Cooperative noninvasive brain stimulation to induce long-term motor plasticity

Thesis submitted as requirement to fulfill the degree „Doctor of Philosophy“ (Ph.D.)

at the
Faculty of Medicine
Eberhard Karls University
Tübingen

by

Hanna, Faber

from

Lauf an der Pegnitz

2018

Dean: Professor Dr. I. B. Autenrieth

1. Reviewer: Professor Dr. U. Ziemann
2. Reviewer: Privatdozent Dr. M. Himmelbach

To my brothers

Samuel & Johannes

A. Table of contents

1 Introduction ... 12

1.1 The motor system ... 12

1.2 Transcranial magnetic stimulation ... 13

1.2.1 History of TMS ... 13

1.2.2 Physiology underlying TMS ... 13

1.2.3 Motor cortex excitability – a reflector of motor learning ... 13

1.2.4 TMS – a method to test effective connectivity ... 14

1.3 Transcranial direct stimulation ... 15

1.3.1 History of tDCS ... 15

1.3.2 TDCS effects on motor cortex excitability ... 15

1.3.3 Proposed physiological mechanisms underlying tDCS ... 17

1.4 The global burden of stroke ... 17

1.4.1 The socioeconomic impact of stroke ... 17

1.4.2 TMS – a predictor for motor recovery after stroke ... 17

1.4.3 TMS – a rehabilitation option after stroke ... 18

1.5 Spike-timing dependent plasticity – a model for learning ... 18

1.5.1 LTP and LTD at the cellular level ... 18

1.5.2 Paired associative stimulation ... 20

1.5.2.1 Peripheral PAS ... 20

1.5.2.2 Corticocortical PAS ... 20

1.5.3 Anatomical and neurophysiological properties of the supplementary motor cortex ... 21

1.5.4 Functional role of the supplementary motor cortex ... 22

1.5.5 Coordinated motor activity after stroke ... 23

1.6 Aim of the study: induction of longer lasting changes in M1 ... 24

1.6.1 Experiment 1: cooperative $PAS_{M1c \rightarrow M1}$ with $PAS_{SMA \rightarrow M1}$ ... 25

1.6.1.1 Novel MRI-navigated multi-coil technique ... 25

1.6.1.2 Rational for the concurrent stimulation of SMA, M1c and M1 ... 26

1.6.1.3 Translation of the cellular concept of cooperativity to the network level ... 26

1.6.2 Experiment 2: cooperative tDCS with $PAS_{SMA \rightarrow M1}$ 27
1.6.2.1 Novel tDCS montage with increased tangential field strength 27
1.6.2.2 Translation of the cellular concept of cooperativity to the network level 28

2 Material and methods 30

2.1 Experiment 1: test of cooperative effects of $PAS_{M1c \rightarrow M1}$ with $PAS_{SMA \rightarrow M1}$ 30
2.1.1 Pilot experiment 30
2.1.1.1 Subjects 30
2.1.1.2 MRI data acquisition 30
2.1.1.3 Recording of motor evoked potentials 30
2.1.1.4 Localization and stimulation of M1 31
2.1.1.5 Localization and stimulation of M1c 32
2.1.1.6 Localization of the SMA 32
2.1.1.7 Stimulation of the supplementary motor area 33
2.1.1.8 Paired associative stimulation 34
2.1.1.9 Time line of experiments 35
2.1.1.10 Data analysis and statistics 36
2.1.2 Main experiment 36
2.1.2.1 Subjects 36
2.1.2.2 Localization of SMA 37
2.1.2.3 Data analysis and statistics 38
2.2 Experiment 2: test of cooperative effects of tDCS with $PAS_{SMA \rightarrow M1}$ 39
2.2.1 Novel tDCS electrode montage with increased tangential electric field component 39
2.2.2 Subjects 43
2.2.3 MRI data acquisition 43
2.2.4 Effective SMA-M1 connectivity 45
2.2.5 Transcranial direct current stimulation 46
2.2.6 Paired associative stimulation 46
2.2.7 Time line of experiments 47
2.2.8 Control experiment 2 49
2.2.9 Data analysis and statistics 50

3 Results **54**

3.1 Experiment 1: cooperative PAS$_{M1c \rightarrow M1}$ with PAS$_{SMA \rightarrow M1}$ **54**

3.1.1 Pilot experiment 54

3.1.1.1 Baseline measures of the pilot experiment 54

3.1.1.2 Effects of cooperative PAS$_{M1c \rightarrow M1}$ with PAS$_{SMA \rightarrow M1}$ stimulation on M1 excitability 55

3.1.2 Main experiment 57

3.1.2.1 Baseline measures of corticospinal excitability 57

3.1.2.2 Online effects of PAS$_{M1c \rightarrow M1}$ with PAS$_{SMA \rightarrow M1}$ 58

3.1.2.3 Effects of cooperative PAS$_{M1c \rightarrow M1}$ with PAS$_{SMA \rightarrow M1}$ 59

3.2 Experiment 2: cooperative tDCS with PAS$_{SMA \rightarrow M1}$ **60**

3.2.1 Baseline measures of corticospinal excitability and effective SMA-M1 connectivity 60

3.2.2 Online-effects of tDCS with PAS$_{SMA\text{-}M1}$ 61

3.2.3 Effects of tDCS with PAS$_{SMA \rightarrow M1}$ 62

3.2.4 Modulation of the PAS$_{SMA \rightarrow M1}$ effect by anodal versus cathodal tDCS. 64

3.2.5 Effects of tDCS on SMA-M1 connectivity 66

3.2.6 Control experiment 2 70

3.2.6.1 Baseline measures of corticospinal excitability 70

3.2.6.2 Exclusive tDCS effects 70

4 Discussion **72**

4.1 Experiment 1: cooperative PAS$_{SMA \rightarrow M1}$ with PAS$_{M1c \rightarrow M1}$ to induce LTP **72**

4.1.1 Simultaneous PAS$_{M1c \rightarrow M1}$ with PAS$_{SMA \rightarrow M1}$ is unable to induce plastic changes 72

4.1.2 PAS$_{SMA \rightarrow M1}$ requires metaplastic priming for successful plasticity induction 73

4.1.3 Methodological consequences of experiment 1 75

4.2 Experiment 2: cooperative tDCS with PAS$_{SMA \rightarrow M1}$ **76**

4.2.1 PAS$_{SMA \rightarrow M1}$ has no effects on M1 excitability 76

4.2.2 PAS $_{SMA \rightarrow M1}$ does not alter effective SMA-M1 connectivity 78

4.2.3 TDCS in the novel montage is unable to change M1 excitability 78

4.2.4 Unidirectional effects of cooperative tDCS with PAS$_{SMA \rightarrow M1}$ 79

4.2.4.1 Cortical geometry takes account for polarity-independent effects. 79

4.2.4.2 Radial symmetry of cortical columns explains polarity-independent tDCS effects 80
4.2.4.3 $PAS_{SMA \rightarrow M1}$ and tDCS interact cooperatively 81
4.2.4.4 Effects on effective SMA-M1 connectivity 82
4.2.4.5 Cooperative effects of tDCS with $PAS_{SMA \rightarrow M1}$ stabilize post-plasticity induction 83
4.2.5 Astrocytic Calcium surges accountable for polarity-independent tDCS effects 84
4.3 Limitations and outlook 85
4.3.1 MEPs as single output measure 85
4.3.2 Limitations of tDCS 86
4.3.3 Participation of other cortical areas 86
4.3.4 Limitations of translational medical research with NIBS 87
4.4 Final conclusion 89

5 Summary 91

6 Bibliography / List of references 93

7 German summary 106

8 Publications 108

9 Declaration of candidates own contribution 109

10 Appendix 110

10.1 Study information and informed consent for experiment 1 110
10.2 Study information and informed consent for experiment 2 124
10.3 Permission for the transfer of MRI-images 145
10.4 Detection of handedness 146
10.5 TMS Screening Form 147
10.6 Expense allowance for study participants 148
10.7 Case report form 149

11 Acknowledgments 150

B. Index of figures

Figure 1 TDCS modulates left primary motor cortex excitability bidirectionally. 16
Figure 2 Induction of LTP and LTD at the cellular and the network level. 19
Figure 3 Readiness potential during voluntary movements of the left hand. 22
Figure 4 Cooperativity at the cellular level. 25
Figure 5 Effects of the tangential component of the electric field induced by tDCS. 28
Figure 6 Localization of the SMA in the pilot experiment 1 33
Figure 7 Experiment 1: experimental procedure. 35
Figure 8 Localization of SMA by neuronavigation. 38
Figure 9 Electric field distribution of the novel experimentally applied montage. 40
Figure 10 Electric field distribution of the “classical” montage. 42
Figure 11 Experiment 2: experimental set-up. 45
Figure 12 Experiment 2: experimental procedure. 48
Figure 13 Control experiment 2: experimental procedure. 50
Figure 14 Pilot experiment 1: effects of cooperative $PAS_{M1c \rightarrow M1}$ with $PAS_{SMA \rightarrow M1}$. 56
Figure 15 Online effects of cooperative $PAS_{M1c \rightarrow M1}$ with $PAS_{SMA \rightarrow M1}$ 58
Figure 16 Cooperative effects of $PAS_{M1c \rightarrow M1}$ with $PAS_{SMA \rightarrow M1}$. 59
Figure 17 Online effects of cooperative tDCS with $PAS_{SMA \rightarrow M1}$. 62
Figure 18 Effects of cooperative tDCS with $PAS_{SMA \rightarrow M1}$. 64
Figure 19 Individual responses to cooperative tDCS with $PAS_{SMA \rightarrow M1}$. 65
Figure 20 Effect sizes of anodal tDCS versus cathodal tDCS. 66
Figure 21 Effective SMA-M1 connectivity post cooperative tDCS with $PAS_{SMA \rightarrow M1}$. . 69
Figure 22 Effects of tDCS without $PAS_{SMA \rightarrow M1}$ in the novel montage. 71

C. Index of tables

Table 1 Pilot experiment 1: baseline measures 54
Table 2 Experiment 1: baseline measures 57
Table 3 Experiment 2: baseline measures 60
Table 4 Control experiment 2: baseline measures 70

D. List of abbreviations

A	anterior
AMT	active motor threshold
BL	baseline
BOLD	blood oxygenation level dependent
CF	contralateral forehead
CM	contralateral primary motor cortex
EEG	electroencephalography
EMG	electromyography
FDI	first dorsal interosseus
fMRI	functional magnetic resonance imaging
I	inferior
ISI	interstimulus interval
L	left
LTD	long-term depression
LTP	long-term potentiation
M1	left primary motor cortex
M1c	right primary motor cortex
MEG	magnetoencephalography
MEP	motor evoked potential
MEP_{1mV}	motor evoked potential elicited with SI_{1mV}
MNI	Montreal Neurological Institute
MRI	magnetic resonance imaging
NIBS	non-invasive brain stimulation
OC	occipital
P	post
P1-5	participant 1-5
P0, P20, P30, P40, P60, P90, P120	measurements at 0, 20, 30, 40, 60, 90 and 120 min post intervention
PAS	paired associative stimulation
PAS 1-3	first, second and third block of $PAS_{SMA \rightarrow M1}$
$PAS_{SMA \rightarrow M1}$	PAS of SMA and M1

$PAS_{M1c \rightarrow M1}$	PAS of M1c and M1
pcTMS	paired coil transcranial magnetic stimulation
PD	Parkinson's disease
PIA	pia mater
PMC	premotor cortex
POM	area posterior to the primary motor cortex
POST 1-3	10, 20 and 30 min after $PAS_{SMA \rightarrow M1}$
PRAEC	precentral
PRE 1-3	10, 20, 30 min before $PAS_{SMA \rightarrow M1}$
PRM	area rostral to the primary motor cortex
QPS	quadro-pulse stimulation
R	right
REC	recording electrode
REF	reference electrode
rmANOVA	repeated measures analysis of variance
RMT	resting motor threshold
RMT_{25mm}	RMT determined with a figure-of-eight coil of 25 mm diameter
RMT_{50mm}	RMT determined with a figure-of-eight coil of 50 mm diameter
rTMS	repetitive transcranial magnetic stimulation
S	superior
S1	bipolar electrode in posterior horizontal cortical layer II/III
S2	bipolar electrode in anterior horizontal cortical layer II/III
S3	bipolar electrode in posterior horizontal cortical layer V
S4	bipolar electrode in vertical cortical layer V
SD	standard deviation
SEM	standard error of the mean
SMA	supplementary motor area
SI_{1mV}	stimulus intensity to elicit a mean MEP peak-to-peak amplitude of on average 1 mV in a block of 30 trials at BL
STDP	spike-timing-dependent plasticity
tDCS	transcranial direct current stimulation
TES	transcranial electric stimulation
TMS	transcranial magnetic stimulation

VCA	vertical commissure anterior
WM	white mater

1 Introduction

1.1 The motor system

The motor system is the exclusive cortical output system (Grillner 2003). Already present at birth, the motor system permanently adapts to the demands of the environment (Schaefer et al. 2017). This ongoing learning activity is the basis for the lifelong acquisition of new motor abilities (Schaefer et al. 2017).

Each movement is linked to a specific pattern of coordinated neuronal activity within the motor network (Purves 2012). The network includes several brain regions, such as the premotor cortex (PMC), supplementary motor area (SMA), parietal motor cortex, basal ganglia, cerebellum, and the final integrator and output system of the brain – the primary motor cortex (Purves 2012). Pyramidal cells, Lamina V motor neurons, form the main output of the primary motor cortex (Ghosh and Porter 1988). Most of their afferent input from other brain regions is direct (Sloper 1973). Compared to other species, even primates, the human neocortex significantly differs in having a higher number of pyramidal cells and more layers (Marin-Padilla 2014). This elaborated structure enables higher motor capabilities due to a longer and more variable information flow cascade (Marin-Padilla 2014).

The majority of human pyramidal motor neurons cross the body to synapse with a second motor neuron in the spinal cord (Purves 2012). This second motor neuron innervates the corresponding muscle fibers and causes limb movements contralateral to the active hemisphere (Purves 2012).

While the motor system's output from the primary motor cortex is visible and easily accessible by transcranial magnetic stimulation (TMS), the sequence of neuronal activation before movement execution needs further technologies such as functional magnetic imaging (fMRI) (Grefkes et al. 2008b), electroencephalography (EEG) (Kornhuber et al. 1965, Lange et al. 2004) and magnetoencephalography (MEG) (Gross et al. 2001) for evaluation.

1.2 Transcranial magnetic stimulation

1.2.1 History of TMS

Merton and Morton (1980) were the first to non-invasively provoke hand movements in healthy participants by transcranial electric stimulation (TES). TES uses voltages up to 2000 V to stimulate the human cortex through the intact skull and causes pain in participants (Barker et al. 1985). This limitation was addressed by Barker et al. (1985). Instead of an electric current to directly activate the primary motor cortex, a magnetic field was used to indirectly induce an electric field in the cortex - the hour of birth of TMS (Barker et al. 1985). The registered muscle potential in response to a single magnetic stimulus is "assumed to be due to the current induced in the tissue by the rapid, time-varying magnetic field" (Barker et al. (1985), p. 1107).

1.2.2 Physiology underlying TMS

While the TMS coil is held tangential to the skull, a fast current, passing through the wire inside the coil, induces a magnetic field surrounding the coil (Siebner and Ziemann 2007). This magnetic field induces an electric field in the cortex (Siebner and Ziemann 2007). The magnetic field activates motor neurons both directly and indirectly via interneurons (Di Lazzaro et al. 2004). Thereby, it causes a muscle response of the target muscle, the motor evoked potential (MEP), which can be registered by surface electromyography (EMG) (Di Lazzaro et al. 2004). It is a measure for neuronal excitability (Hallett 2000). A rise in threshold for MEP induction of the resting muscle (RMT) reflects a rise in excitability in a core of neurons and their local density (Hallett 2000). TMS allows the assessment of the motor system at the cortical as well as at the spinal level (Müller and Ziemann 2007). Placed over the skull, the TMS coil activates the first motor neuron in the motor cortex (Müller and Ziemann 2007). Placed over the backbone, the TMS coil directly excites the axons of the second motor neurons in the spinal cord (Müller and Ziemann 2007). Thus, TMS is integral to evaluate motor function in brain research and an important tool in neurological diagnostics (Hallett 2007).

1.2.3 Motor cortex excitability – a reflector of motor learning

Motor learning at the cortical level is mirrored by an increase in MEP amplitude and can noninvasively be evaluated by TMS (Muellbacher et al. 2001, Muellbacher et al.

2002, Ziemann et al. 2004). After motor practice, the primary motor cortex shows increased excitability, which indicates enhanced neuronal activity in the primary motor cortex (Muellbacher et al. 2002). During the acquisition of a new motor task, MEP amplitude elicited by TMS over the primary motor cortex increases (Muellbacher et al. 2001). Contrarily, TMS stimulation of the brainstem or the spinal cord shows no increase in MEP amplitude (Muellbacher et al. 2001). After consolidation of the motor task, maximal force generation remains at the acquired higher level, while MEP amplitude returns to baseline level (Muellbacher et al. 2001).

1.2.4 TMS – a method to test effective connectivity

Kujirai et al. (1993) combine a subthreshold conditioning stimulus, which is a TMS stimulus that is unable to elicit a motor response or a MEP in the target muscle, with a test stimulus above threshold. Depending on the interstimulus interval (ISI) between the pulses, this pairing results either in increase or decrease of the MEP amplitude compared to the amplitude elicited by the test stimulus alone (Kujirai et al. 1993, Ziemann et al. 1996). Short intervals between the stimuli (< 5 ms) cause inhibition (Kujirai et al. 1993), while intervals (> 8 ms) result in facilitation (Ziemann et al. 1996). These paired-pulse protocols (pcTMS) help to gain insight into the interneuronal influence on motor cortex excitability (Ziemann et al. 1996). PcTMS can also test effective connectivity within the motor system (Arai et al. 2012, Civardi et al. 2001, Ferbert et al. 1992, Lafleur et al. 2016). For this application, a conditioning pulse is given to a brain area and a test stimulus is applied over the primary motor cortex. Effective connectivity is expressed as the ratio of the resulting MEP size to the MEP size, which is produced by the test stimulus on its own (Arai et al. 2012). Brain research uses this technique to test the effective connectivity between various parts of the motor system, i.e. M1 and contralateral right motor cortex (M1c) (Ferbert et al. 1992), SMA and M1 (Arai et al. 2012) or PMC and M1 (Civardi et al. 2001). Recently, a test battery of pcTMS protocols was developed to quickly evaluate effective connectivity of the motor system in clinical practice (Lafleur et al. 2016) (Faber et al. 2017a).

1.3 Transcranial direct stimulation

1.3.1 History of tDCS

Fritsch and Hitzig (1870) revolutionized physiology in showing that direct electrical stimulation of the dissected brain can cause limb movements and that the stimulation site determines the character of the movement. Depending on the polarity on the surface, direct current stimulation increases or decreases spontaneous and evoked activity in cats (Creutzfeldt et al. 1962). In rats transcortical polarizing current shifts both evoked and spontaneous firing depending on the used polarity (Bindman et al. 1964). A temporary change of activity in a dense neuronal surrounding is able to induce long-lasting synaptic modifications in the brain (Bindman et al. 1962, Gartside 1968).

1.3.2 TDCS effects on motor cortex excitability

In humans, tDCS induces equivalent excitability changes (Nitsche and Paulus 2000). The "classical" tDCS montage places one electrode over the motor hot spot of the target muscle defined by TMS, and a second electrode over the contralateral eyebrow (Nitsche and Paulus 2000). Anodal stimulation commonly refers to a placement of the anode over M1, while cathodal stimulation is defined by a placement of the cathode over M1 (Nitsche and Paulus 2000) (Figure 1).

Anodal tDCS increases excitability (Nitsche and Paulus 2000). Cathodal tDCS reduces excitability (Ardolino et al. 2005, Nitsche and Paulus 2000) (Figure 1). Analogous to the early direct current studies in animals, these effects are attributed to changes in neuronal membrane potential (Nitsche and Paulus 2000). Anodal tDCS depolarizes underlying cortical neurons, whereas cathodal tDCS hyperpolarizes them (Nitsche and Paulus 2000). Using different tDCS montages, subsequent studies have induced similar changes in M1 excitability with tDCS of the posterior parietal cortex (Rivera-Urbina et al. 2015) or tDCS over the cerebellum with decreased inhibitory tone in case of cathodal stimulation and an increase of inhibitory tone in case of anodal stimulation (Galea et al. 2009).

