-TMS EEG period of pre-drug (blue) and post-drug (red) measures for carbamazepine (CBZ, top left), tiagabine (TGB, top right), brivaracetam (BRV, bottom left), and placebo (PBO, bottom right). CBZ mainly increased beta band power, while TGB caused enormous power increases in all frequency bands (delta, theta, alpha, and beta). TGB-induced changes in delta and beta bands were particularly strong. There were no drug-induced power changes in other drug conditions or for other frequency bands. Power spectra are plotted for channel Cz, and asterisks indicate significant drug-related changes ($p < 0.05$). T-statistic maps plotted versus placebo (drug(post-pre) vs. placebo(post-pre)), and channels forming significant clusters are marked with asterisks in the t-statistic maps.

Supplementary material

Table S1. Localization (significant electrodes) of carbamazepine effects on TEP amplitudes

RMT adjusted	vs. baseline	P25	over the left MC	▼	#	C3, Fz, Cz, C1, FC3
		P25	over the right POC	▼	*	P4, O2, P8, Iz, CP6, TP10, Oz, O1, P7, Pz, TP9, P2, P6, PO8, Poz
		N100	over the right MC	▼	#	C4, Fz, Cz, Pz, C1
		N100	over the left POC	▼	*	P3, O1, O2, T7, P7, Iz, CP5, TP9, TP10, PO4, C5, P5, TP7, PO7, POz, Oz
		P180	over the right MC	▼	*	C3, C4, P3, P4, Cz, Pz, FC1, CP1, F1, C1, P2, FC3, FCz
	vs. placebo	P25	over the left MC	▼	*	F3, C3, F7, Fz, Cz, FC1, CP1, FC5, CP5, F1, C1, FC3
		P25	over the right POC	▼	*	P4, O2, T8, P8, Iz, CP6, TP10, Oz, O1, TP9, P6, PO8, POz, PO3, PO4, TP8, PO7
		N100	over the right MC	▼	#	F4, FC2, CP2, FC6, F2
		N100	over the left POC	▼	#	O2, Iz, TP10, C5, P5, TP7, Oz
		P180	over the right MC	▼	*	C4, P4, Fz, Cz, FC2, CP2, CP6, F2, C1, FC3
RMT unadjusted	vs. baseline	P25	over the left MC	▼	#	F3, C3, F7, Fz, Cz, FC1, CP1, FC5, CP5, F1, C1, FC3
		P25	over the right POC	▼	*	P4, O2, P8, Iz, CP6, TP10, Oz, Pz, P2, P6, PO8, POz
		N45	over the right MC	▲	#	P4, O2, P8, Iz, CP6, TP10, Oz
		N45	over the left	▲	#	C3, CP5, C1, FC3

			MC			
		P180	over the right MC	▼	*	C3, C4, P3, P4, Cz, Pz, FC1, CP1, F1, C1, P2, FC3, FCz
	vs. placebo	P25	over the left MC	▼	*	F3, C3, FC1, CP1, FC5, CP5, F1, C1, FC3, Cz
		P25	over the right POC	▼	*	C4, P4, O2, T8, P8, Iz, CP6, TP10, CP4, PO4, C6, P6, TP8, Oz
		N45	over the left MC	▲	*	F3, C3, P3, FC1, FC5, CP5, C1, FC3
		N45	over the right MC	▲	*	F4, C4, P4, O2, T8, P8, Iz, FC2, CP2, FC6, CP6, TP10, F2, Fpz, Oz
		P180	over the right POC	▼	*	C4, P4, Fz, Cz, FC2, CP2, FC6, CP6, F2, C1

MC, Motor Cortex; POC, Parieto-Occipital Cortex; ▲, increase; ▼, decrease; #, $p < 0.1$; *, $p < 0.05$.

Table S2. Localization (significant electrodes) of brivaracetam effects on TEP amplitude

vs. baseline	N100	over the right MC	▼	*	F4, C4, Cz, FC2, CP2, FC6, CP6, F2, Fz
	P180	over the right MC	▼	#	C3, C4, P3, P4, Cz, Pz, FC1, CP1, F1, C1, P2, FC3, FCz
vs. placebo	N100	over the right MC	▼	*	F4, C4, Cz, FC2, CP2, FC6, CP6, F2, Fz
	P180		-	-	-