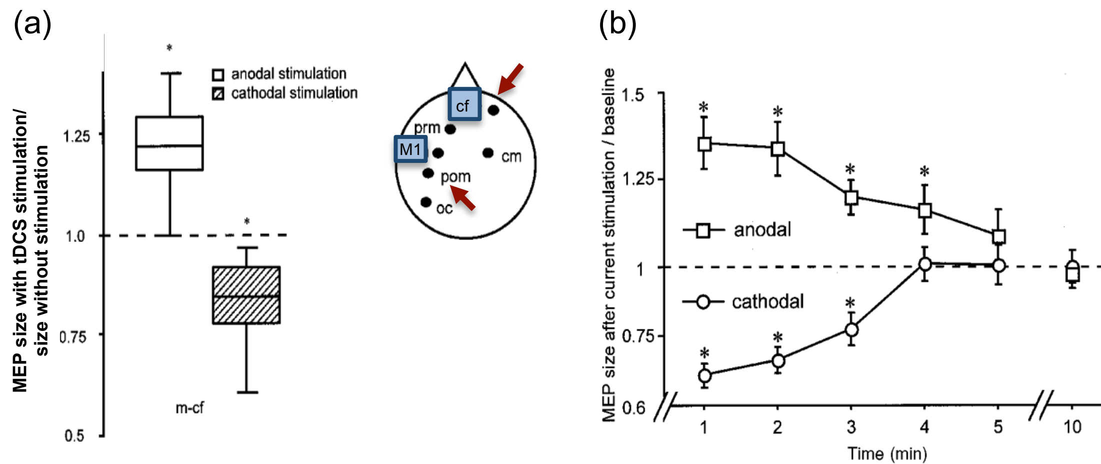


Figure 1 TDCS modulates left primary motor cortex excitability bidirectionally.

a) First report on bidirectional excitability changes induced by transcranial direct current stimulation (tDCS) of the human brain. Anodal tDCS (anode placed over the left primary motor cortex (M1), cathode placed over the contralateral forehead (cf)) increased M1 excitability in n = 10 participants. Contrarily, cathodal tDCS (same electrode position as anodal tDCS with reversed polarities) decreased M1 excitability. Importantly, these effects were only found with stimulation over M1. In control experiments, varied electrode positions caused no changes in M1 excitability (not illustrated here). Blue squares indicate the electrode position, which significantly changed motor evoked potential (MEP) amplitude (electrodes over M1 and cf). Red arrows indicate an electrode position comparable to our montage in experiment 2 (electrodes over cf and an area posterior to the primary motor cortex (pom)). The box plots display the range from the 25th to 75th percentile of the normalized MEP amplitudes during stimulation divided by the normalized MEP amplitudes without stimulation. The horizontal line illustrates the median. The error bars cover the 10th to 90th percentiles. Significant differences between the MEPs with and without stimulation are indicated by asterisks (two-tailed t-test, paired samples, $p < 0.05$) (after (Nitsche and Paulus 2000)). (b) Displayed are the polarity-dependent effects after 5 min of 1 mA anodal tDCS (squares) and cathodal tDCS (circles). Anodal tDCS increased MEP amplitude, while cathodal tDCS decreased MEP amplitude compared to baseline. Mean MEP ± standard error of the mean (SEM) normalized to baseline (y-axis) in dependence of the time (x-axis, in min) of n = 19 participants. Significant MEP amplitude changes post tDCS compared to baseline are indicated by asterisks (two-tailed t-test, paired samples, $p < 0.05$). Abbreviations: cm = contralateral primary motor cortex, oc = occipital, prm = area rostral to the primary motor cortex (from (Nitsche and Paulus 2000)).

1.3.3 Proposed physiological mechanisms underlying tDCS

TMS and tDCS differ in their effects on brain activity. While TMS activates neurons in a constraint region (Di Lazzaro et al. 2004, Opitz et al. 2011, Thielscher et al. 2011), tDCS influences brain activity in a much broader area between the electrodes (Miranda et al. 2013, Opitz et al. 2015). Unlike TMS, tDCS is unable to activate pyramidal neurons. TDCS induces excitability changes in neurons in shifting the membrane potential (Nitsche and Paulus 2000). It affects the probability of spontaneous neuronal firing (Bikson et al. 2004). In this way, tDCS modulates cortical activity (Bergmann et al. 2016).

1.4 The global burden of stroke

1.4.1 The socioeconomic impact of stroke

According to the atlas of heart disease and stroke published by the World Health Organization in 2004, stroke is one leading cause for acquired disability (Mackay et al. 2004). Better blood pressure control reduced the incidence of stroke and better treatment options lowered the mortality rate after a brain insult (Mozaffarian et al. 2016). Nevertheless, stroke remains the only disease with ongoing significant rise of the age-standardized rate of years lived with disability in the US (Murray et al. 2013). It has a huge socioeconomic impact with the loss of earnings due to disability as highest cost contributor (Brown et al. 2006, Mozaffarian et al. 2016). This highly motivates the search for new rehabilitation methods (Hummel and Gerloff 2012). One promising approach for stroke rehabilitation is the use TMS, tDCS and other noninvasive brain stimulation (NIBS) methods (Adeyemo et al. 2012).

1.4.2 TMS – a predictor for motor recovery after stroke

In clinical practice, TMS is used to anticipate motor recovery after stroke (Chen and Winstein 2009, Coupar et al. 2012). The presence of MEPs and somatosensory evoked potentials are the most significant predictors of upper limb recovery (Chen and Winstein 2009, Coupar et al. 2012). Somatosensory evoked potentials are the electrical potentials, which are created in the somatosensory system after peripheral nerve stimulation (Mauguiere et al. 1999). Upper limb recovery directly relates to the later independency in activities of daily life (Coupar et al. 2012). Especially in severely impaired patients, neurophysiological measures, such as MEPs evoked by TMS, are

important prognostic markers, as active participation is not necessary (Hendricks et al. 2002, Stinear 2010).

1.4.3 TMS – a rehabilitation option after stroke

Beyond predicting motor outcome, TMS promotes motor rehabilitation (Adeyemo et al. 2012, Khedr et al. 2005). Repetitive TMS (rTMS) applied over the affected hemisphere in acute stroke patients significantly increases the effects of standard physical and medical therapy (Khedr et al. 2005). However, effect size and duration of TMS and other noninvasive stimulation techniques are limited (Dimyan and Cohen 2011, Lefaucheur et al. 2017). Therefore, various stimulation techniques, i.e. peripheral nerve stimulation and tDCS (Celnik et al. 2009) or bilateral rTMS (Takeuchi et al. 2009), are combined to maximize effects.

1.5 Spike-timing dependent plasticity – a model for learning

1.5.1 LTP and LTD at the cellular level

Hebb (1949) states in his neuropsychological postulate: "when an axon of cell *A* is near enough to excite a cell *B* and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that *A*'s efficiency, as one of the cells firing *B*, is increased" ((Hebb 1949), p.62). Indeed, basic neuroscience proves that neuronal connections can be weakened (long-term depression, LTD) (Dan and Poo 2006) or strengthened (long-term potentiation, LTP) dependent on the specific timing of presynaptic and postsynaptic neuronal activity (Bliss and Lomo 1973, Dan and Poo 2006). Repeated pairing of presynaptic followed by postsynaptic stimulation induces LTP (Bliss and Lomo 1973, Dan and Poo 2006). In contrast, repeated pairing of postsynaptic stimulation with subsequent presynaptic stimulation causes LTD. This process is called spike-timing dependent plasticity (STDP) (Markram et al. 2011) (Figure 2a) (Faber et al. 2017a).

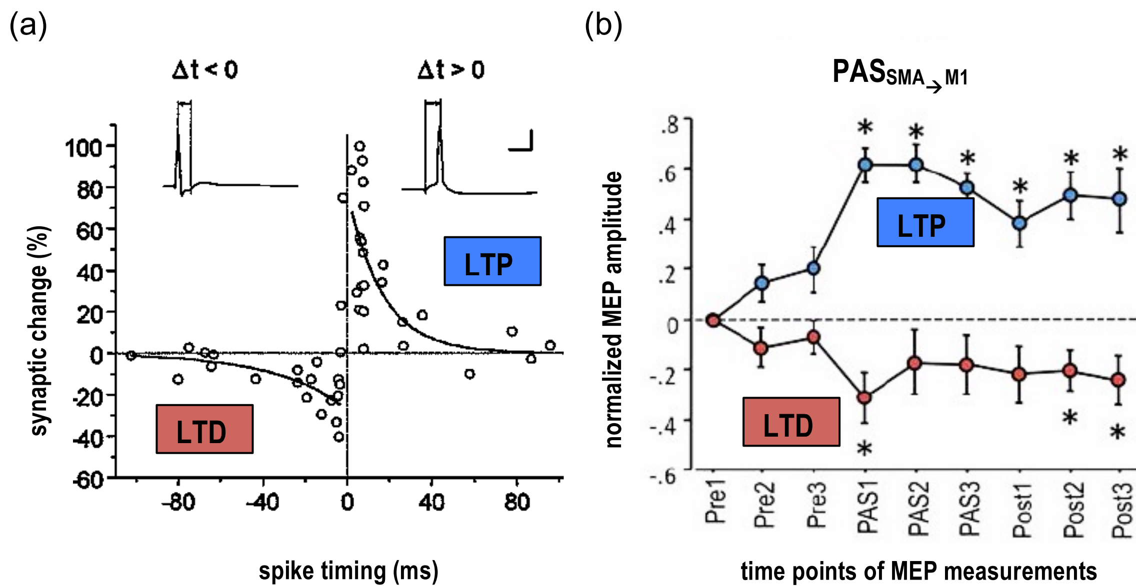


Figure 2 Induction of LTP and LTD at the cellular and the network level.

(a) The induction of long-term potentiation (LTP) and long-term depression (LTD) at the cellular level critically depended on the exact timing of pre- and postsynaptic spikes between hippocampal glutamatergic neurons in culture. Spike timing (Δt) is defined as the interval between the peak of the excitatory postsynaptic potential and the peak of the postsynaptic action potential (x-axis, in ms). The y-axis displays the synaptic change in %. This is the change of the excitatory postsynaptic current amplitude at 20-30 min after repetitive spiking. An exponential function is used to fit both LTP and LTD (after (Bi and Poo 2001)). (b) At the network level corticocortical paired associative stimulation (PAS) of the supplementary motor area (SMA) and the left primary (M1) ($PAS_{SMA \rightarrow M1}$) increased M1 excitability significantly if SMA stimulation preceded bilateral primary motor cortex stimulation by 6 ms (interstimulus interval (ISI) = - 6 ms) (blue circles). M1 excitability decreased if SMA stimulation followed bilateral primary motor cortex stimulation by 15 ms (ISI = 15 ms) (red circles). Note: induction of both LTP-like and LTD-like plasticity by $PAS_{SMA \rightarrow M1}$ was only possible if it was primed by near-synchronous bilateral primary motor cortex stimulation (ISI = 0.8 ms). Displayed are ln-transformed motor evoked potentials (MEP) amplitudes (means ± standard error of the mean (SEM), n = 7) normalized to Pre1 (y-axis) in dependence to the time points of measurements (x-axis). Asterisks indicate significant MEP changes compared to Pre1 ($p < 0.05$ according to post hoc two-tailed t-tests) Abbreviations: Pre1-3 = 10, 20, 30 min before $PAS_{SMA \rightarrow M1}$, PAS 1-3 = first, second and third block of $PAS_{SMA \rightarrow M1}$, Post 1-3 = 10, 20 and 30 min after $PAS_{SMA \rightarrow M1}$ (after (Arai et al. 2011)).

1.5.2 Paired associative stimulation

1.5.2.1 Peripheral PAS

In 2000, Stefan et al. introduced paired associative stimulation (PAS) (Stefan et al. 2000). PAS consists of the repeated pairing of low-frequency electrical stimulation of somatosensory afferents (e.g. of the median nerve at the wrist) with TMS over the contralateral primary motor cortex (Stefan et al. 2000). Similar to the cellular level, the time interval between the two stimuli determines the induction of LTP-like or LTD-like plasticity (Arai et al. 2011, McDonnell et al. 2007, Stefan et al. 2000, Wolters et al. 2003). If the median nerve stimulus precedes the TMS pulse by about 10 ms, the MEP size decreases in a LTD-like manner (Wolters et al. 2003). The median nerve stimulus reaches the cortex about 15 ms after the TMS pulse (Wolters et al. 2003). By contrast, peripheral stimulation 25 ms before the TMS pulse causes a long-lasting (> 30 min) LTP-like increase in primary motor cortex excitability (Stefan et al. 2000). In this situation, the median nerve stimulus excites the cortex either concurrently or shortly before the transcranial magnetic stimulus (Wolters et al. 2003). PAS is topographically specific which means the excitability changes occur in the representation area of the specific hand muscle stimulated by TMS and are absent in other body representations of the primary motor cortex (Stefan et al. 2000). LTP-like plasticity further increases by the consecutive application of PAS protocols in an adequate time window (Müller-Dahlhaus et al. 2015).

1.5.2.2 Corticocortical PAS

Analogous to peripheral PAS, the repeated pairing of two TMS pulses over connected brain areas (corticocortical PAS) changes connectivity in an STDP-like way (Hallett 2007, Müller-Dahlhaus et al. 2010, Rizzo et al. 2009). PAS of SMA and M1 ($PAS_{SMA \rightarrow M1}$) modulates M1 excitability bidirectionally (Arai et al. 2011) (Figure 2b). This PAS-induced excitability modulation critically depends on the ISI between SMA and M1 stimulation. Repeated associative SMA-before-M1 stimulation at an ISI of 6 ms induces a LTP-like M1 excitability increase (Arai et al. 2011). M1-before-SMA stimulation at an ISI of 15 ms induces a LTD-like M1 excitability decrease (Arai et al. 2011) (Figure 2b). In the same manner TMS of other brain areas, such as cerebellum (Lu et al. 2012), posterior parietal cortex (Veniero et al. 2013) and the ventral premotor

cortex (Buch et al. 2011) bidirectionally modulates primary motor cortex excitability, depending on the interstimulus interval (Koch et al. 2013, Veniero et al. 2013). This form of cortical plasticity shows compatible features with the STDP, defined on the cellular level (Müller-Dahlhaus et al. 2010). Corticocortical and peripheral PAS additionally share the same characteristics, i.e. timing and topographical specificity and interaction of consecutive PAS protocols (see Section 1.5.2.1) (Faber et al. 2017a).

1.5.3 Anatomical and neurophysiological properties of the supplementary motor cortex

The supplementary motor cortex consists of pre-SMA, supplementary eye field and the SMA proper from rostral to caudal (Nachev et al. 2008). It is located in the dorsomedial frontal cortex, posterior to the anterior cingulate cortex and anterior to the leg representation of the primary motor cortex (Picard and Strick 1996, Picard and Strick 2001). The SMA, also referred to as SMA proper, is the most posterior part of the supplementary motor cortex. In human imaging studies SMA is divided from pre-SMA based on the vertical anterior commissural (VCA) line, which is a vertical line through the anterior commissure (Arai et al. 2012, Picard and Strick 1996, Vorobiev et al. 1998). In humans, SMA has a somatotopic organization, meaning each area of the body is represented at a specific region of SMA (Fink et al. 1997, Yazawa et al. 2000). It is densely connected to hand knob area of primary motor cortex (Vergani et al. 2014) and both brain areas connect to the same parts of the striatum (Lehericy et al. 2004a). Electrical stimulation of SMA leads to limb movements even after primary motor cortex elimination (Penfield 1950). In monkeys, SMA possesses reciprocal connections to the primary motor cortex (Liu et al. 2002, Luppino et al. 1994) and contributes to the corticospinal tract (Luppino et al. 1994). By contrast, pre-SMA has strong connections to the prefrontal cortex but not to the primary motor cortex (Liu et al. 2002). It shows diffuse, rather than somatotopic, activity during the readiness potential or *Bereitschaftspotential* (Shibasaki and Hallett 2006, Yazawa et al. 2000). This is a negative potential that can be detected by EEG up to 1 s before movement execution (Kornhuber et al. 1965, Nachev et al. 2008, Shibasaki and Hallett 2006). Its maximum amplitude is centrally in the midline (Shibasaki and Hallett 2006). The first part of the readiness potential can be bilaterally registered over pre-SMA and SMA (Shibasaki and Hallett 2006). The later, sharp negatively rising component shows exact somatotopy

and is maximal over the lateral premotor cortex and the primary motor cortex (Shibasaki and Hallett 2006) (Figure 3). In line with the temporal structure of brain activity before movement execution, disruption of motor activity by high-frequency rTMS over the primary motor cortex immediately increases the error rate in a sequential finger movement task (Gerloff et al. 1997). Contrarily, rTMS over SMA rises the error rate not until 1 s after the beginning of the rTMS intervention (Gerloff et al. 1997).

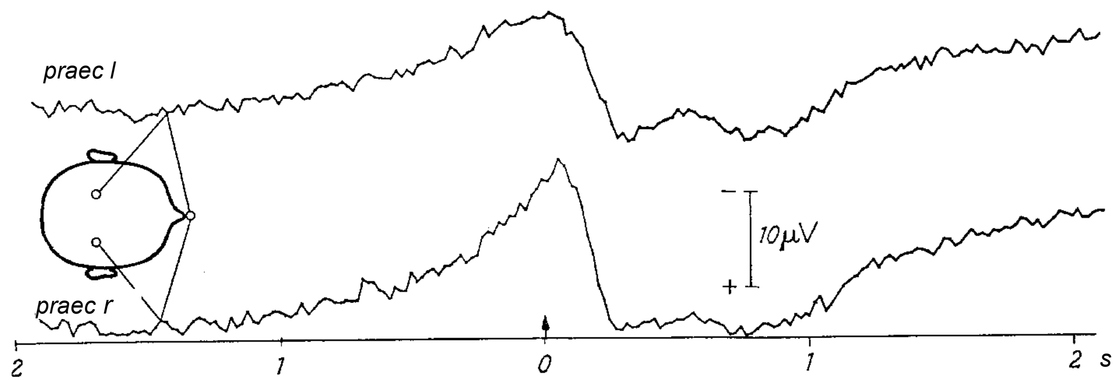


Figure 3 Readiness potential during voluntary movements of the left hand.

Electroencephalography (EEG) recordings during voluntary movements of the left hand in a healthy participant. Unipolar EEG recording of right and left precentral region against the nose with negative value at the top (y-axis, in μV) in dependence of the time (x-axis, in s). The arrow indicates movement onset. Before movement execution a negative potential occurred in the precentral region. It had its maximum over the contralateral, right hemisphere. This potential is called the readiness potential or *Bereitschaftspotential*. Displayed is the average of 512 EEG recordings during voluntary movements of the left hand of one individual. Abbreviations: l = left, praec = precentral, r = right (after (Kornhuber et al. 1965)).

1.5.4 Functional role of the supplementary motor cortex

The supplementary motor cortex is important for self-initiated movements (Nachev et al. 2008). Neurosurgical resection of SMA results in contralateral akinesia, which is the loss of voluntary muscle activity (Laplane et al. 1977). Akinesia is a key symptom of Parkinson's disease (PD). In PD the dopaminergic neurons in the basal ganglia degenerate (Purves 2012). The supplementary motor cortex has a strong connection to the basal ganglia (Lehericy et al. 2004a, Vergani et al. 2014). Repetitive high-frequency transcranial magnetic stimulation over SMA significantly increases motor

abilities in PD patients (Hamada et al. 2008b). The early phase of the readiness potential, which is associated with supplementary motor cortex activation, shows reduced amplitude in PD patients (Dick et al. 1989). Besides improving motor abilities of PD patients, the intake of a precursor of dopamine, levodopa, increases the amplitude of the early but not the late phase of the readiness potential (Dick et al. 1987, Purves 2012). SMA initiates movements but also prevents involuntary movement execution (Sumner et al. 2007). A short presentation of a graspable object leads to activity in SMA proper (Sumner et al. 2007). A healthy brain network suppresses subsequent hand movement, while patients with supplementary motor cortex lesions reach for the object (Sumner et al. 2007). Furthermore, neurons of SMA encode the specific timing of movement sequences (Tanji and Shima 1994). High-frequency rTMS over SMA increases the error rate only in complex finger movements, whereas rTMS over the primary motor cortex augments the error rates in complex and scale finger movements (Gerloff et al. 1997). In monkeys reversible cooling of SMA increases the error rate in motor tasks (Tanji et al. 1985). Disturbing neuronal activity in the supplementary motor cortex increases reaction time (Nakamura et al. 1999, Tanji et al. 1985). In conclusion, the supplementary motor cortex is functionally relevant for movement execution and motor learning with rising activity depending on the complexity of a motor task (Nachev et al. 2008).

1.5.5 Coordinated motor activity after stroke

Movements of the right hand are linked to activity in the contralateral left M1 (Purves 2012). Human fMRI during unimanual fist clenching of the right hand show a rise in effective connectivity between the SMA, the PMC and M1 in the left, contralateral, hemisphere and in M1 activity (Grefkes et al. 2008a). At the same time, neuronal activity in the ipsilateral M1c is inhibited (Grefkes et al. 2008a).

Patients with a residual hand paresis after subcortical stroke have decreased resting state connectivity from parietal cortex to the primary motor cortex and supplementary motor cortex (Inman et al. 2012). Movements of the paretic hand reveal decreased effective SMA to primary motor cortex connectivity and increased inhibitory coupling from contralesional to ipsilesional primary motor cortex (Grefkes et al. 2008b). The connectivity change between ipsilesional SMA and the primary motor cortex positively correlates with the level of motor impairment of the paretic hand (Grefkes et al. 2008b).

After stroke, the grade of motor recovery of the paretic hand from acute to subacute state after stroke correlates with the normalization of the connections in the SMA-PMC-M1 network and between M1c and M1 (Rehme et al. 2011). Downregulation of the increased inhibitory coupling from contralesional primary motor cortex to ipsilesional primary motor cortex by low-frequency, inhibitory rTMS over contralesional primary motor cortex increases the maximal voluntary fist-clenching rate of the paretic hand (Grefkes et al. 2010). Additionally, it enhances effective connectivity between ipsilesional SMA and primary motor cortex (Grefkes et al. 2010). In the healthy, $PAS_{SMA \rightarrow M1}$ can bidirectionally modulate M1 excitability long-term (see Section 1.5.2.2) (Faber et al. 2017a).

1.6 Aim of the study: induction of longer lasting changes in M1

In this thesis, we aimed to develop methods that can induce plasticity in M1 more effectively than hitherto possible. Thereby we were inspired by basic research at the cellular level (Sjöström and Häusser 2006, Sjöström et al. 2001). In their work a concept named cooperativity rescues LTP induction of a subthreshold stimulus (Sjöström et al. 2001) (Figure 4). At the cellular level, a presynaptic stimulus that is subthreshold to cause synaptic LTP, results in a significant increase in synaptic strength, if it is either (1) paired with an extracellular stimulus or (2) postsynaptic depolarization (Sjöström et al. 2001). To transfer those findings to the systems level of human cortex, we conducted two experiments. Experiment 1 examined, whether the cooperative pairing of PAS of M1c to M1 ($PAS_{M1c \rightarrow M1}$) (Rizzo et al. 2011, Rizzo et al. 2009) with $PAS_{SMA \rightarrow M1}$ (Arai et al. 2011) increases M1 excitability. Experiment 2 tested, if cooperative application of anodal tDCS during $PAS_{SMA \rightarrow M1}$ (Arai et al. 2011), contrarily to cathodal or sham tDCS, increases M1 excitability and SMA-M1 connectivity (Faber et al. 2017a).

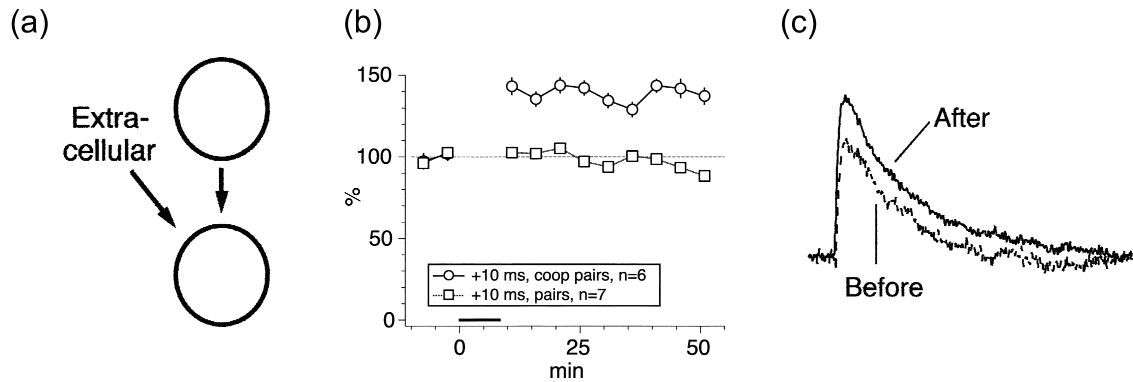


Figure 4 Cooperativity at the cellular level.

(a) Cooperative application of an extracellular stimulus with the pre-before-post spiking of Lamina V cells (white circles) could rescue the induction of long-term potentiation (LTP). 50 pairs of paired stimulation were applied. Simultaneous delivery of a unitary and an extracellular stimulus 10 ms before postsynaptic firing rescued LTP induction. (b) The pre-before-post stimulation alone caused no LTP (squares, n = 7). Contrarily, cooperative extracellular stimulation overcame this threshold and induced LTP (circles, n = 6). Y-axis: change in excitatory postsynaptic amplitude in reference to baseline in %. X-axis: time in min. (c) Displayed is the time curve of the amplitude of the unitary excitatory potential. Cooperative stimulation resulted in a 46% increase in the amplitude of the unitary excitatory postsynaptic ($p < 0.01$) (from (Sjöström et al. 2001)).

1.6.1 Experiment 1: cooperative $PAS_{M1c \rightarrow M1}$ with $PAS_{SMA \rightarrow M1}$

1.6.1.1 Novel MRI-navigated multi-coil technique

At the system level, bilateral priming of the motor cortex (M1 and M1c) before $PAS_{SMA \rightarrow M1}$ is necessary to induce STDP-like plasticity (Arai et al. 2011). Notably, the act of priming itself does not change M1 excitability (Arai et al. 2011). Experiment 1 aimed to induce and systematically test cooperative STDP-like effects without bilateral motor cortex priming by employing a novel time- and site-specific magnetic resonance imaging (MRI) navigated multi-coil PAS technique. It enabled the stimulation of two or more inputs to the target M1 at the same time with target M1 stimulation. The ratio behind this approach was the physiological situation: hand movements involve dynamic coupling of a multitude of projections of motor areas rather than one brain region with the active primary motor cortex (Grefkes et al. 2008a). We cooperatively applied $PAS_{M1c \rightarrow M1}$ (Rizzo et al. 2011, Rizzo et al. 2009) and $PAS_{SMA \rightarrow M1}$ (Arai et al. 2011) and expected stronger and longer enduring STDP-like effects compared to the individual application of both PAS protocols.

1.6.1.2 Rational for the concurrent stimulation of SMA, M1c and M1

Why did we stimulate SMA and M1c concordantly with M1 and not other structures of the motor network? SMA plays an important role for self-initiated hand movements (Nachev et al. 2008) (see Section 1.5.3 and 1.5.4). During voluntary movements coupling of neuronal activity between SMA and target M1 increases while, on the other hand, the coupling between M1c and M1 decreases (Grefkes et al. 2008a). Additionally, reestablishing a good connectivity between those connections during neurorehabilitation is important for a good motor outcome (Grefkes et al. 2010, Rehme et al. 2011) (see Section 1.5.5). One hemisphere's primary motor cortex drives movements of the contralateral hand (Purves 2012). Of note, it also suppresses unwanted ipsilateral hand activity in inhibiting the contralateral primary motor cortex (Stinear and Byblow 2003, Stinear and Byblow 2004). As a consequence, lesions of the primary motor cortex result in contralateral motor impairment and cortical imbalance between the hemispheres (Hummel and Cohen 2006, Hummel and Gerloff 2012). The normalization of increased inhibitory coupling between contralesional and ipsilesional primary motor cortex by low-frequency rTMS over contralesional primary motor cortex improves maximal fist clenching rate of the paretic hand of stroke patients (Grefkes et al. 2010). Treatments after stroke aim to either increase excitability of the ipsilesional or decrease excitability of the contralesional hemisphere to regain this cortical balance (Hummel and Cohen 2006, Hummel and Gerloff 2012).

1.6.1.3 Translation of the cellular concept of cooperativity to the network level

An important precondition to translate the cellular concept of cooperativity to the network level (Sjöström and Häusser 2006, Sjöström et al. 2001) is the simultaneous application of two protocols that are on its own subthreshold to cause a significant excitability change in M1: (1) $PAS_{SMA \rightarrow M1}$ without bilateral near-simultaneous primary motor cortex priming is incapable to enhance M1 excitability (Arai et al. 2011); (2) $PAS_{M1c \rightarrow M1}$ does not induce significant MEP changes (Rizzo et al. 2011, Rizzo et al. 2009). Experiment 1 compared a PAS condition with adequate timing (condition 1) to a control condition with inadequate timing to induce changes (condition 2) (Arai et al. 2011, Rizzo et al. 2009). We expected a rise in M1 excitability exclusively in the condition 1 (see Section 2.1.1.8).

1.6.2 Experiment 2: cooperative tDCS with $PAS_{SMA \rightarrow M1}$

1.6.2.1 Novel tDCS montage with increased tangential field strength

The “classical” tDCS montage mainly causes tangential fields even under the electrode (Nitsche and Paulus 2000, Rahman et al. 2013). The tangential component primarily affects neurons with a parallel orientation to the current flow (Rahman et al. 2013) (Figure 5). The impact of direct current stimulation on axon terminals is two to three times higher compared to somas (Rahman et al. 2013). Transmitter release enhances if the soma is directed towards the cathode and the axon terminal is oriented towards the anode. Presynaptic hyperpolarization increases action potentials and results in enhanced transmitter release (Hubbard and Willis 1962a, Hubbard and Willis 1962b). On the other hand, depolarization decreases action potentials and transmitter release (Hubbard and Willis 1962c) (Figure 5).

The location of SMA is in the central frontal region of the cortex (Lehericy et al. 2004b). The hand area of M1 is localized more posterior and lateral in the precentral gyrus (Lehericy et al. 2004b). Hence, we assumed in a simplistic model, that the connecting fibers between SMA and M1 run from anterior-medial to posterior-lateral in parallel to the cortical surface and the induced tangential field. Anodal stimulation would result in somatic depolarization and dendritic hyperpolarization and increase synaptic efficiency (Rahman et al. 2013).

Modeling indicates that the tangential field component of the electric field is highest in front of the tDCS electrodes (Miranda et al. 2013). We modeled the electric field during tDCS and created based on this data a novel tDCS montage to induce higher tangential field strength in M1, at the location where SMA-M1 axon terminals end. In hyperpolarizing axon terminals, the enhanced tangential current flow during anodal tDCS in our novel montage would increase synaptic strength of the SMA-M1 connection more effectively than hitherto possible (for further details, see Section 2.2.1) (Faber et al. 2017a).

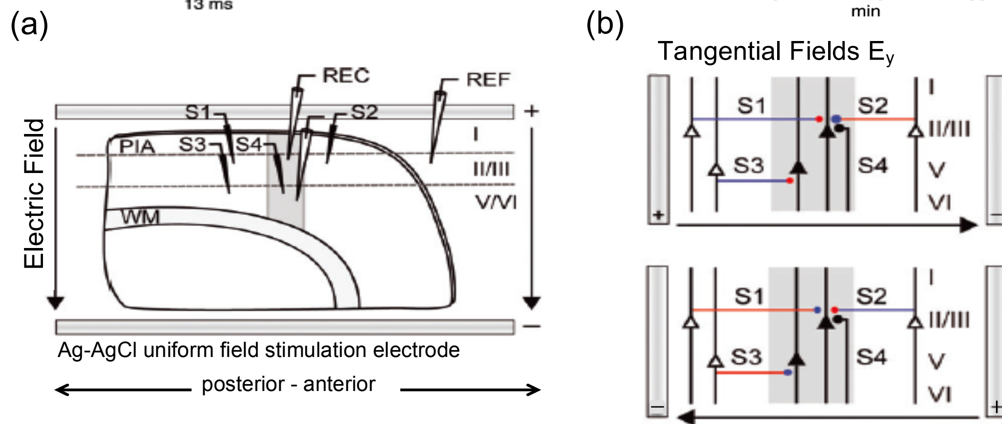


Figure 5 Effects of the tangential component of the electric field induced by tDCS.

(a) Experimental set-up: a constant current passed through parallel Ag-AgCl wires in a bath across the rat motor cortex slice and generated uniform extracellular electric fields. In order to examine different synaptic pathways, bipolar electrodes in four different locations in the grey matter (S1-S4) were used for stimulation (posterior horizontal layer II/III (S1); anterior horizontal layer II/III (S2); posterior horizontal layer V (S3); and vertical layer V to II/III (S4)). The stimulation electrode was always positioned 500 μm from the recording electrode (REC). (b) Expected polarization pattern of specific synaptic pathways due to the induced tangential field E_y, which is thought to be responsible for the tDCS effects. The direct current field depolarizes (red), hyperpolarizes (blue) or does not affect (black) somas, dendrites and axons. Triangles indicate pyramidal cells. Circles indicate somas of afferent input. Abbreviations: PIA = pia mater, REF = reference electrode, WM = white matter (from Rahman et al. (2013)).

1.6.2.2 Translation of the cellular concept of cooperativity to the network level

In experiment 2 our hypothesis was that cooperative anodal tDCS in our novel montage, in contrast to cathodal or sham tDCS, with $PAS_{SMA \rightarrow M1}$ would rise M1 excitability and SMA-M1 connectivity (Arai et al. 2012). At the cellular level, LTP induction by pairing of pre-synaptic and post-synaptic action potentials of excitatory synapses often requires, that the presynaptic action potential causes a sufficiently high excitatory postsynaptic potential (Sjöström and Häusser 2006, Sjöström et al. 2001). Cooperative depolarization during paired pre- and postsynaptic stimulation lowers the threshold for LTP induction (Sjöström and Häusser 2006, Sjöström et al. 2001). TDCS polarizes the human cortex (see Section 1.3). An essential requirement for cooperativity is the pairing of two stimulation protocols that are subthreshold to increase M1 excitability. We cooperatively applied (1) $PAS_{SMA \rightarrow M1}$, a PAS protocol that needs prior

bilateral near-synchronous primary motor cortex stimulation to rise M1 excitability (Arai et al. 2011) with (2) a short tDCS protocol in a more posterior tDCS montage which would not induce plasticity in M1 (Nitsche and Paulus 2000). To confirm subthreshold tDCS stimulation we additionally conducted a control experiment, which evaluated the effects of the application of tDCS alone in our novel montage (Faber et al. 2017a).

2 Material and methods

2.1 Experiment 1: test of cooperative effects of $PAS_{M1c \rightarrow M1}$ with $PAS_{SMA \rightarrow M1}$

2.1.1 Pilot experiment

2.1.1.1 Subjects

Five subjects participated in a pilot experiment (mean age ± standard error of the mean (SEM), 29.3 ± 1.6 years, age range 24-34; 4 females). They were screened for contraindications to TMS (Rossi et al. 2011). Participants with a history of neurological or psychiatric disease, use of CNS active drugs or any other drugs (including nicotine and alcohol) were excluded. The study conformed to the latest version of the Declaration of Helsinki and was approved by the local Ethics Committee of the Medical Faculty of the Eberhard Karls University Tübingen, Germany (Project-number: 654/2012BO1).

2.1.1.2 MRI data acquisition

All subjects participated in MRI experiments before. The acquired anatomical T_1-weighted images were used in our neuronavigation system.

2.1.1.3 Recording of motor evoked potentials

The participants were seated in a comfortable reclining chair with a pillow placed under the right arm. Participants were asked to stay awake and keep their eyes open during the experiment. Due to the influence of even slight involuntary muscle activation on corticospinal excitability (Ridding et al. 1995) and plasticity induction (Müller-Dahlhaus and Ziemann 2015), subjects were instructed to relax both arms and hands during the whole experiment – except during the measurement of the active motor threshold. We used Ag-AgCl cup electrodes in a belly-tendon montage to record MEPs from the resting first dorsal interosseous (FDI) of the right hand. We monitored complete muscle relaxation visually by high-gain EMG (50µV/division) during the measurements. We amplified, band-pass filtered (20Hz - 2kHz; D360 amplifier, Digitimer, Hertfordshire, UK) and digitized the EMG raw signal at a rate of 10kHz (CED Micro 1401; Cambridge Electronic Design, Cambridge, UK) for offline analysis (Arai et al. 2012) (Faber et al. 2017a).

2.1.1.4 Localization and stimulation of M1

We applied focal TMS over the hand area of the left M1 to detect the motor hot spot of the right FDI at the beginning of each session (Arai et al. 2012). This is the TMS coil position that resulted consistently in the largest MEPs at a slightly suprathreshold stimulus intensity (Müller-Dahlhaus and Ziemann 2015). It was registered with a marker on the individual MRI in the neuronavigation system (Localite GmbH, St. Augustin, Germany) and marked with a pen on the scalp to guarantee a fix coil position during the whole experiment (Arai et al. 2012). We used a figure-of-eight coil (diameter of each wing, 50 mm; handle orthogonal to coil plane) that was connected to a Magstim 200^2 magnetic stimulator with a monophasic current waveform (The Magstim Company, Carmarthenshire, Wales, UK) (Arai et al. 2012). We held the coil tangential to the scalp, with an angle of the coil junction of 45° away from the midline, to induce a current directed from posterior-lateral to anterior-medial in M1 (Arai et al. 2012). This current direction mainly activates corticospinal neurons transsynaptically by excitatory interneurons (Di Lazzaro et al. 2008, Rossini et al. 2015). We defined RMT as the minimal stimulus intensity, which elicited MEPs > 50 μV in at least five out of ten consecutive trials (Groppa et al. 2012, Rossini et al. 1994, Rossini et al. 2015). We used the relative frequency method to determine RMT to the nearest 1% of maximum stimulator output (MSO) (Groppa et al. 2012). RMT was determined using a figure-of-eight coil with a diameter of 50 mm of each wing and handle orthogonal to coil plane (RMT_{50mm}) (Arai et al. 2012). In the next step, we determined SI_{1mV} in the right FDI. SI_{1mV} is the stimulus intensity to elicit mean MEP peak-to-peak amplitude of on average 1mV in a block of 30 trials at baseline (BL) (Arai et al. 2012). This number of trials gives a reliable estimation of the corticospinal excitability (Cuypers et al. 2014). The inter-trial interval in all MEP measurements of this study varied randomly between 4 and 6 s to reduce the subject's anticipation of the next trial. We used a small 'branding iron' figure-of-eight coil (diameter of each wing, 25 mm) to measure RMT (RMT_{25mm}) and the active motor threshold (AMT) of the right FDI (Arai et al. 2012). AMT was defined as the lowest stimulator intensity, that caused in at least five out of ten successive trials during a slight voluntary isometric contraction of the FDI (~10% of maximal voluntary strength) MEPs of > 200 μV (Groppa et al. 2012, Rossini et al. 1994, Rossini et al. 2015) (Faber et al. 2017a).

2.1.1.5 Localization and stimulation of M1c

To detect the motor hot spot of the left FDI in M1c we proceeded in the same way as for the right FDI (see Section 2.1.1.4). We used a second figure-of-eight coil (diameter of each wing, 50mm; handle orthogonal to coil plane) that was connected to another Magstim 200^2 magnetic stimulator with a monophasic current waveform (The Magstim Company, Carmarthenshire, Wales, UK) (Arai et al. 2012). We marked the position with a pen on the scalp to guarantee the same coil position throughout the experiment and determined RMT and SI_{1mV} of the left FDI (Arai et al. 2012). A detailed description of the procedure can be found under Section 2.1.1.4.

2.1.1.6 Localization of the SMA

Based on the individual MRI, we drew a line between anterior and posterior commissure (anterior-posterior commissure line) (Arai et al. 2012). The VCA line, which is the vertical line on the anterior-posterior commissure line through the anterior commissure, is a standardized anatomical separator of pre-SMA and SMA proper (Arai et al. 2012, Picard and Strick 1996, Vorobiev et al. 1998). The figure-of-eight coil was centered over the intersection between the subject's scalp and the VCA line and in tangential orientation to the surface of the subject's head (Figure 6) (Arai et al. 2012). The coil position was marked at the subject's head. The distance between coil and the vertex (CZ) in cm was noted to confirm stable positioning between sessions (distance CZ) (Table 1) (Arai et al. 2012).

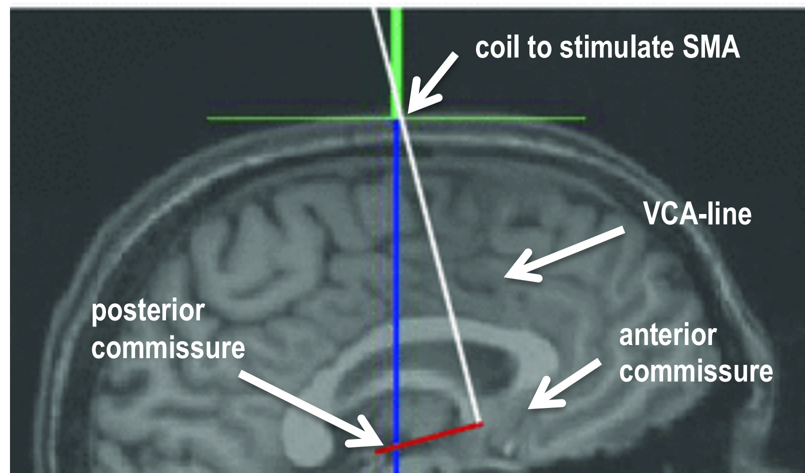


Figure 6 Localization of the SMA in the pilot experiment 1.

Sagittal image through the midline of a subject's head. For the anatomical separation of supplementary motor area (SMA) from pre-SMA, we used the vertical commissure anterior line (VCA-line) (white line). This is the line through the anterior commissure that is orthogonal to the line that connects anterior and posterior commissure (red line). The target of the coil to stimulate the supplementary motor area (SMA) was the intersection between the VCA-line and the head's surface. The coil orientation was tangential to the head's surface with induced current from medial to lateral (green line). The blue line displays the orthogonal projection of the intersection of the SMA coil (after (Arai et al. 2012)).

2.1.1.7 Stimulation of the supplementary motor area

We used a small figure-of-eight coil (diameter of each wing, 25 mm, handle orthogonal to coil plane) connected to a Magstim 200^2 magnetic stimulator with a monophasic current waveform (The Magstim Company, Carmarthenshire, Wales, UK) to stimulate the left SMA proper (Arai et al. 2012). The coil was held tangential to the scalp with an angle of the coil junction perpendicular to the midline (Arai et al. 2012). This resulted in an induced current direction from medial to lateral in left SMA (Arai et al. 2012). The stimulator intensity for SMA stimulation was set at 140% AMT as determined over M1 (for description of AMT acquisition, see Section 2.1.1.4) (Arai et al. 2012). Previous experiments of our group confirm these parameters as most effective for SMA stimulation (Arai et al. 2012, Arai et al. 2011) (Faber et al. 2017a).

2.1.1.8 Paired associative stimulation

We compared two different PAS conditions (Figure 7). Condition 1 consisted of the supposed optimal ISIs for cooperative $PAS_{M1c \rightarrow M1}$ with $PAS_{SMA \rightarrow M1}$. The parameters were taken from two previous studies, which showed effective paired associative stimulation of the single connections: $PAS_{SMA \rightarrow M1}$ (ISI = 6 ms) (Arai et al. 2011, Civardi et al. 2001) and $PAS_{M1c \rightarrow M1}$ (ISI = 8 ms) (Rizzo et al. 2011, Rizzo et al. 2009). In a control condition 2 SMA, M1 and M1c were stimulated at ISIs unsuitable to induce LTP-like excitability in M1 (SMA → M1: ISI = - 3 ms (Arai et al. 2011); M1c → M1: ISI = -1 ms (Rizzo et al. 2009)).

Each PAS condition consisted of one block of 50 trials with an inter-trial interval of 5 s. M1 was stimulated at intensity SI_{1mV} (right FDI), SMA at intensity of 140% AMT as determined over M1 (right FDI) and M1c at SI_{1mV} intensity (left FDI) (Arai et al. 2011). Whenever the individual anatomy did not allow optimal positioning of all three coils, we ensured stable positioning of the SMA coil as defined by the neuronavigation system for two reasons: Firstly, the figure-of-eight-coil used for SMA stimulation was the smallest and therefore, most focal coil (Thielscher et al. 2015). Secondly, SMA stimulation does not cause a direct readout such as MEP amplitude, which would be an easy detectible indicator for even slight deviations from the hot spot. Consequently, stable positioning of the SMA coil was particularly critical. We slightly deviated from the optimal coil orientation for M1 and M1c stimulation, whenever unavoidable, but kept the coil position (Di Lazzaro et al. 2004). As a result, we had to increase the stimulation intensity to elicit MEPs of on average 1 mV peak-to-peak amplitude (Di Lazzaro et al. 2004). For details concerning the localization and stimulation of M1, M1c and SMA see Sections 2.1.1.4 – 2.1.1.7.