MC, Motor Cortex; ▼, decrease; #, $p < 0.1$; *, $p < 0.05$

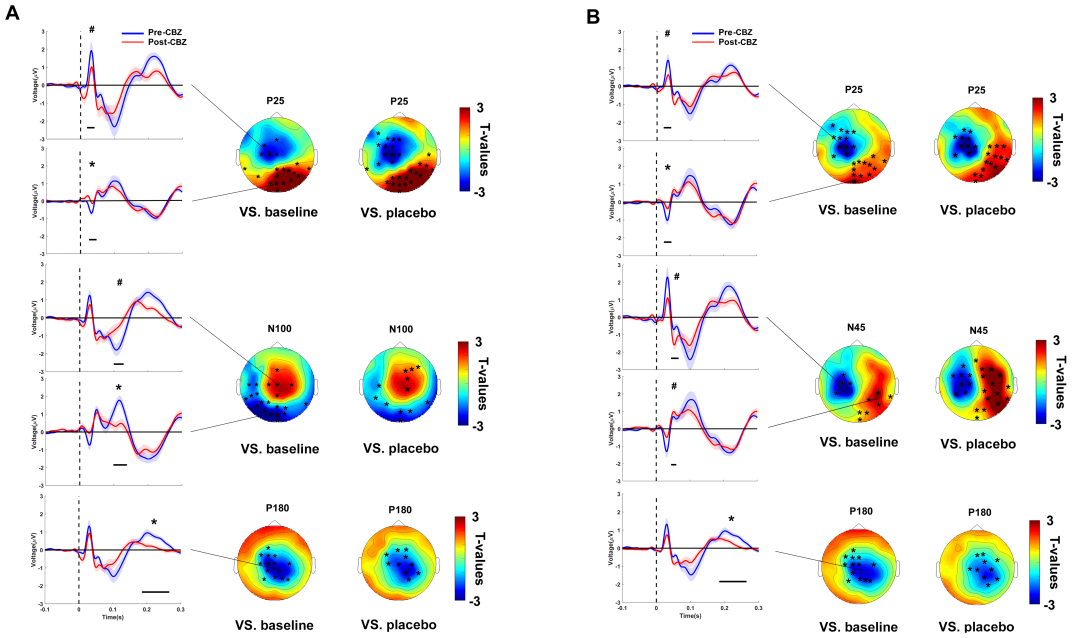
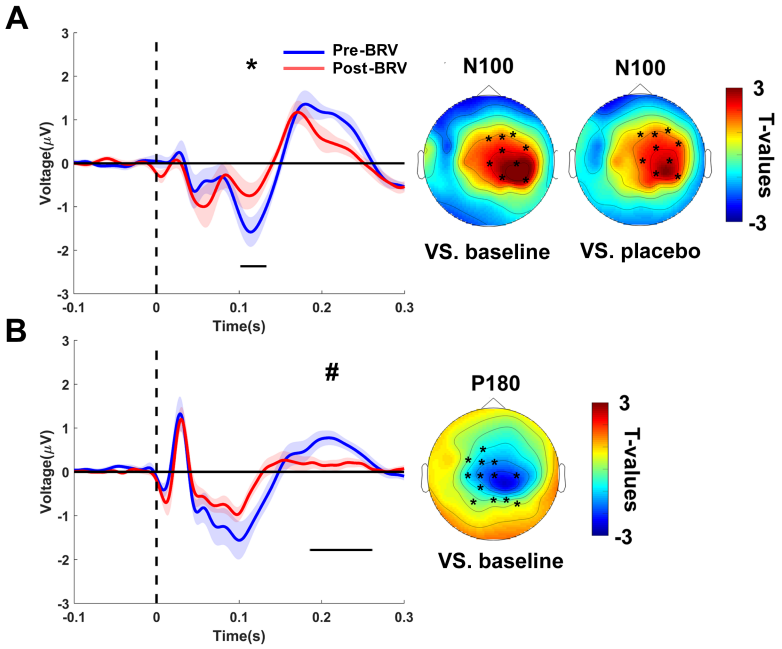


Figure S1. Carbamazepine induced changes of TEPs. (A) TEPs recorded *with* adjusted TMS intensity. (B) TEPs recorded *without* adjusted TMS intensity. CBZ suppressed the P25 and P180 with and without adjusting TMS intensity. In contrast, without adjustment, there was an increase in N45 potential, which was no longer significant when adjusting TMS intensity. Moreover, there was a significant decrease in the N100 potential only after adjusting the intensity for CBZ-induced RMT increases. TEPs (shadings: ± 1 SEM) plotted as grand averages of those channels constituting a significant cluster of differences versus baseline (post-CBZ – pre-CBZ), separately for pre-drug (blue) and post-drug (red) measures. Horizontal black bars underneath the TEPs denote the significant clusters in time (*$p < 0.05$, $^{\#}p < 0.1$). T-statistic maps of the TEP amplitude were plotted versus placebo (CBZ(post-pre) vs. placebo(post-pre)) and versus baseline (post-CBZ – pre-CBZ). Channels constituting a significant cluster of changes vs. baseline or vs. placebo are marked by asterisks in the corresponding t-statistic maps.