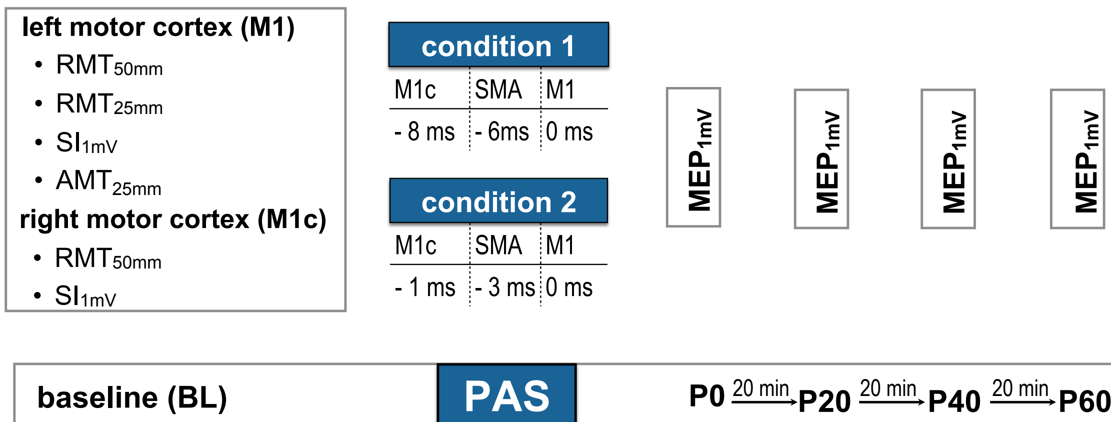


Figure 7 Experiment 1: experimental procedure.

Time line of experiment: paired associative stimulation (PAS) consisted of 50 pulses of PAS of the supplementary motor area (SMA), the right motor cortex (M1c) and the left motor cortex (M1). The tables display the used interstimulus intervals (ISI) in ms for stimulation of SMA, M1c and M1 (Arai et al. 2011, Rizzo et al. 2009). The ISIs varied between conditions 1 and 2. We determined the stimulus intensity to elicit a mean motor evoked potential (MEP) peak-to-peak amplitude of on average 1mV in a block of 30 trials at BL (SI_{1mV}) in the right first dorsal interosseus muscle (FDI) at baseline (BL). This stimulus intensity was used to elicit motor evoked potentials (MEP_{1mV}) immediately (P0), and 20 min (P20), 40 min (P40) and 60 min (P60) after the PAS intervention to assess the effects of the PAS. In the condition 1 the ISIs for the triple stimulation of SMA, M1c and M1 were optimal to induce cooperative LTP-like plasticity changes in M1, while in the control condition 2 the ISIs were inadequate to induce a LTP-like excitability increase (Arai et al. 2011, Rizzo et al. 2009). Abbreviations: AMT = active motor threshold, BL = baseline, RMT_{25mm} = resting motor threshold using 25 mm figure-of-eight coil, RMT_{50mm} = resting motor threshold using 50 mm figure-of-eight coil.

2.1.1.9 Time line of experiments

The readouts of the experiment are displayed in Figure 7. At BL we measured RMT_{50mm}, RMT_{25mm}, SI_{1mV} and AMT for M1 (right FDI) and RMT_{50mm} and SI_{1mV} for M1c (left FDI). Readouts were obtained 0, 20, 40, 60 min after the application of cooperative $PAS_{M1c \rightarrow M1}$ with $PAS_{SMA \rightarrow M1}$ (P0, P20, P40, P60). We used the same stimulus intensity as determined at BL (SI_{1mV}) throughout all post-intervention measurements. We obtained 30 MEPs at a rate of 0.1 Hz with a random inter-trial interval variation of 25% to limit anticipation of the next trial. Cooperative $PAS_{M1c \rightarrow M1}$ with $PAS_{SMA \rightarrow M1}$ consisted of 5 min stimulation with the ISIs of either condition 1 or 2,

described under Section 2.1.1.8 (Figure 7). Each subject participated in condition 1 and condition 2. Sessions in a given individual were separated by at least 3 days to limit carry-over effects. The experiments were performed in a pseudo-randomized, double-blinded crossover design.

2.1.1.10 Data analysis and statistics

The 10% smallest and largest MEPs were excluded from analysis to protect data from outliers.

Baseline measures of cortical excitability

One-way repeated measures analysis of variance (rmANOVA) was performed to test for variability of the baseline measures.

Time course of MEP amplitude

The data was normally distributed according to the Shapiro-Wilk test. We performed a two-way rmANOVA to test for an effect of PAS condition on SI_{1mV} with the within-subject effects of CONDITION (two levels: condition 1, condition 2) and TIME (five levels: BL, P0, P20, P40, P60).

Whenever Mauchly’s test indicated a violation of sphericity, we used the Greenhouse-Geisser correction. Data was analyzed using software (SPSS ver. 22.0.0.01 for Mac; SPSS Inc.). A p-value < 0.05 was considered significant. All data were displayed as mean ± SEM.

2.1.2 Main experiment

2.1.2.1 Subjects

Fourteen subjects participated in experiment 1 (mean age ± SEM, 26.8 ± 0.9 years, age range 22 - 34; 4 females). One subject was excluded from analysis as experiments were performed based on a head model instead of the individual MRI-images. Two other subjects cancelled participation due to scheduling conflicts. All subjects were right-handed according to the Edinburgh Handedness Inventory (Oldfield 1971) and were screened for contraindications to TMS (Rossi et al. 2011). Participants with a history of neurological or psychiatric disease, use of CNS active drugs or use of any other drugs

(including nicotine and alcohol) were excluded (Arai et al. 2012). The study conformed to the latest version of the Declaration of Helsinki and was approved by the local Ethics Committee of the Medical Faculty of the Eberhard Karls University Tübingen, Germany (Project-number: 654/2012BO1).

2.1.2.2 Localization of SMA

We identified the target SMA proper anatomically based on the individual MRI. We took coordinates for SMA proper from previous functional magnetic resonance studies (x = 0, y = - 6, z = 69; Montreal Neurological Institute (MNI) coordinate system) (Zhang et al. 2012). These coordinates were taken from the high-level seeds of the study (Zhang et al. 2012). Their placement 10 mm below cortical surface most likely corresponds to the TMS stimulation site in the brain (Zhang et al. 2012). For calculating the optimal coil position, we used the entry-target-function of the Localite system (Localite GmbH, St. Augustin, Germany), which automatically calculates the nearest point of the head's surface to a specific brain region (Figure 8). We indicated the location for SMA stimulation with a pen on the scalp to double check stable positioning during the experiment (Arai et al. 2012). MRI acquisition, MEP recording, localization and stimulation of M1 and M1c, the PAS protocol as well as experimental procedure were the same as in the pilot experiment – please see the Sections 2.1.1.2 – 2.1.1.5 and 2.1.1.7 - 2.1.1.8 for further details (Faber et al. 2017a).

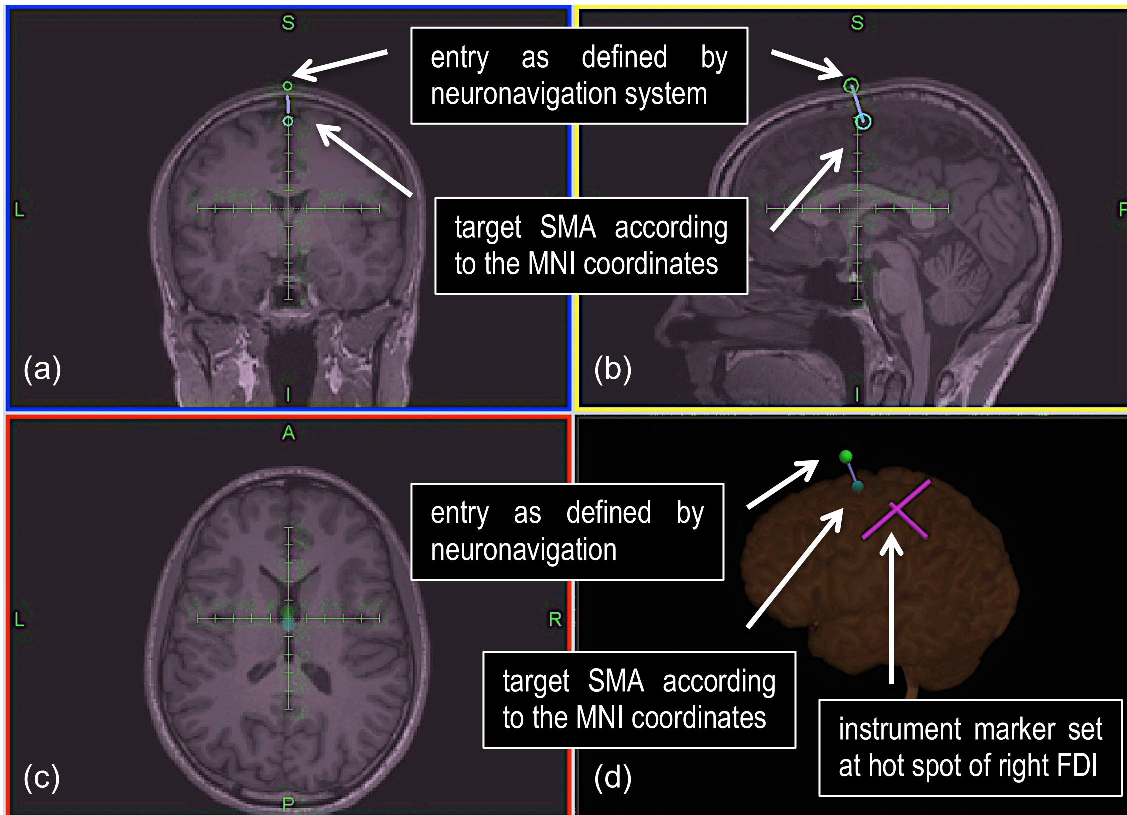


Figure 8 Localization of SMA by neuronavigation.

Screenshot of the neuronavigation system that was used for targeting the supplementary motor area (SMA) (Localite GmbH, St. Augustin, Germany). The target SMA (blue circle) was defined on the coordinates in the Montreal Neurological Institute (MNI) system (x = 0, y = - 6, z = 69; MNI coordinate system) (Zhang et al. 2012). The entry (green circle) was calculated by the software. It is defined as the closest point to the target SMA on the head's surface. The hot spot of the right first dorsal interosseus (FDI) on the left primary motor cortex (M1) was defined by transcranial magnetic stimulation (TMS) and marked with an instrument marker (pink cross in (d)). (a) Coronal, (b) sagittal and (c) axial two-dimensional views and (d) three-dimensional view of the brain. The green crosshairs in (a) - (c) define the planes of the other two-dimensional views (a) - (c). Abbreviations: A = anterior, I = inferior, L = left, P = posterior, R = right, S = superior.

2.1.2.3 Data analysis and statistics

The 10% smallest and largest MEPs were excluded from analysis to protect data from outliers. The data set was not normally distributed according to the Shapiro-Wilk test. For this reason, we used logarithmized data.

Baseline measures of cortical excitability

Variability of the baseline measures was evaluated by one-way rmANOVA.

Online effects of cooperative $PAS_{M1c \rightarrow M1}$ with $PAS_{SMA \rightarrow M1}$ on M1 excitability

The 50 MEPs recorded during cooperative $PAS_{M1c \rightarrow M1}$ with $PAS_{SMA \rightarrow M1}$ were split in BINs of 10 (5 BINs total) (Fuhl et al. 2015). We normalized the data to BIN 1 (Fuhl et al. 2015). A two-way rmANOVA was performed to test for an effect of CONDITION with the within-subject effects of CONDITION (two levels: condition 1, condition 2) and BIN (five levels: BIN 1-5) (Fuhl et al. 2015).

Effects of cooperative $PAS_{M1c \rightarrow M1}$ with $PAS_{SMA \rightarrow M1}$ on M1 excitability

To test for effects of cooperative $PAS_{M1c \rightarrow M1}$ with $PAS_{SMA \rightarrow M1}$ on M1 excitability we proceeded like in the pilot experiment (see Section 2.1.1.10).

Whenever Mauchly's test indicated a violation of sphericity we used the Greenhouse-Geisser correction. Data were analyzed using software (SPSS ver. 22.0.0.01 for Mac; SPSS Inc.). A p-value < 0.05 was considered significant. All data were displayed as mean ± SEM.

2.2 Experiment 2: test of cooperative effects of tDCS with $PAS_{SMA \rightarrow M1}$

2.2.1 Novel tDCS electrode montage with increased tangential electric field component

Modeling of the electric field on the human brain during tDCS reveals the biggest magnitude of the tangential component between the electrodes, while it is comparably small under the electrodes (Miranda et al. 2013, Opitz et al. 2015, Salvador et al. 2015). Therefore, we placed the tDCS electrode 3 cm posterior to M1 to increase the tangential field strength in M1. This electrode montage also protected the tDCS electrode from uncontrolled heating and current induction by the TMS coil. The electric field simulations were performed with Simnibs (Thielscher et al. 2015, Windhoff et al. 2013), using the example head model provided with the software. We modeled absolute electric field strength as well as its radial and tangential components during anodal and cathodal tDCS of the experimentally applied montage (Figure 9) and the "classical" montage (Nitsche and Paulus 2000) (Figure 10). This is the montage commonly used for M1 stimulation (Nitsche and Paulus 2000) (Faber et al. 2017a).

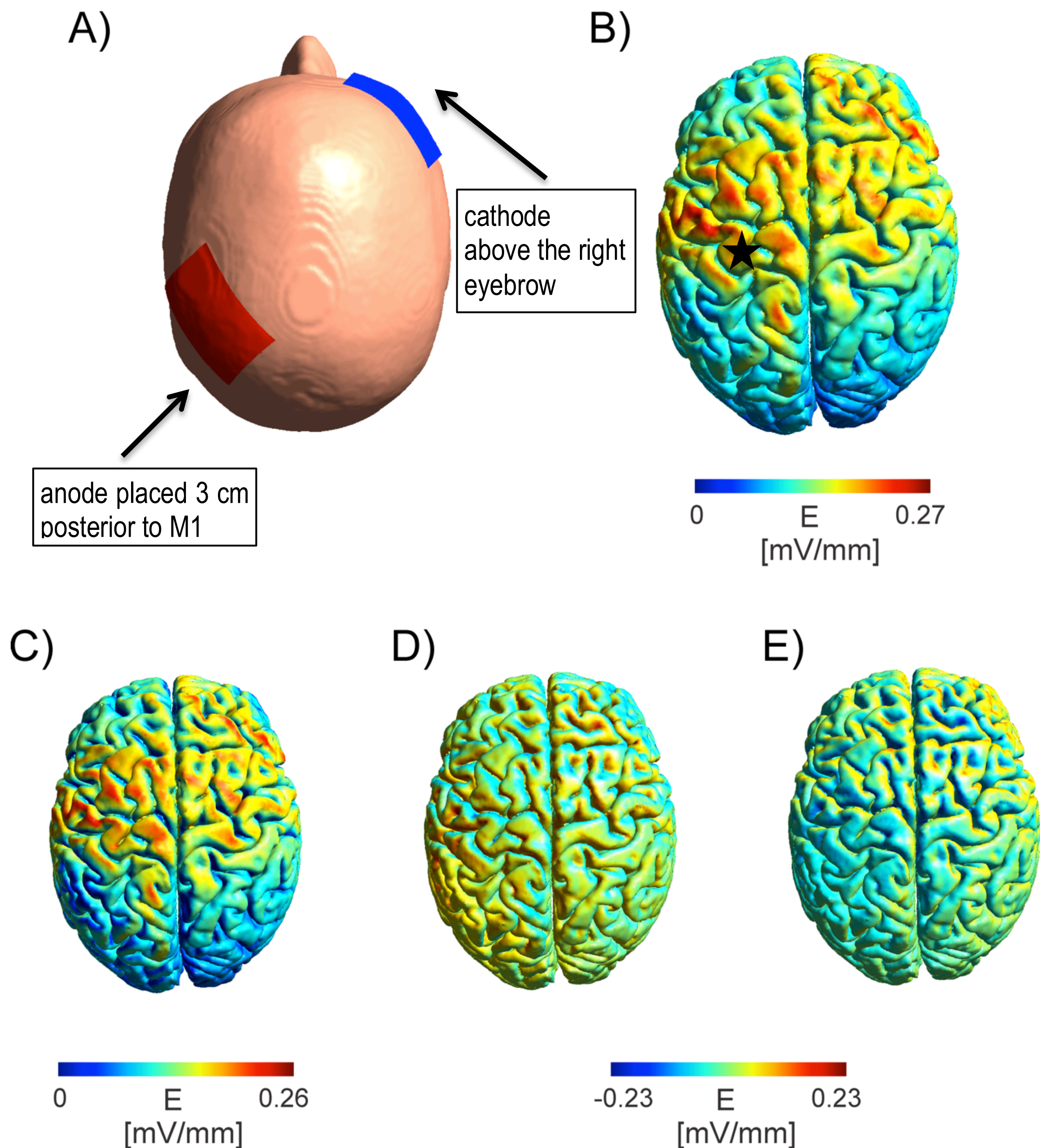


Figure 9 Electric field distribution of the novel experimentally applied montage.

A) Electrode montage used for experiment 2. The anode (red) was shifted 3 cm behind the left primary motor cortex (M1) and the cathode (blue) was placed over the contralateral supraorbital region. As we defined the current direction by the electrode close to M1, this electrode arrangement refers to anodal stimulation. B) Display of the absolute electric field strength with high values in the regions between the electrodes. In particular, at the gyrus precentralis the electric field strength was augmented compared to the "classical" montage (Figure 10 B). C) Tangential electric field strength with highest values at superficial cortical regions. It is enhanced at the gyrus precentralis compared to the "classical" montage (Figure 10 C). D) Display of the inflowing (positive values) and outflowing (negative values) components of the perpendicular electric field, when the anode is placed behind M1. At the motor cortex both inflowing and outflowing components occurred simultaneously. E) Display of

the inflowing and outflowing components of the perpendicular electric field, when the cathode is placed behind M1. It had in- and outflowing currents in M1 with the reversed pattern compared to anodal stimulation. All electric field simulations were made with Simnibs on the program's example head model (Thielscher et al. 2015, Windhoff et al. 2013). In figures B - E the electric field strength E (in mV/mm scaled for a stimulation intensity of 1 mA) is color-coded with blue indicating low values and red indicating high values of electric field strength. Asterisk indicates hand area of M1. A broader analysis of the effects of electrode position in reference to M1 can be found in (Opitz et al. 2015) (after (Faber et al. 2017a)).

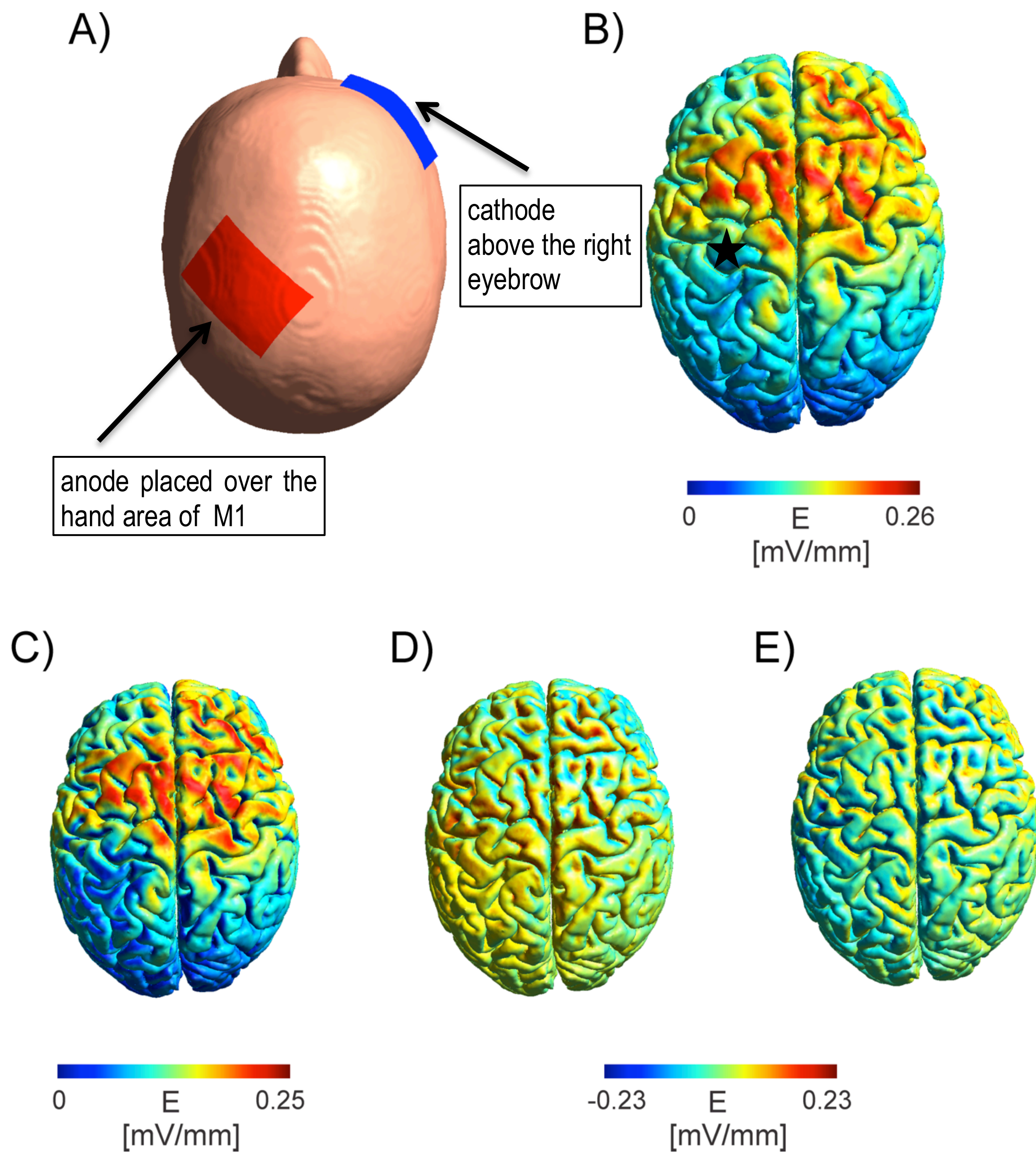


Figure 10 Electric field distribution of the "classical" montage.

A) In the conventional montage for tDCS motor cortex stimulation the anode (red) is directly placed over left primary motor cortex (M1) and the cathode (blue) is placed over the contralateral supraorbital region for anodal stimulation (cathodal stimulation with reversed polarity of electrodes) (Nitsche and Paulus 2000). B) The absolute electric field strength has high values in the regions between the electrodes. The electric field strength at M1 was decreased compared to our experimentally applied montage (Figure 9 B). C) Display of the tangential electric field strength with strongest values at cortical crowns. Note its reduction in M1 compared to the experimentally applied montage (Figure 9 C). D) Display of inflowing (positive values) and outflowing (negative values) components of the perpendicular electric field in the "classical" montage during anodal stimulation. At the motor cortex both

inflowing and outflowing components occurred simultaneously. E) Display of inflowing and outflowing components of the perpendicular electric field, when the cathode is directly positioned over M1 (cathodal stimulation). Compared to the experimentally applied montage the perpendicular electric field strength in M1 was enhanced in the "classical" montage (Nitsche and Paulus 2000). All electric field simulations were made with Simnibs on the program's example head model (Thielscher et al. 2015, Windhoff et al. 2013). In all figures, the electric field strength E (in mV/mm scaled for a stimulation intensity of 1 mA) is color-coded with blue indicating low values and red indicating high values of electric field strength. Asterisk indicates hand area of M1. A broader analysis of the effects of electrode position in reference to M1 can be found in (Opitz et al. 2015) (after (Faber et al. 2017a)).

2.2.2 Subjects

Twenty subjects were enrolled in the experiment. Three subjects participated in the pilot experiment to develop the experimental set-up. Two subjects canceled participation due to scheduling problems. Fifteen healthy male subjects (age range: 24 - 31 years; mean age ± SEM: 25.8 ± 0.61 years) were included in the final analysis of the study. Female participants were excluded as menstrual cycle-related effects on cortical excitability can potentially interfere with TMS measures of excitability and TMS-induced plasticity (Inghilleri et al. 2004, Smith et al. 2002, Smith et al. 1999). All experiments were performed in the afternoon to minimize diurnal variability of PAS effects due to fluctuations of cortisol levels over the day (Sale et al. 2008). All subjects were right-handed according to the Edinburgh Handedness Inventory (laterality score (mean ± standard deviation (SD)): 0.85 ± 0.21) (Oldfield 1971). Subjects were screened for contraindications to TMS (Rossi et al. 2011) and underwent a physical examination before each experiment. Subjects with a history of neurological or psychiatric disease, use of CNS active drugs or use of any other drugs (including nicotine and alcohol) were excluded (Arai et al. 2012).

The study conformed to the latest version of the Declaration of Helsinki and was approved by the local Ethics Committee of the Medical Faculty of the Eberhard Karls University Tübingen, Germany (Project number: 046/2014BO2). All subjects provided written informed consent prior to participation (Faber et al. 2017a).

2.2.3 MRI data acquisition

We acquired navigated high-resolution T1-weighted anatomical images (MPRAGE; magnetization-prepared rapid gradient echo) to be used for our neuronavigation system

for participants, who had no anatomical T1-images from previous studies. We used a 3T scanner (Magnetom Prisma^{fit}, syngo MR D13D; Siemens) and the following parameters TR = 2300 ms, TI = 900 ms, TE = 4.18 ms, flip angle = 9°, matrix size = 256 x 256 x 176, resolution = 1x1x1 mm.

For details concerning the recording of MEPs, the localization and stimulation of M1 and localization and stimulation of M1c see Sections 2.1.1.3 - 2.1.1.5. For the localization and stimulation of SMA we preceded as outlined in Sections 2.1.2.2 and 2.1.1.7 (Faber et al. 2017a).

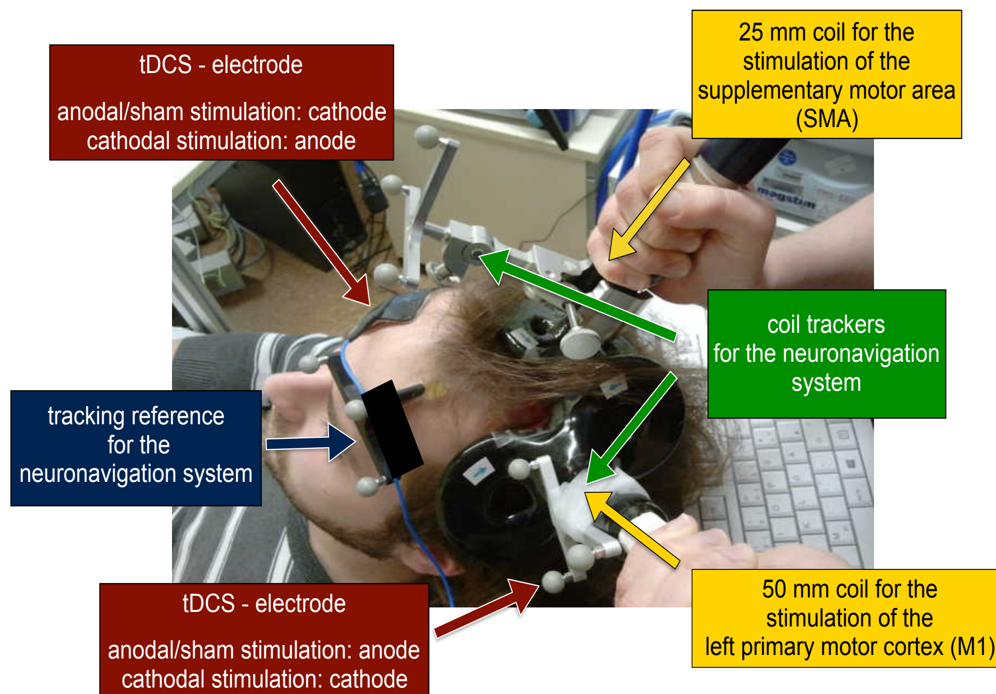


Figure 11 Experiment 2: experimental set-up.

We used a neuronavigation system (Localite GmbH, St. Augustin, Germany) during the simultaneous, cooperative application of transcranial direct current stimulation (tDCS) and the paired associative stimulation (PAS) of the connection between supplementary motor area (SMA) and the left primary motor cortex (M1) ($PAS_{SMA \rightarrow M1}$). One tDCS electrode was placed over the right eyebrow, the other tDCS electrode 3 cm behind M1 (indicated in red). We defined the stimulation polarity by the electrode close to M1. A figure-of-eight coil with outer diameter of 50 mm was used for M1 stimulation and a figure-of-eight coil with outer diameter of 25 mm for SMA stimulation (indicated in yellow). We attached a tracker of the neuronavigation system to each coil to control for stable positioning throughout the experiment (indicated in green). The tracking reference to localize the participant's head is marked in blue (after (Faber et al. 2015, Faber et. al. 2016))

2.2.4 Effective SMA-M1 connectivity

We tested effective SMA-M1 connectivity in line with a previously developed pcTMS protocol (Arai et al. 2012). We stimulated the left SMA with the induced current directing from medial to lateral and stimulus intensity of 140% AMT as measured over M1 (see Section 2.1.1.4) with a 25-mm figure-of-eight-coil. SMA stimulation was followed by M1 stimulation at ISI = 6 ms with the induced current in anterior-medial direction at SI_{1mV}. If necessary, the M1 stimulus intensity was readjusted to ensure average peak-to-peak MEP amplitude of 1 mV of the unconditioned MEPs before each

measurement of effective SMA-M1 connectivity. At each time point (BL, P0 - P120), 30 unconditioned and 30 conditioned MEPs were recorded (Arai et al. 2012).

2.2.5 Transcranial direct current stimulation

We used a battery-driven constant current stimulator (neuroConn, Ilmenau, Germany) and a pair of electrodes (35 cm^2) covered with TEN 20 electrode paste (Weaver and Company, Colorado, USA) to deliver 1 mA tDCS with a current density = 0.0286 mA/cm^2. We delivered two blocks of 5 min anodal or cathodal stimulation separated by a 5-min interval. Five min of tDCS neither cause persistent changes in M1 excitability assessed with TMS (Nitsche and Paulus 2000) nor in functional connectivity within the motor network in fMRI (Amadi et al. 2014). This failure to induce overt plasticity was an important prerequisite for our study. We aimed to test whether the cooperative STDP effects seen at the cellular level translate to the network level (Sjöström and Häusser 2006, Sjöström et al. 2001): two NIBS protocols - $PAS_{SMA \rightarrow M1}$ and tDCS - both insufficient to affect excitability by its own - would cause cooperative STDP-like effects if administered simultaneously.

We used 30 s of anodal tDCS with current intensity of 1 mA for sham stimulation (Gandiga et al. 2006). The tDCS current intensity was ramped up and down over 10 s for on- and offset during anodal, cathodal and sham stimulation (Gandiga et al. 2006). One electrode was attached above the orbita on the right forehead, contralateral to the stimulated M1 like in the "classical" montage (Nitsche and Paulus 2000). The position of the stimulating electrode for the left M1 varied: it was placed 3 cm posterior to the motor hot of the right FDI as determined by TMS (Figure 9) (Faber et al. 2017a).

2.2.6 Paired associative stimulation

For $PAS_{SMA \rightarrow M1}$ we adapted a protocol that was recently developed to boost M1 excitability (Arai et al. 2011). For a more effective SMA stimulation, we deviated from the current direction from anterior to posterior, to medial to lateral (Arai et al. 2012). We applied two instead of three blocks of 50 trials of $PAS_{SMA \rightarrow M1}$ to use a protocol that on its own is subthreshold to potentiate M1 excitability long-term. Besides, we forwent bilateral priming of the primary motor cortex, which is necessary to significantly increase M1 excitability (Arai et al. 2011). We applied $PAS_{SMA \rightarrow M1}$ in two blocks of 50 trials with an inter-trial interval of 6 s with 25% of variability and an inter-block

interval of 5 min for coil cooling. SMA simulation was performed 6 ms before M1 stimulation with an induced current orientation from medial to lateral and a stimulus intensity of 140% AMT (see Sections 2.1.2.2 and 2.1.1.7). M1 was stimulated at SI_{1mv} and an induced current direction from posterior-lateral to anterior-medial (see Section 2.1.1.4) (Faber et al. 2017a).

2.2.7 Time line of experiments

MEP amplitude and effective SMA-M1 connectivity (Arai et al. 2012) were measured at BL, P0, P30, P60, P90, and P120 after $PAS_{SMA \rightarrow M1}$. We determined SI_{1mV} at BL and used it for MEP_{1mV} measurements at P0-P120. We acquired 30 MEPs at a rate of 0.1 Hz with an intertrial variation of 25% to reduce the anticipation of the next trial. For cooperative PAS, two 5-min blocks of tDCS and $PAS_{SMA \rightarrow M1}$ with an inter-block interval of 5 min were applied simultaneously (Figures 11-12). All subjects attended three sessions (PAS + anodal tDCS, PAS + cathodal tDCS, PAS + sham tDCS) in a pseudo-randomized, double-blinded crossover design. To restrain carry-over effects, two subsequent sessions in each subject lay at least 1 week apart (Faber et al. 2017a).

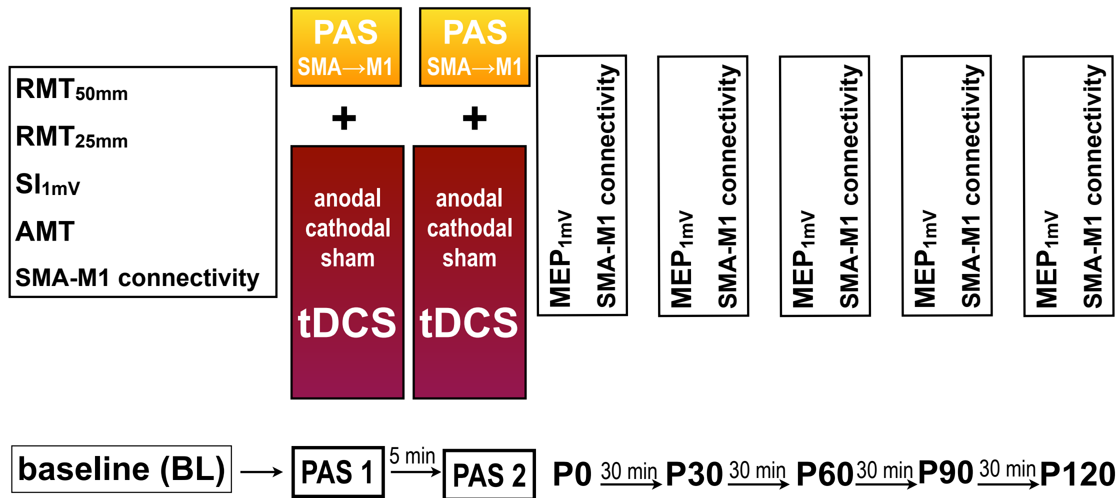


Figure 12 Experiment 2: experimental procedure.

Time line of experiment 2. Baseline measurements at the target muscle of the right hand (RMT_{50mm}, SI_{1mV}, RMT_{25mm}, AMT and effective SMA-M1 connectivity) were followed by the intervention. It consisted of the application of two 5 min blocks of transcranial direct current stimulation (tDCS) and paired associative stimulation (PAS) of SMA and M1 ($PAS_{SMA \rightarrow M1}$) separated by an interblock interval of 5 min. TDCS was applied at an intensity of 1 mA with one electrode 3 cm posterior to the M1 hand representation and the other electrode over the contralateral eye brow. $PAS_{SMA \rightarrow M1}$ consisted of the paired associative stimulation of the SMA-M1 connection with SMA stimulation 6 ms before M1 stimulation (Arai et al. 2011, Arai et al. 2012). To test effective SMA-M1 connectivity (SMA-M1 connectivity), 30 conditioned and 30 unconditioned motor evoked potentials (MEPs) were applied (Arai et al. 2012). For the conditioned MEPs, SMA was stimulated 6 ms before M1 at a stimulus intensity of 140% AMT with induced current direction from medial to lateral (Arai et al. 2012). Unconditioned MEPs resulted from stimulation of M1 alone (Arai et al. 2012). For the measurements of effective SMA-M1 connectivity, we readjusted the stimulator intensity to elicit MEPs of on average 1 mV peak-to-peak amplitude at P0 - P120 (Arai et al. 2012). The readouts at P0 - P120 consisted of MEP_{1mV} and effective SMA-M1 connectivity (Arai et al. 2012). Abbreviations: AMT = active motor threshold, BL = baseline, M1 = left primary motor cortex, MEP_{1mV} = MEP elicited with SI_{1mV}, P0, P30, P60, P90 and P120 = measurements at 0, 30, 60, 90 and 120 min post intervention, PAS 1 = first block of cooperative tDCS with $PAS_{SMA \rightarrow M1}$, PAS 2 = second block of cooperative tDCS with $PAS_{SMA \rightarrow M1}$, RMT_{25mm} = resting motor threshold using 25 mm figure-of-eight coil, RMT_{50mm} = resting motor threshold using 50 mm figure-of-eight coil, SI_{1mV} = stimulus intensity to elicit a mean MEP peak-to-peak amplitude of on average 1 mV in a block of 30 trials at BL, SMA = supplementary motor area (after (Faber et al. 2017a)).

2.2.8 Control experiment 2

A control experiment was performed to test the effects of tDCS alone (i.e., without PAS $_{SMA \rightarrow M1}$) (Figure 13). Thirteen male participants (age range: 23-28 years, mean age (mean $\pm$ SEM): 25.2 $\pm$ 1.6) took part in this experiment. They were right-handed according to the Edinburgh handedness inventory (laterality index (mean $\pm$ SD): 0.89 $\pm$ 0.10) (Oldfield 1971). Three subjects canceled participation due to problems with their schedules. Female participants were excluded as menstrual cycle-related effects on cortical excitability potentially interfere with measures of excitability and TMS-induced plasticity (Inghilleri et al. 2004, Smith et al. 2002, Smith et al. 1999). All experiments were performed in the afternoon to minimize diurnal variability of PAS effects due to fluctuations of cortisol levels over the day (Sale et al. 2008). Subjects were screened for contraindications to TMS and underwent a physical examination before each experiment (Rossi et al. 2011). Subjects with a history of neurological or psychiatric disease, use of CNS active drugs or use of any other drugs (including nicotine and alcohol) were excluded. The study conformed to the latest version of the Declaration of Helsinki and was approved by the local Ethics Committee of the Medical Faculty of the Eberhard Karls University Tübingen, Germany (Project number: 046/2014BO2). All subjects provided written informed consent prior to participation.

TDCS was performed in three experimental conditions (anodal, cathodal and sham tDCS). The same electrode montage and stimulation parameters were used as in experiment 2 (for a detailed description see Section 2.2.5 (Figure 9) (Faber et al. 2017a, Faber et al. 2017b).

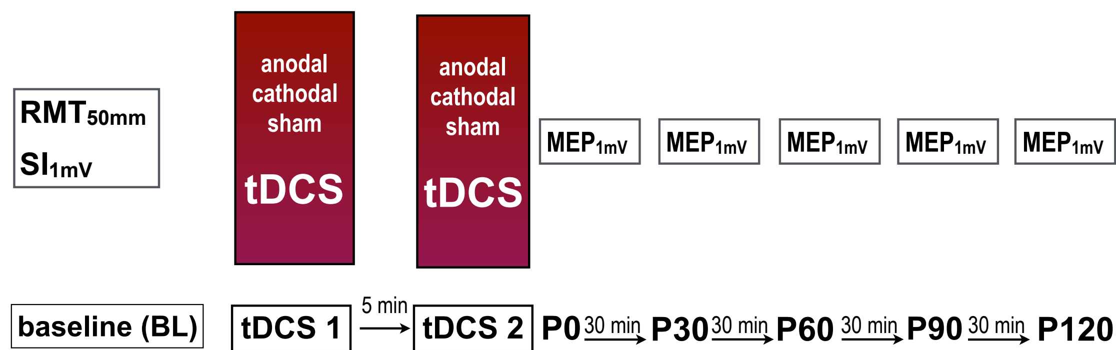


Figure 13 Control experiment 2: experimental procedure.

Time line of control experiment 2. After measuring RMT_{50mm} and SI_{1mV} of the right first dorsal interosseus (FDI) at baseline (BL), we applied two 5-min blocks of transcranial direct current stimulation (tDCS) with an inter-block interval of 5 min (tDCS 1, tDCS 2). At P0 - P120 we stimulated at SI_{1mV} and used MEP_{1mV} of the FDI as readout. Abbreviations: MEP = motor evoked potential, MEP_{1mV} = MEP elicited with SI_{1mV}, P0, P30, P60, P90 and P120 = measurements at 0, 30, 60, 90 and 120 min post intervention, RMT_{50mm} = resting motor threshold using 50 mm figure-of-eight stimulating coil, SI_{1mV} = stimulus intensity to elicit a mean MEP peak-to-peak amplitude of on average 1 mV in a block of 30 trials at BL (Faber et al. 2017a, Faber et al. 2017b) (after (Faber et al. 2017a)).

2.2.9 Data analysis and statistics

10% of the highest and lowest MEPs of each time point were excluded from analysis to protect the data from outliers. As the data set was not normally distributed according to Shapiro-Wilk test, we used the ln-transformed data. Whenever Mauchly's test indicated a violation of sphericity, we used the Greenhouse-Geisser correction.

Experiment 2

Baseline measure of cortical excitability

We performed one-way rmANOVA to test for variability of the baseline measures.

Online-effects of tDCS on $PAS_{SMA \rightarrow M1}$

To evaluate the online-effect of tDCS on $PAS_{SMA \rightarrow M1}$, we took the 100 conditioned pulses during the two $PAS_{SMA \rightarrow M1}$ blocks (SMA was always stimulated 6 ms before M1). We split the recorded MEPs in consecutive order in BINs of ten and averaged each of these BINs (Fuhl et al. 2015). We normalized the BINs to BIN 1 and performed a rmANOVA with the within-subject factors CONDITION (three levels: PAS + anodal

tDCS, PAS + sham tDCS, PAS + cathodal tDCS) and BIN (ten levels: BIN 1 – 10) (Fuhl et al. 2015).

Main effect of TIME and CONDITION

To test for effects of TIME and CONDITION on MEP amplitude, we conducted a two-way rmANOVA with the within-subject effects of TIME (six levels: BL, P0, P30, P60, P90, P120) and CONDITION (three levels: PAS + anodal tDCS, PAS + cathodal tDCS, PAS + sham tDCS).