1159

1160 **Figure S2.** Brivaracetam induced changes of TEPs. (A) BRV decreased the
1161 N100 compared to both baseline and placebo contralateral to the stimulation
1162 site. (B) There was also a trend-wise reduction in P180 potential only when
1163 compared to baseline. TEPs (shadings: ± 1 SEM) plotted for grand averages of
1164 those channels constituting a significant cluster of differences versus baseline
1165 (post-BRV – pre-BRV), separately for pre-drug (blue) and post-drug (red).
1166 Horizontal black bars underneath the TEPs denote significant clusters in time
1167 (*$p < 0.05$, $^{\#}p < 0.1$). T-statistic maps of the TEP amplitude were plotted versus
1168 placebo (BRV(post-pre) vs. placebo(post-pre)) and versus baseline (post-BRV –
1169 pre-BRV). Channels constituting a significant cluster of changes vs. baseline or
1170 vs. placebo are marked by asterisks in the corresponding t-statistic maps.

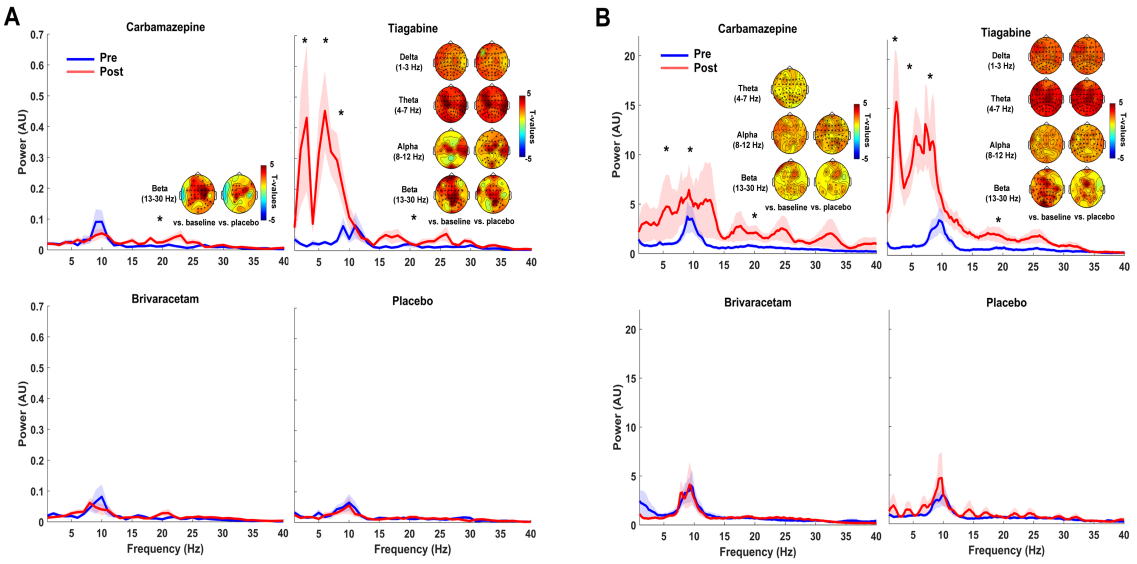


Figure S3. Drug-induced changes of spontaneous oscillatory power. Grand average power spectra (shadings: ± 1 SEM) are plotted for (A) the pre-TMS period and (B) the eyes open resting state EEG recording for pre-drug (blue) and post-drug (red) measures, separately for carbamazepine (CBZ), tiagabine (TGB), brivaracetam (BRV), and placebo. CBZ increased the power of spontaneous oscillations in the beta band during the pre-TMS period, and in theta, alpha, and beta bands for the resting state EEG recording. TGB caused particularly strong effects in all frequency bands (delta, theta, alpha and beta) in both pre-TMS periods and resting state EEG recordings, that were most pronounced in delta and theta bands. There were no drug-induced changes in other drug conditions or other frequency bands. Power spectra are plotted for channel Cz, and asterisks indicate significant drug related changes ($p < 0.05$). T-statistic maps are plotted versus placebo (drug(post-pre) vs. placebo(post-pre)) and versus baseline (post-drug – pre-drug), and channels forming significant clusters of increased power are marked with asterisks in the corresponding t-statistic maps.