Modulatory effects of anodal and cathodal tDCS

The graph of experiment 2 indicates, that anodal and cathodal tDCS, irrespective of tDCS polarity, reversed or at least diminished the $PAS_{SMA \rightarrow M1}$ effects of the sham-tDCS condition (Figure 18). We therefore performed the following additional data-driven analyses. To quantify the effect size of anodal and cathodal tDCS induced changes on MEP amplitude of $PAS_{SMA \rightarrow M1}$ (absolute tDCS-effect), we calculated the absolute anodal and cathodal tDCS effect of the ln-transformed data with the following procedure. For the absolute anodal tDCS effect, we took the grand average of normalized MEP amplitude at all time points after $PAS_{SMA \rightarrow M1}$ + anodal tDCS minus the grand average of normalized MEP amplitudes at all time points after $PAS_{SMA \rightarrow M1}$ + sham tDCS $\left(\frac{\sum P_{i_{anodal}}}{5BL_{anodal}} - \frac{\sum P_{i_{sham}}}{5BL_{sham}}\right)$ with $i = 0, 30,\ 60,\ 90,\ 120$. For the computation of the absolute effect of cathodal tDCS we proceeded accordingly. We calculated the grand average of normalized MEP amplitudes at all time points after $PAS_{SMA \rightarrow M1}$ + cathodal tDCS minus the grand average of normalized MEP amplitudes at all time points after $PAS_{SMA \rightarrow M1}$ + sham tDCS $\left(\frac{\sum P_{i_{cathodal}}}{5BL_{cathodal}} - \frac{\sum P_{i_{sham}}}{5BL_{sham}}\right)$, with $i = 0, 30,\ 60,\ 90,$ 120. To further evaluate the effect size and the relationship between the different tDCS polarities on $PAS_{SMA \rightarrow M1}$, a linear regression analysis was performed. We used the Spearman correlation, as data were not normally distributed.

Effective SMA-M1 connectivity

Effective SMA-M1 connectivity was expressed as the ratio between the mean conditioned over the mean unconditioned MEP (Arai et al. 2012). 10% highest and

lowest of unconditioned and conditioned MEPs were excluded from our analysis to protect data from outliers. To test for an effect of CONDITION and TIME on SMA-M1 connectivity, we conducted a two-way rmANOVA with the within-subject effects of CONDITION (three levels: PAS + anodal tDCS, PAS + cathodal tDCS, PAS + sham tDCS) and TIME (six levels: BL, P0, P30, P60, P90, P120). As the TIME*CONDITION interaction was significant, the following post-hoc analysis was conducted: in order to compare two PAS conditions, we performed three two-way rmANOVA with the within-subject effects of CONDITION (two levels: always containing two out of the following conditions: PAS + cathodal tDCS, PAS + anodal tDCS or PAS + sham tDCS) and TIME (six levels: BL, P0, P30, P60, P90, P120). In case of significance we compared the respective two conditions at each time point using the paired t-test. We used the Bonferroni correction for multiple testing.

Control experiment 2

Baseline measure of cortical excitability

One-way rmANOVA was performed to test for variability of the baseline measures.

Main effect of TIME and CONDITION

We conducted a two-way rmANOVA with the within-subject effects of CONDITION (three levels: anodal tDCS, cathodal tDCS, sham tDCS) and TIME (six levels: BL, P0, P30, P60, P90, P120) to test for the effects of the different stimulation protocols on MEP amplitude.

Immediate tDCS effects

Previous experiments using similarly brief tDCS protocols report short-lasting polarity-dependent MEP alterations (Nitsche and Paulus 2000, Nitsche and Paulus 2001). Anodal tDCS increases and cathodal tDCS decreases MEP amplitude (Nitsche and Paulus 2000, Nitsche and Paulus 2001). To compare immediate tDCS effects, we normalized the MEP amplitude acquired after anodal tDCS at P0 to the MEP amplitudes acquired after sham tDCS at PO. For cathodal tDCS the same procedure was performed. The MEP amplitude acquired after cathodal tDCS at P0 was normalized to the MEP amplitudes acquired after sham tDCS at P0. We performed a

paired t-test on the normalized data in the two conditions to test for differences between the tDCS conditions.

Software (SPSS ver. 22.0.0.01 for Mac; SPSS Inc.) was used for data analysis of experiment 2. We displayed all data as means ± SEM. A p-value < 0.05 was considered significant (Faber et al. 2017a, Faber et al. 2017b).

3 Results

3.1 Experiment 1: cooperative $PAS_{M1c \rightarrow M1}$ with $PAS_{SMA \rightarrow M1}$

3.1.1 Pilot experiment

3.1.1.1 Baseline measures of the pilot experiment

The baseline measures did not differ between condition 1 and 2 (Table 1).

Table 1 Pilot experiment 1: baseline measures

Group mean data (mean ± standard error of the mean (SEM), n = 5) of MEP_{1mV} and AMT in M1 in mV and MEP_{1mV} in M1c in mV and the distance CZ of SMA from the vertex in cm at baseline in the two experimental conditions. Condition 1: SMA stimulation 6 ms before M1 stimulation and M1c stimulation 8 ms before M1 stimulation. Control condition 2: SMA stimulation 3 ms before M1 stimulation and M1c stimulation 1 ms before M1 stimulation. RmANOVA did not reveal a significant effect of condition for any of the baseline measures of corticospinal excitability (all $p > 0.05$). Abbreviation: AMT = active motor threshold determined over M1, CZ = vertex, distance CZ = distance between SMA and CZ in cm, FDI = first dorsal interosseus, M1 = left primary motor cortex, M1c = right primary motor cortex, MEP = motor evoked potential, MEP_{1mV} = motor evoked potential elicited with SI_{1mV}, %MSO = percentage of maximum stimulator output, SI_{1mV} = stimulus intensity to elicit a mean MEP peak-to-peak amplitude of on average 1 mV in a block of 30 trials at BL in the FDI, TMS = transcranial magnetic stimulation, rmANOVA = repeated measures analysis of variance.

pilot experiment 1				
	M1		M1c	SMA
	MEP_{1mV} (mV)	AMT (%MSO)	MEP_{1mV} (mV)	distance CZ (cm)
condition 1	1.00 ± 0.06	50.00 ± 3.67	1.16 ± 0.08	3.30 ± 0.34
condition 2	0.99 ± 0.07	50.20 ± 3.29	1.01 ± 0.10	3.50 ± 0.27
F	0.02	0.05	1.80	1.00
p	0.90	0.83	0.25	0.37

3.1.1.2 Effects of cooperative $PAS_{M1c \rightarrow M1}$ with $PAS_{SMA \rightarrow M1}$ stimulation on M1 excitability

The rmANOVA revealed no effect of CONDITION ($F_{1, 5} = 0.068$, $p = 0.805$), TIME ($F_{4, 20} = 1.566$, $p = 0.222$) or CONDITION*TIME ($F_{1.90, 9.49} = 0.481$, $p = 0.623$) interaction (Figure 14) on MEP excitability after cooperative $PAS_{M1c \rightarrow M1}$ with $PAS_{SMA \rightarrow M1}$.

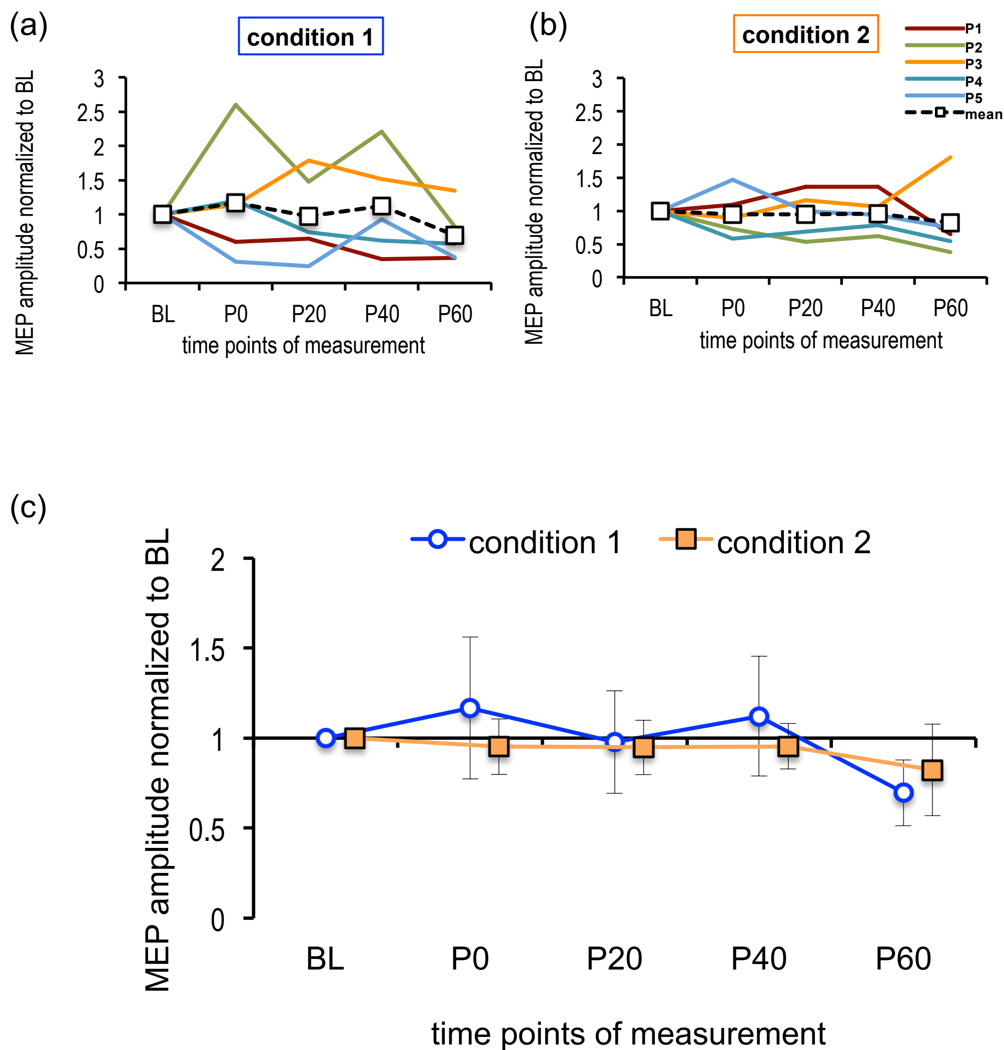


Figure 14 Pilot experiment 1: effects of cooperative $PAS_{M1c\rightarrow M1}$ with $PAS_{SMA\rightarrow M1}$.

(a,b) Single subject data (colored lines (P1-5)) and overall mean (black dashed line) in condition 1 (a) and condition 2 (b) from the pilot experiment (n = 5). Displayed is the individual mean MEP amplitude at baseline (BL) and immediately (P0), 20 min (P20), 40 min (P40) and 60 min (P60) after paired associative stimulation (PAS) of the supplementary motor area (SMA), the left primary motor cortex (M1) and the right primary motor cortex (M1c) ($PAS_{M1c\rightarrow M1}$ with $PAS_{SMA\rightarrow M1}$). Note the variance across subjects in condition 1 (c) Time course of mean MEP amplitude in condition 1 (blue) and condition 2 (orange) at BL, P0, P20, P40 and P60. The two PAS conditions did not result in significant M1 excitability change. Displayed is the mean ± standard errors of the mean (SEM) for n = 5 participants. The x-axis shows time points of measurements. The y-axis the MEP amplitude normalized to BL. Abbreviations: MEP = motor evoked potential, P1-5 = participant 1-5, $PAS_{M1c\rightarrow M1}$ = paired associative stimulation of M1c and M1, $PAS_{SMA\rightarrow M1}$ = paired associative stimulation of SMA and M1.

3.1.2 Main experiment

3.1.2.1 Baseline measures of corticospinal excitability

None of the one-way rmANOVAs to compare the baseline measures of corticospinal excitability was significant (Table 2). Baseline measures for RMT_{50mm}, RMT_{25mm} were acquired in only 9 of 11 subjects. As the other baseline parameters did not differ between the two experimental conditions in all 11 subjects, we included all subjects in the main analysis.

Table 2 Experiment 1: baseline measures

Group mean data (mean ± standard error of the mean (SEM), n = 11) of the baseline measures in condition 1 (SMA stimulation 6 ms and M1c stimulation 8 ms before M1 stimulation) and condition 2 (SMA stimulation 3 ms and M1c stimulation 1 ms before M1 stimulation) (Arai 2011). One-way rmANOVA of each baseline measure of cortical excitability did not reveal a significant effect of condition (all $p > 0.05$). Note: Resting motor threshold using the 50 mm figure-of-eight coil (RMT_{50mm}) and resting motor threshold using 25 mm figure-of-eight coil (RMT_{25mm}) were acquired in only 9 of 11 subjects. Abbreviations: AMT = active motor threshold, M1 = left primary motor cortex, M1c = right primary motor cortex, MEP = motor evoked potential, MEP_{1mV} = motor evoked potential elicited with SI_{1mV}, %MSO = percentage of maximum stimulator output, SI_{1mV} = stimulus intensity to elicit a mean MEP peak-to-peak amplitude of on average 1 mV in a block of 30 trials at BL, SMA = supplementary motor area, rmANOVA = repeated measures analysis of variance.

experiment 1						
	M1				M1c	
	RMT_{50mm} (%MSO)	MEP_{1mV} (mV)	RMT_{25mm} (%MSO)	AMT (%MSO)	RMT_{50mm} (%MSO)	MEP_{1mV} (mV)
condition 1	31.33 ± 1.67	0.89 ± 0.07	57.56 ± 2.49	41.09 ± 1.95	35.89 ± 1.16	1.01 ± 0.06
condition 2	30.59 ± 1.61	1.02 ± 0.06	56.20 ± 2.72	41.36 ± 1.68	33.70 ± 1.35	1.03 ± 0.06
F	0.57	2.57	0.59	0.05	3.82	0.08
p	0.47	0.14	0.46	0.82	0.09	0.78

3.1.2.2 Online effects of $PAS_{M1c \rightarrow M1}$ with $PAS_{SMA \rightarrow M1}$

There were no significant effects of CONDITION ($F_{1, 10} = 2.272$, $p = 0.163$), BIN ($F_{4, 40} = 0.401$, $p = 0.806$) or the CONDITION*BIN ($F_{4, 40} = 0.834$, $p = 0.512$) interaction. That indicated, that the MEP amplitudes recorded during the two CONDITIONs of cooperative $PAS_{M1c \rightarrow M1}$ with $PAS_{SMA \rightarrow M1}$ did not vary (Figure 15).

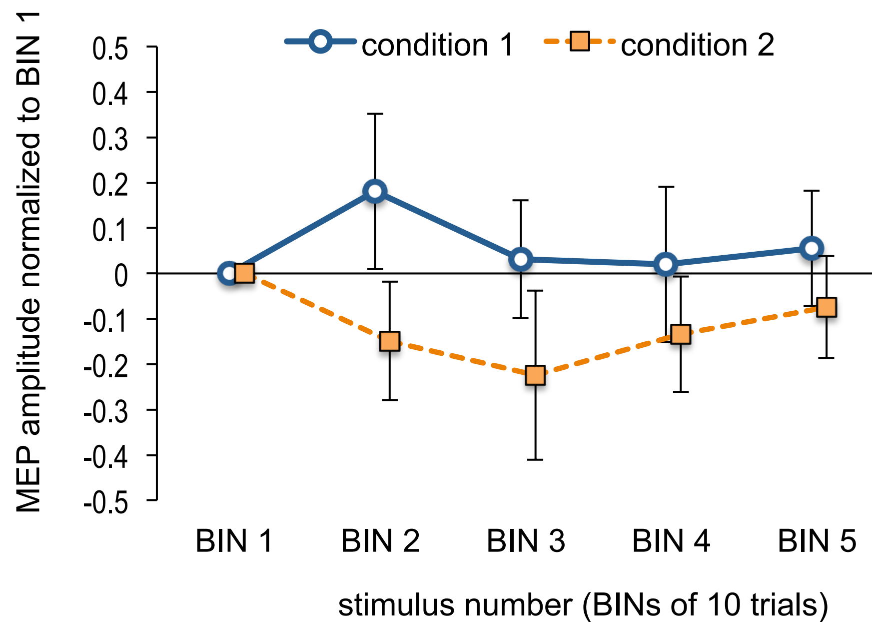


Figure 15 Online effects of cooperative $PAS_{M1c \rightarrow M1}$ with $PAS_{SMA \rightarrow M1}$

The experimental conditions did not show a significant effect in the within-subject factors BIN, CONDITION nor CONDITION*BIN interaction. Motor evoked potentials (MEPs) were measured during the application of $PAS_{M1c \rightarrow M1}$ with $PAS_{SMA \rightarrow M1}$. The MEPs were logarithmized, cumulated in bins of 10 and normalized to BIN 1 during the intervention (Fuhl et al. 2015). Condition 1 (blue): Supplementary motor area (SMA) stimulation 6 ms before left primary motor cortex (M1) stimulation and right primary motor cortex (M1c) stimulation 8 ms before M1 stimulation (Arai 2011). Condition 2 (orange): SMA stimulation 3 ms before M1, M1c stimulation 1 ms before M1 stimulation (Arai 2011). Each data point represents mean ± standard error of the mean (SEM) of 10 consecutive MEP trials during PAS for n = 11. The x-axis represents the stimulus number in BINs of 10 trials. The y-axis represents the mean MEP amplitude normalized to BIN 1 (Fuhl et al. 2015). Abbreviations: PAS = paired associative stimulation, $PAS_{M1c \rightarrow M1}$ = paired associative stimulation of M1c and M1, $PAS_{SMA \rightarrow M1}$ = paired associative stimulation of SMA and M1.

3.1.2.3 Effects of cooperative $PAS_{M1c \rightarrow M1}$ with $PAS_{SMA \rightarrow M1}$

The rmANOVA indicated a trend towards an effect of CONDITION ($F_{1, 10} = 3.492$, $p = 0.091$), while both TIME ($F_{4, 40} = 0.684$, $p = 0.607$) and TIME*CONDITION ($F_{4, 40} = 1.887$, $p = 0.132$) were not significant. The linear contrast of the TIME*CONDITION interaction showed a trend towards significance $F_{1, 10} = 3.775$, $p = 0.081$ (Figure 16).

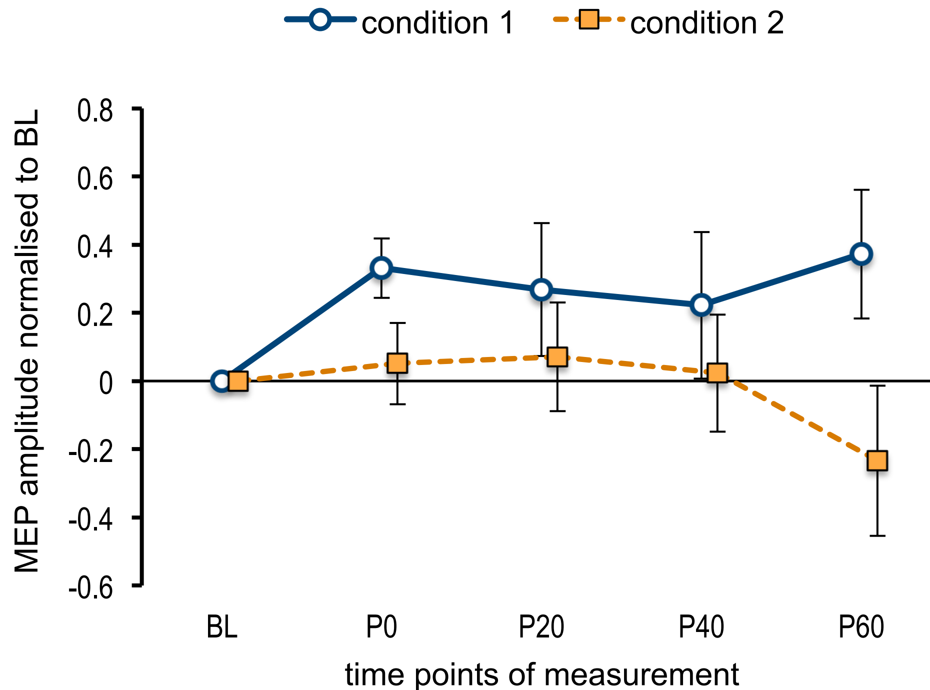


Figure 16 Cooperative effects of $PAS_{M1c \rightarrow M1}$ with $PAS_{SMA \rightarrow M1}$.

Display of the mean logarithmic MEP amplitudes normalized to baseline (BL) (y-axis) in dependence of time after the stimulation (x-axis, in min). Condition 1: supplementary motor area (SMA) stimulation 6 ms before left primary motor cortex (M1) stimulation and right primary motor cortex (M1c) stimulation 8 ms before M1 (blue). Condition 2: SMA stimulation 3 ms before M1 and M1c stimulation 1 ms before M1 (orange). All data are means of ln MEP ± standard error of the mean (SEM). Abbreviations: P0, P20, P40, P60 = measuring points 0, 30, 60 min after cooperative tDCS with $PAS_{SMA \rightarrow M1}$, PAS = paired associative stimulation, $PAS_{M1c \rightarrow M1}$ = paired associative stimulation of M1c and M1, $PAS_{SMA \rightarrow M1}$ = paired associative stimulation of SMA and M1.

3.2 Experiment 2: cooperative tDCS with $PAS_{SMA \rightarrow M1}$

3.2.1 Baseline measures of corticospinal excitability and effective SMA-M1 connectivity

The rmANOVAs to analyze the differences between the measures of corticospinal excitability in three experimental conditions at BL revealed not significance (Table 3) (Faber et al. 2017a).

Table 3 Experiment 2: baseline measures

Group mean data (mean ± standard error of the mean (SEM), n = 15) of the baseline measures in the three experimental conditions (PAS + anodal tDCS, PAS + cathodal tDCS, PAS + sham tDCS). One-way rmANOVA did not reveal a significant effect of condition for any of the baseline measures (all $p > 0.05$). Abbreviations: AMT = active motor threshold, effective SMA-M1 connectivity = ratio between the mean conditioned over the mean unconditioned MEP: for the conditioned MEPs SMA stimulation at 140% AMT preceded M1 stimulation by 6 ms, whereas for the unconditioned MEPs only M1 was stimulated (Arai 2012), M1 = left primary motor cortex, M1c = right primary motor cortex, MEP = motor evoked potential, MEP_{1mV} = motor evoked potential elicited with SI_{1mV}, PAS = paired associative stimulation, %MSO = percentage of maximum stimulator output, RMT_{25mm} = resting motor threshold using 25 mm figure-of-eight coil, RMT_{50mm} = resting motor threshold using 50 mm figure-of-eight coil, SI_{1mV} = stimulus intensity to elicit a mean MEP peak-to-peak amplitude of on average 1 mV in a block of 30 trials at BL, SMA = supplementary motor area, tDCS = transcranial direct current stimulation, polarity defined by the motor cortex electrode, rmANOVA = repeated measures analysis of variance (after (Faber et al. 2017a)).

experiment 2					
	RMT_{50mm} (%MSO)	RMT_{25mm} (%MSO)	AMT (%MSO)	MEP_{1mV} (mV)	effective SMA-M1 connectivity (%)
PAS + anodal tDCS	37.27 ± 1.57	57.80 ± 2.22	45.33 ± 2.47	1.20 ± 0.06	1.02 ± 0.07
PAS + cathodal tDCS	36.40 ± 1.43	56.40 ± 2.27	44.07 ± 2.43	1.06 ± 0.05	0.98 ± 0.03
PAS + sham tDCS	36.67 ± 1.52	58.27 ± 2.12	44.67 ± 2.16	1.09 ± 0.07	1.00 ± 0.05
F	1.14	0.95	0.74	2.47	0.08
p	0.34	0.40	0.49	0.10	0.92

3.2.2 Online-effects of tDCS with PAS_{SMA-M1}

The analysis of the online effects of tDCS with PAS_{SMA-M1} revealed a significant within-subject factor TIME ($F_{4.63, 64.80} = 2.595$, $p = 0.037$). The within-subject factor CONDITION ($F_{1.42, 19.83} = 0.572$, $p = 0.516$) and CONDITION*TIME ($F_{6.27, 87.77} = 0.818$, $p = 0.563$) were not significant (Figure 17).

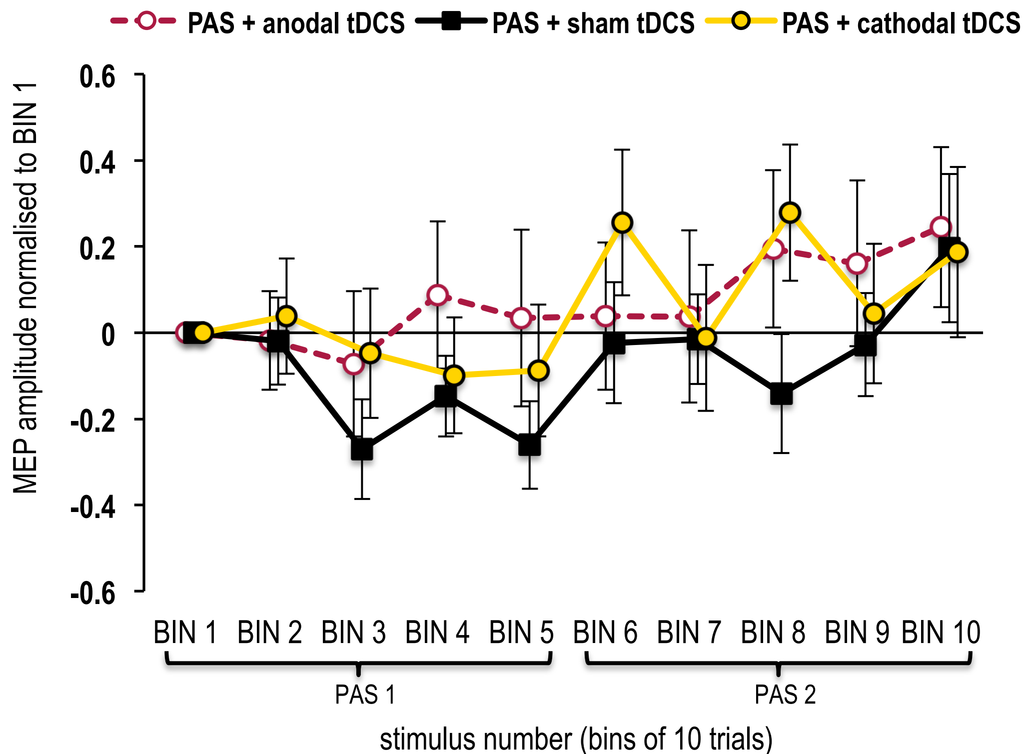


Figure 17 Online effects of cooperative tDCS with $PAS_{SMA \rightarrow M1}$.

The MEPs measured during the application of two blocks of PAS + tDCS were split in BINs of 10 consecutive MEPs (2x5 BINs per PAS intervention) (Fuhl et al. 2015). The graph shows an increase of MEP amplitude over the time course of the intervention irrespective of experimental condition (PAS + sham tDCS, PAS + anodal tDCS, PAS + cathodal tDCS). Each data point represents the mean ± standard error of the mean (SEM) of the logarithmic mean of 10 consecutive MEP amplitudes for n = 15 participants normalized to BIN 1 (y-axis) in dependence of the stimulus number of the whole PAS interventions (x-axis, bins of 10 trials) (Fuhl et al. 2015). Abbreviations: MEP = motor evoked potential, PAS = paired associative stimulation, PAS 1 = first PAS block, PAS 2 = second PAS block, $PAS_{SMA \rightarrow M1}$ = PAS of SMA and M1, tDCS = transcranial direct current stimulation.

3.2.3 Effects of tDCS with $PAS_{SMA \rightarrow M1}$

There were no significant effects of TIME ($F_{5, 70} = 1.05$, $p = 0.40$), CONDITION ($F_{2, 28} = 0.88$, $p = 0.43$), or CONDITION*TIME ($F_{10, 140} = 1.48$, $p = 0.16$) on MEP amplitude. A graph with x-axis as time points of measurement and y-axis as MEP amplitude displayed a trend for a MEP increase in the PAS + sham tDCS condition over time (Figure 18). Compared to the PAS + sham tDCS, both anodal and cathodal tDCS led to an increase in MEP amplitude at P0 and to a decrease in MEP amplitude at all later

time points (P60, P90, P120). Only at P30 anodal and cathodal tDCS showed opposing effects. PAS + anodal tDCS increased and PAS + cathodal tDCS decreased MEP amplitude in reference to PAS + sham tDCS condition. These results suggested in contrast to our original hypothesis, that anodal and cathodal tDCS stimulation did not follow opposing but rather similar time courses in reference to PAS + sham tDCS condition. Contrast analysis verified this finding by a significant quadratic contrast of the CONDITION*TIME interaction ($F_{1, 14} = 8.829$, $p = 0.01$) (Faber et al. 2017a).

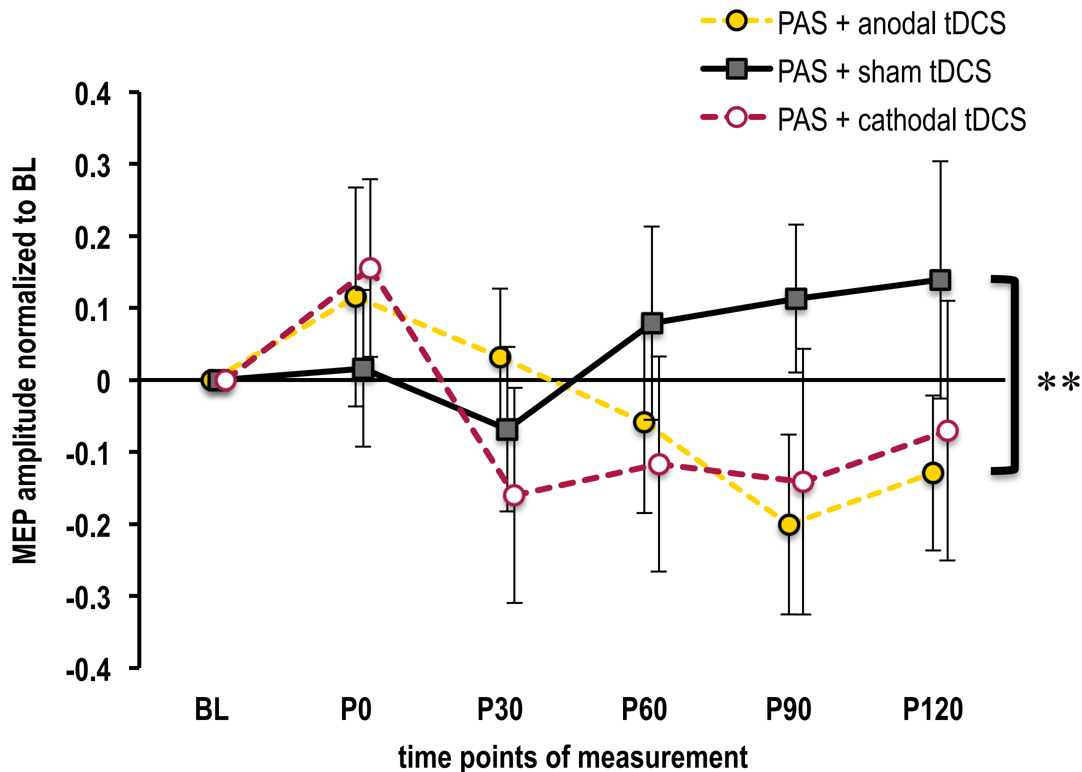


Figure 18 Effects of cooperative tDCS with $PAS_{SMA \rightarrow M1}$.

Transcranial direct current stimulation (tDCS) suppressed or even reversed the effect of paired associative stimulation (PAS) (PAS + sham tDCS, indicated in grey). Contrarily to our original hypothesis, anodal (indicated in yellow) and cathodal (indicated in red) tDCS altered $PAS_{SMA \rightarrow M1}$ in a similar manner. TDCS showed a tendency to reverse the $PAS_{SMA \rightarrow M1}$ effects at the later time points (P60 - P120). Data shows the mean ± standard error of the mean (SEM) for n = 15 normalized to baseline (BL) (y-axis) in dependence of the time points of measurements post intervention (x-axis, in min). Asterisks indicate significance at p = 0.01. Abbreviations: M1 = left primary motor cortex, MEP = motor evoked potential, P0, P30, P60, P90, P120 = measuring points 0, 30, 60, 90, 120 min after cooperative tDCS with $PAS_{SMA \rightarrow M1}$, $PAS_{SMA \rightarrow M1}$ = paired associative stimulation of SMA and M1, SMA = supplementary motor area (after (Faber et al. 2017a)).

3.2.4 Modulation of the $PAS_{SMA \rightarrow M1}$ effect by anodal versus cathodal tDCS.

Both anodal and cathodal tDCS shifted the PAS effect in the same direction in thirteen of the fifteen tested subjects (86,7%). The data of the majority of subjects (13 / 15) indicated, that if the absolute anodal tDCS effect on $PAS_{SMA \rightarrow M1}$ was repressive, then the absolute effect of cathodal tDCS effect on $PAS_{SMA \rightarrow M1}$ was repressive as well. If the

absolute anodal tDCS effect on $PAS_{SMA \rightarrow M1}$ was reinforcing, the absolute effect of cathodal tDCS effect on $PAS_{SMA \rightarrow M1}$ was reinforcing. In contrast, the absolute anodal tDCS and absolute cathodal tDCS effect showed opposing directions in only 2/15 subjects (13.3%) (Figure 19). This distribution differed significantly from equality ($p = 0.004$). Moreover, the Spearman correlation indicated a significant correlation of the size of the absolute modulatory effect of anodal tDCS on $PAS_{SMA \rightarrow M1}$ and the absolute modulatory effect of cathodal tDCS on $PAS_{SMA \rightarrow M1}$ at $p < 0.05$ ($r_s = 0.525$, $p = 0.044$) (Figure 20) (Faber et al. 2017a).

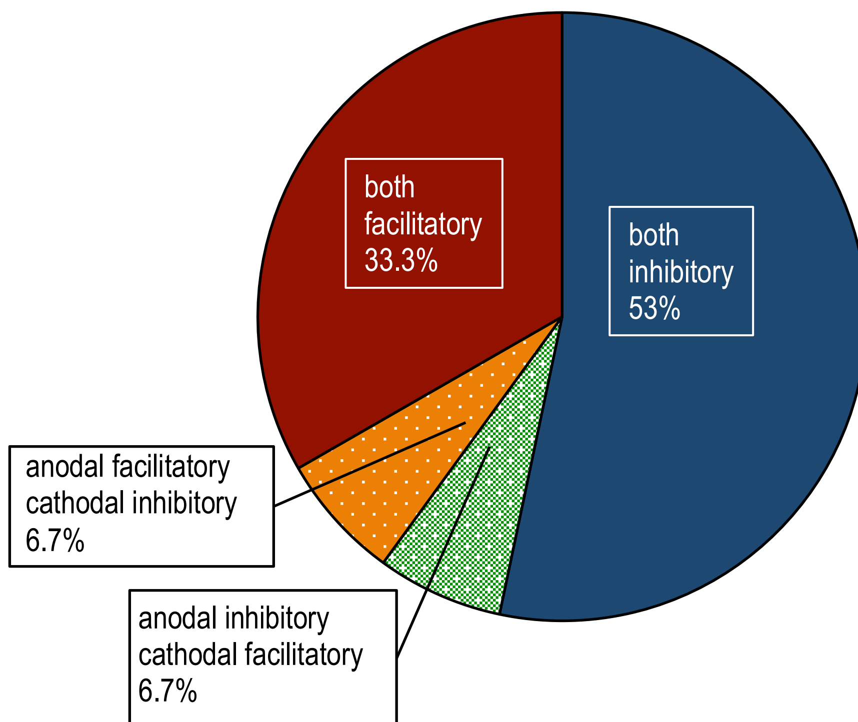


Figure 19 Individual responses to cooperative tDCS with $PAS_{SMA \rightarrow M1}$.

Pie chart displaying the modulatory effects of anodal and cathodal transcranial direct current stimulation (tDCS) during $PAS_{SMA \rightarrow M1}$. Anodal and cathodal tDCS modulated $PAS_{SMA \rightarrow M1}$ effect in identical direction in the majority of participants (13/15 = 86.7%) (indicated in red (both tDCS effects facilitatory) and blue (both tDCS effects inhibitory)). Modifications in opposite directions were only observed in 2/15 (13.3%) of participants (indicated in orange (anodal facilitatory and cathodal inhibitory) and green (anodal inhibitory and cathodal facilitatory). Abbreviations: M1 = left primary motor cortex, PAS = paired associative stimulation, $PAS_{SMA \rightarrow M1}$ = PAS of SMA and M1, SMA = supplementary motor area (after (Faber et al. 2017a)).

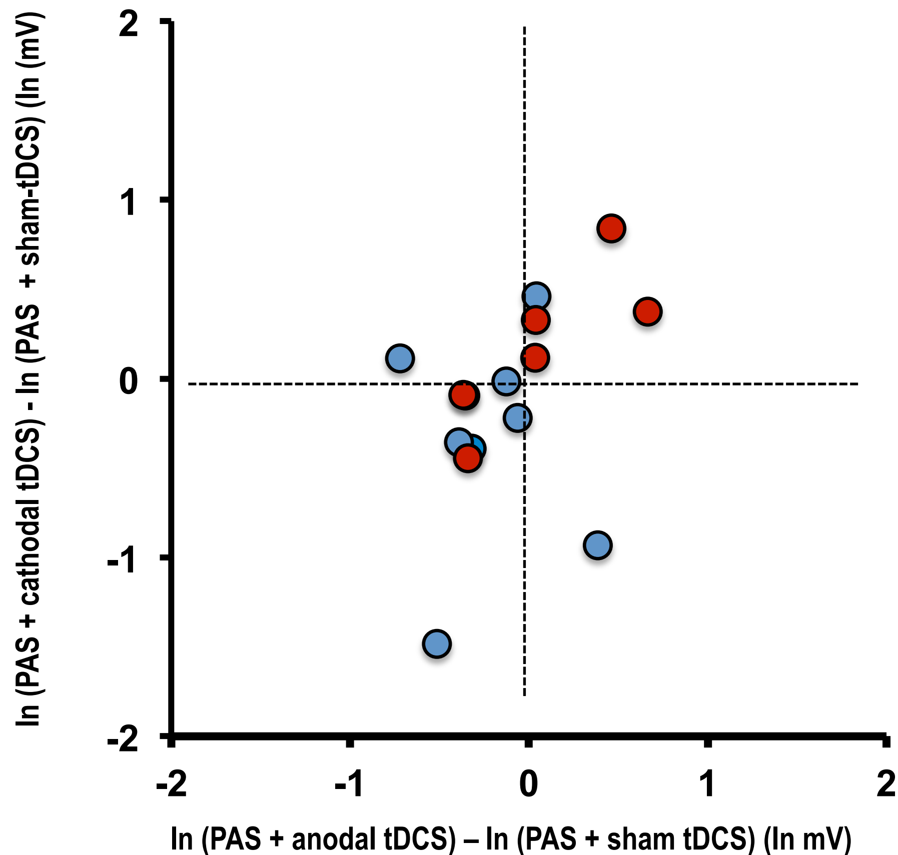


Figure 20 Effect sizes of anodal tDCS versus cathodal tDCS.

Correlation of the individual $PAS_{SMA \rightarrow M1}$ effects (average of ln-transformed MEP amplitudes over all post-PAS measurements minus baseline) corrected for the effect of $PAS_{SMA \rightarrow M1}$ on MEP amplitudes in the PAS + sham tDCS condition after PAS + anodal tDCS (x-axis, in ln mV) and after PAS + cathodal tDCS (y-axis, in ln mV) for n = 15 participants. Note that the magnitude of these effects correlated significantly $r_s = 0.525$, p = 0.044. Blue data points indicate an increase of MEP amplitude in the PAS + sham tDCS condition. Red data points indicate decrease of MEP amplitude in the PAS + sham tDCS condition. Abbreviations: M1 = left primary motor cortex, PAS = paired associative stimulation, $PAS_{SMA \rightarrow M1}$ = PAS of SMA and M1, SMA = supplementary motor area, tDCS = transcranial direct current stimulation (after (Faber et al. 2017a)).

3.2.5 Effects of tDCS on SMA-M1 connectivity

The rmANOVA, comparing the effects of PAS + anodal tDCS, PAS + cathodal tDCS, PAS + sham tDCS on effective SMA-M1 connectivity, disclosed neither an effect of CONDITION ($F_{1.43, 20.00} = 0.087$, p = 0.854) nor TIME ($F_{2.47, 34.57} = 0.328$, p = 0.766). The CONDITION*TIME interaction on MEP amplitude was significant

($F_{10, 140}$ = 1.904, p = 0.049) (Figure 21). To further analyze this effect, we conducted three rmANOVAs having the same within-subject factor TIME (six levels: BL, P0, P30, P60, P90, P120) but differing in the within-subject factor CONDITION (two levels): (1) PAS + anodal tDCS vs. PAS + sham tDCS, (2) PAS + cathodal tDCS vs. PAS + sham tDCS, (3) PAS + anodal tDCS vs. PAS + cathodal tDCS.

The rmANOVA comparing the CONDITIONs PAS + cathodal tDCS vs. PAS + sham tDCS revealed a significant TIME*CONDITION interaction ($F_{5, 70}$ = 4.041, p = 0.003). TIME ($F_{5, 70}$ = 0.204, p = 0.96) and CONDITION ($F_{1, 14}$ = 0.03, p = 0.865) were not significant. Two-tailed paired t-test at each time point revealed that the TIME*CONDITION interaction was due to differences at P30 (t(14) = -2.629, p = 0.020) and P60 (t(14) = 2.198, p = 0.045). The conditions did not differ at the other time points (P0 (t(14) = 0.303, p = 0.766), P90 (t(14) = 0.905, p = 0.381) and P120 (t(14) = 0.975, p = 0.346). Note that the observed differences did not survive the Bonferroni correction for multiple comparisons at any time point.

The rmANOVA to analyze the effects of PAS + anodal tDCS and PAS + sham tDCS showed no effect of TIME ($F_{5, 70}$ = 0.498, p = 0.777), CONDITION ($F_{1, 14}$ = 0.112, p = 0.743), or CONDITION*TIME ($F_{5, 70}$ = 1.132, p = 0.352). The evaluation of the effects of PAS + anodal tDCS vs. PAS + cathodal tDCS demonstrated no effect of TIME ($F_{5, 70}$ = 1.70, p = 0.146), CONDITION ($F_{1, 14}$ = 0.076, p = 0.786) or CONDITION*TIME ($F_{5, 70}$ = 0.881, p = 0.499) (Figure 21).

Contrast analysis of the rmANOVA, comparing the effects of PAS + anodal tDCS, PAS + cathodal tDCS, PAS + sham tDCS on effective SMA-M1 connectivity with the within-subject factors CONDITION (three levels: PAS + anodal tDCS, PAS + sham tDCS and PAS + cathodal tDCS) and TIME (six levels: BL, P0, P30, P60, P90, P120) displayed a non-significant linear contrast of the CONDITION*TIME interaction ($F_{1, 14}$=0.239, p = 0.644) and a trend towards a significant quadratic contrast in the CONDITION*TIME interaction ($F_{1,14}$ = 3.142, p = 0.098).

In summary, the data on SMA-M1 connectivity displayed a significant CONDITION *TIME interaction between the three experimental conditions. Additional analyses showed, that this significance was based on a significant CONDITION*TIME interaction between PAS + cathodal tDCS and PAS + sham tDCS at P30 and P60. A graph displaying time points of measurement after $PAS_{SMA \rightarrow M1}$ on the x-axis and effective SMA-M1 connectivity normalized to BL on the y-axis suggested, that anodal and cathodal tDCS modulate $PAS_{SMA \rightarrow M1}$ in the same instead of opposite directions. This result was further supported by a trend towards significance of the quadratic contrast of the CONDITION*TIME interaction. Findings suggested unidirectional effects of anodal and cathodal tDCS on $PAS_{SMA \rightarrow M1.}$

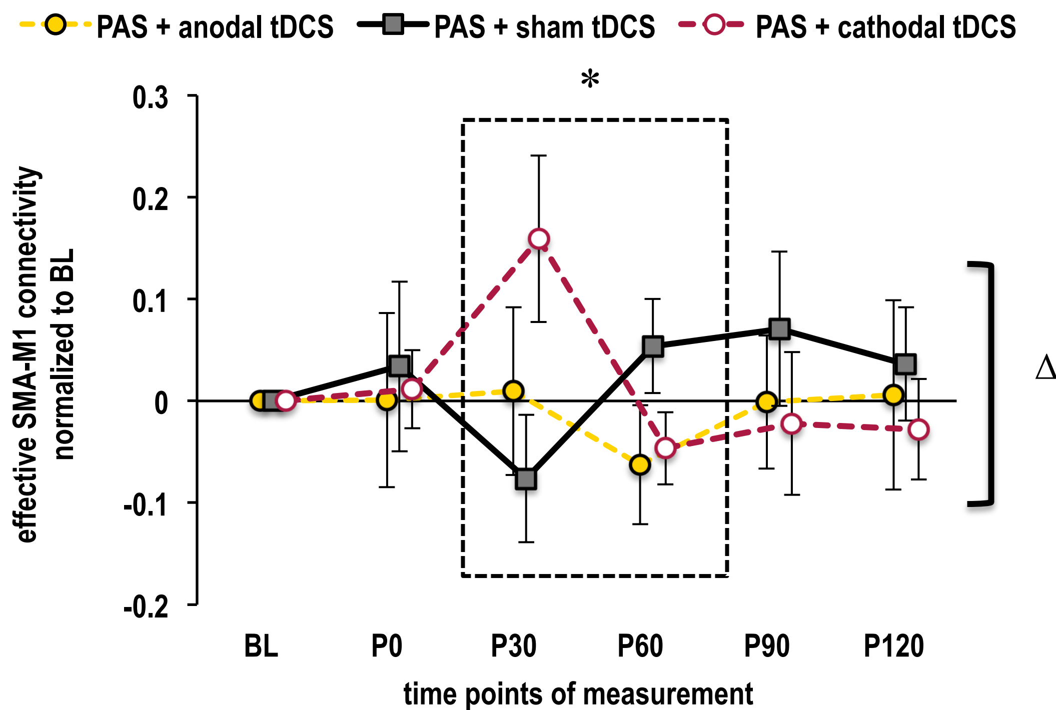


Figure 21 Effective SMA-M1 connectivity post cooperative tDCS with $PAS_{SMA \rightarrow M1}$.

Transcranial direct current stimulation (tDCS) suppressed or even reversed the effect of paired associative stimulation (PAS) on SMA-M1 connectivity (PAS + sham tDCS, indicated in grey). Anodal (red) and cathodal (yellow) tDCS altered effective SMA-M1 connectivity in the same direction in contrast to our original hypothesis. Effective connectivity is defined as the mean of the conditioned over the unconditioned MEP amplitude at each timed point (Arai 2012). The x-axis displays the time points of measurement after the PAS intervention in min. The y-axis shows effective SMA-M1 connectivity normalized to baseline (BL). Data points represent the mean ± standard error of the mean (SEM) for n = 15 subjects. * indicates a significant effect of the TIME*CONDITION interaction at $p < 0.05$ due to differences of PAS_{SMA-M1} + sham tDCS and PAS_{SMA-M1} + cathodal tDCS at time points P30 and P60. Δ indicates a trend towards significance for the within-subject quadratic contrast between PAS_{SMA-M1} + cathodal tDCS, PAS_{SMA-M1} + anodal tDCS and PAS_{SMA-M1} + sham tDCS ($p < 0.1$). Abbreviations: P0, P30, P60, P90, and P120 = measurements at 0, 30, 60, 90 and 120 min post intervention.

3.2.6 Control experiment 2

3.2.6.1 Baseline measures of corticospinal excitability

None of the one-way rmANOVAs, which compared the baseline measures of corticospinal excitability, revealed significance (Table 4) (Faber et al. 2017a, Faber et al. 2017b).

Table 4 Control experiment 2: baseline measures

Group mean data (mean ± standard error of the mean (SEM), n = 10) of the RMT_{50mm} and MEP_{1mV} at baseline in the three experimental conditions (anodal, cathodal or sham transcranial direct current stimulation (tDCS)). F- and p-value result from the one-way rmANOVA checking for differences between the experimental conditions in each baseline parameter. Note: p never reached significance (all $p > 0.05$). Abbreviation: %MSO = percentage of maximum stimulator output, RMT_{50mm} = resting motor threshold determined with the 50 mm figure-of-eight coil, MEP = motor evoked potential, MEP_{1mV} = motor evoked potential elicited with, rmANOVA = repeated measures analysis of variance, SI_{1mV} = stimulus intensity to elicit a mean MEP peak-to-peak amplitude of on average 1 mV in a block of 30 trials at BL (Faber et al. 2017a, Faber et al. 2017b) (after (Faber et al. 2017a)).

control experiment 2		
	RMT_{50mm} (%MSO)	MEP_{1mV} (mV)
anodal tDCS	35.70 ± 1.60	1.06 ± 0.03
cathodal tDCS	36.10 ± 1.67	0.98 ± 0.04
sham tDCS	37.30 ± 1.93	0.96 ± 0.06
F	2.59	1.53
p	0.10	0.24

3.2.6.2 Exclusive tDCS effects

Control experiment 2 evaluated the isolated tDCS impact on MEP amplitude. The rmANOVA showed no effects of TIME ($F_{5, 45} = 1.86$, $p = 0.12$), CONDITION ($F_{2, 18} = 0.26$, $p = 0.77$), or CONDITION*TIME ($F_{10, 90} = 1.50$, $p = 0.15$) on MEP amplitude. In contrast to experiment 2, the quadratic contrast of the Condition*TIME interaction was not significant ($F_{1, 9} = 1.95$, $p = 0.20$) (Figure 22). The two-tailed paired t-test

comparing the immediate aftereffects between the anodal and cathodal tDCS was not significant ($t(9) = -0.442$, $p = 0.669$) (Faber et al. 2017a, Faber et al. 2017b).

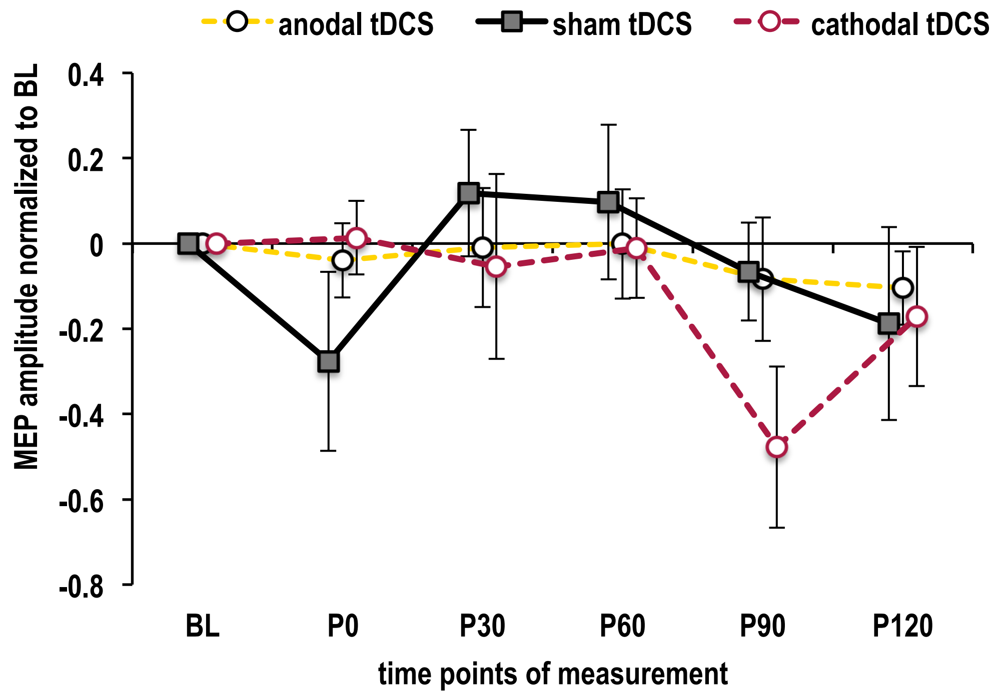


Figure 22 Effects of tDCS without $PAS_{SMA \rightarrow M1}$ in the novel montage.

The effects of anodal, cathodal and sham transcranial direct current stimulation (tDCS) on M1 excitability showed no difference. Displayed is the mean MEP amplitude ± standard error of the mean (SEM) of n = 10 subjects elicited at SI_{1mV} normalized baseline (y-axis) in dependence of the time (x-axis, in min) after the two blocks of anodal (yellow), cathodal (red) or sham (grey) tDCS. Abbreviation: BL = baseline, MEP = motor evoked potential, P0, P30, P60, P90, and P120 = measurements at 0, 30, 60, 90 and 120 min post intervention, SI_{1mV} = stimulus intensity to elicit a mean MEP peak-to-peak amplitude of on average 1mV in a block of 30 trials at BL (Faber et al. 2017a , Faber et al. 2017b) (after (Faber et al. 2017a)).

4 Discussion

4.1 Experiment 1: cooperative $PAS_{SMA \rightarrow M1}$ with $PAS_{M1c \rightarrow M1}$ to induce LTP

In Experiment 1 we hypothesized that cooperative $PAS_{M1c \rightarrow M1}$ (Rizzo et al. 2011, Rizzo et al. 2009) during $PAS_{SMA \rightarrow M1}$ (Arai et al. 2011) would successfully induce LTP. $PAS_{SMA \rightarrow M1}$ only induces LTP in humans, if bilateral near-synchronous priming of M1 and M1c precedes $PAS_{SMA \rightarrow M1}$ (Arai et al. 2011). Inspired by the cellular level, where cooperative pairing with an extracellular stimulus enables subthreshold stimulation to induce LTP (Sjöström and Häusser 2006, Sjöström et al. 2001), we aimed to transfer the concept of cooperativity to the network level. We expected that condition 1 (SMA stimulation 6 ms and M1c stimulation 8 ms before M1 stimulation) would increase M1 excitability, while control condition 2 (SMA stimulation 3 ms and M1c stimulation 1 ms before M1 stimulation) would have no effects on M1 excitability (Arai et al. 2012, Arai et al. 2011, Rizzo et al. 2011, Rizzo et al. 2009).

4.1.1 Simultaneous $PAS_{M1c \rightarrow M1}$ with $PAS_{SMA \rightarrow M1}$ is unable to induce plastic changes

The concurrent application of $PAS_{SMA \rightarrow M1}$ and $PAS_{M1c \rightarrow M1}$ was unable to induce an LTP-like excitability increase in M1. Our experiment demonstrated that neither condition 1 (SMA stimulation 6 ms and M1c stimulation 8 ms before M1 stimulation), nor control condition 2 (SMA stimulation 3 ms and M1c stimulation 1 ms before M1 stimulation) changed M1 excitability significantly. Additionally, bin analysis of PAS online effects did not to reveal any facilitatory effects during the applied protocols (Figure 15).

Firstly, this lack of effects could be due to the chosen ISIs for SMA and M1c stimulation in the present experiment. However, this is unlikely. Paired associative stimulation of SMA and M1 with an ISI = 6 ms resulted in an increase of M1 excitability (Arai et al. 2012, Arai et al. 2011). This effect was absent at an ISI = 3 ms (Arai et al. 2012, Arai et al. 2011). A conditioning TMS stimulus applied over the opposite motor cortex 8 ms before TMS stimulation diminishes MEP amplitude of a test stimulus over the contralateral primary motor cortex (Ferbert et al. 1992). Additionally, cortical-cortical PAS of M1c and M1 enhances M1 excitability at an

ISI = 8 ms (Rizzo et al. 2011, Rizzo et al. 2009), but not at an ISI = 1 ms (Rizzo et al. 2009). This effect was absent in an asymptomatic patient with callosal agenesis (Rizzo et al. 2009). Those studies demonstrate that PAS protocols with the chosen ISIs are capable to modulate excitability of the motor system (Arai et al. 2011, Ferbert et al. 1992, Rizzo et al. 2009).

Secondly, one might argue that a single PAS block is insufficient to cause a long-term excitability increase in M1. Arai et al. (2011) induced LTP in using three blocks of $PAS_{SMA \rightarrow M1}$. Yet already the first $PAS_{SMA \rightarrow M1}$ block increased M1 excitability significantly (see Figure 2 in (Arai et al. 2011)). This indicates that the number of PAS blocks is not responsible for the absence of LTP-induction in the presented experiment.

Finally, $PAS_{SMA \rightarrow M1}$ significantly rises M1 excitability, if primed by near-synchronous bilateral M1 stimulation (ISI = 0.8 ms) (Arai et al. 2011). By contrast, concurrent near-synchronous bilateral M1 stimulation at an ISI of 0.8 ms during $PAS_{SMA \rightarrow M1}$ does not change M1 excitability (Arai et al. 2011). Likewise the concurrent application of $PAS_{M1c \rightarrow M1}$ during $PAS_{SMA \rightarrow M1}$ did not alter M1 excitability in experiment 1.

In summary, based on the current knowledge on $PAS_{M1c \rightarrow M1}$ and $PAS_{SMA \rightarrow M1}$, the utilized experimental protocols are able to induce plasticity (Arai et al. 2011, Rizzo et al. 2011, Rizzo et al. 2009).

4.1.2 $PAS_{SMA \rightarrow M1}$ requires metaplastic priming for successful plasticity induction

Results of experiment 1 demonstrated that the coincident application of two potentially excitability-increasing protocols (Arai et al. 2011, Rizzo et al. 2009) did not synergistically rise M1 excitability. $PAS_{SMA \rightarrow M1}$ after bilateral near-synchronous priming of the primary motor cortex increases M1 excitability, whereas the same bilateral motor cortex stimulation during $PAS_{SMA \rightarrow M1}$ does not influence M1 excitability (Arai et al. 2011). Why do we see different effects with the same combination of pcTMS protocols?

One explanation is that at the systems level homeostatic mechanisms inhibit an excitability boost generated by simultaneously applied possibly excitability raising protocols (Abbott and Nelson 2000, Abraham 2008, Abraham and Bear 1996, Hulme et al. 2013, Karabanov et al. 2015, Müller-Dahlhaus and Ziemann 2015). Homeostatic plasticity preserves neuronal activity within physiological boundaries (Turrigiano and Nelson 2000). Thereby homeostatic mechanisms counterbalance plasticity induction to

prevent synaptic over-excitation and synaptic silencing, and are an essential prerequisite for ongoing learning (Bienenstock et al. 1982, Hulme et al. 2013).
Secondly, prior near-simultaneous bilateral primary motor cortex stimulation could induce metaplastic changes and lower the susceptibility for M1 excitability rise (Arai et al. 2011, Karabanov et al. 2015, Müller-Dahlhaus and Ziemann 2015). Metaplasticity is defined as the adaptability of synaptic plasticity on prior network activity (Abraham and Bear 1996, Bienenstock et al. 1982). It is a major determinant for plasticity induction at the cellular level (Abraham and Bear 1996, Bienenstock et al. 1982). Correlated pre- and postsynaptic activity modifies synaptic connections (Abraham 2008, Bienenstock et al. 1982). This synaptic plasticity is bidirectional – low postsynaptic activity lowers synaptic strength and causes LTD (Bienenstock et al. 1982). Postsynaptic activity above a sliding threshold rises synaptic strength and induces LTP (Bienenstock et al. 1982). Metaplasticity states that this sliding threshold varies depending on the former network activity (Abraham and Bear 1996, Bienenstock et al. 1982). If neurons previously fired at a low level, LTP is more likely (Abraham 2008, Bienenstock et al. 1982). By contrast, prior elevated neuronal activity reduces probability for LTP induction (Abraham 2008, Bienenstock et al. 1982).
Various experiments provide evidence for homeostatic metaplastic mechanisms at the network level (Bienenstock et al. 1982, Müller-Dahlhaus and Ziemann 2015). A pivotal work uses quadro-pulse stimulation (QPS) to demonstrate the transferability of the BCM model at the cortical level (Hamada et al. 2008a). QPS repetitively applies a sequence of four TMS pulses over 30 min (Hamada et al. 2007). High previous network activity by a LTP-inducing QPS protocol, shifts the sliding threshold towards higher level of postsynaptic activity and reduces probability for LTP-induction (Hamada et al. 2008a). By contrast low previous network activity, induced by a possible LTD-inducing protocol, makes LTP easier to attain (Hamada et al. 2008a). Priming with a LTD-inducing protocol rises the effects of a LTP-inducing PAS protocol, while priming with a LTP-inducing protocol attenuates successive LTP-like induction (Müller and Ziemann 2007). LTP is thought to be a reflector for learning (Cooke and Bliss 2006, Hebb 1949). Previous Motor learning, that involves the primary motor cortex, reduces LTP-induction but promotes LTD in rats (Rioult-Pedotti et al. 2000). Comparably, in healthy human participants, motor learning prior to PAS lessens

LTP-like and promotes LTD-like plasticity induction (Ziemann et al. 2004). As metaplasticity does not necessarily change excitability (Abraham and Bear 1996, Hamada et al. 2008a), a subthreshold stimulus, i.e. bilateral near-synchronous priming of the primary motor cortex, could interfere with subsequent plasticity induction in a metaplastic manner (Arai et al. 2011, Cincotta et al. 2005).

If $PAS_{M1c\rightarrow M1}$ provokes metaplastic alterations comparable to bilateral near-synchronous priming of the primary motor cortex (Arai et al. 2011), the consecutive application of $PAS_{M1c\rightarrow M1}$ and $PAS_{SMA\rightarrow M1}$ could induce LTP-like effects in M1. This assumption seems reasonable since bilateral near-synchronous priming of the primary motor cortex, is also subthreshold to cause a LTP-like M1 excitability increase (Arai et al. 2011, Cincotta et al. 2005). Additionally, $PAS_{M1c\rightarrow M1}$ interferes with the SMA-M1-M1c network in lowering intracortical inhibition between concordant regions in M1 (Ferbert et al. 1992, Rizzo et al. 2009). At last, concurrent application of both protocols with $PAS_{SMA\rightarrow M1}$ did not change excitability (cf. results of experiment 1 and (Arai et al. 2011, Cincotta et al. 2005, Rizzo et al. 2009)).

To further explore this hypothesis, future experiments could test, whether $PAS_{M1c\rightarrow M1}$ prior to $PAS_{SMA\rightarrow M1}$ increases M1 excitability. In this thesis, we did not further explore the metaplasticity induced by $PAS_{M1c\rightarrow M1}$, as we aimed to transfer the conception of cooperativity to the network level. A key requisite of cooperativity is the coincidence of applied stimuli (Sjöström and Häusser 2006, Sjöström et al. 2001). In contrast, metaplastic alterations occur prior to plasticity induction (Abraham 2008). For this reason, we did not further investigate to which extent $PAS_{M1c\rightarrow M1}$ induces metaplastic alterations.

4.1.3 Methodological consequences of experiment 1

TMS has effects on brain activity (Di Lazzaro et al. 1998, Siebner and Ziemann 2007). However neuronal activity in a living individual is influenced by various factors. It is important to minimize experimental variability. In experiment 1 we included female participants. As the menstrual cycle influences cortical activity (Smith et al. 2002, Smith et al. 1999), we made female sex an exclusion criterion for experiment 2. Fluctuations of cortisol levels during time of day affect plasticity induction (Sale et al. 2008). Thus, all session in experiment 2 were conducted in the afternoon.

In experiment 2, coil position and orientation were controlled by a neuronavigation system and MNI-coordinates were used for a homogenous SMA localization across subjects (Zhang et al. 2012). We chose this better-controlled method for SMA localization after the pilot experiment 1: two stimulation conditions did not reveal any significant differences, and neither one did induce LTP-like plasticity. However, data indicated a higher inter-subject variability in condition 1 compared to a control condition 2. As this could be assigned either to the stimulation protocol or incidental fluctuations owing to the comparatively small sample of n = 5, we increased the sample and used a new method for SMA localization based on MNI-coordinates of the individual MRI (for details, see Section 2.1.2.2). Condition 1 and condition 2 showed the biggest differences one hour after stimulation (Figure 16). We reasoned that cooperative effects might appear at later time points post stimulation and lengthened measuring time of aftereffects for up to two hours after the intervention in experiment 2.

4.2 Experiment 2: cooperative tDCS with $PAS_{SMA \rightarrow M1}$

TDCS polarizes the cortex of the human brain (Miranda et al. 2013, Nitsche and Paulus 2000, Opitz et al. 2011). At the cellular level cooperative depolarization concurrent with subthreshold stimulation rescues LTP induction (Sjöström and Häusser 2006, Sjöström et al. 2001). This study thought to verify that cooperative, simultaneous anodal tDCS with $PAS_{SMA \rightarrow M1}$, in contrast to cathodal or sham tDCS, increases M1 excitability and effective SMA-M1 connectivity. We compared three experimental conditions in a highly controlled, double-blinded, randomized crossover design: anodal tDCS (two 35 cm^2 electrodes, anode 3 cm posterior to M1 hand area, cathode over contralateral frontopolar cortex, 1 mA, 2 x 5 min), cathodal (reversed polarity) or sham tDCS with neuronavigated $PAS_{SMA \rightarrow M1}$. Effective SMA-M1 connectivity was evaluated by a recently developed pcTMS protocol (for details see (Arai et al. 2012)). Effects on M1 excitability and effective SMA-M1 connectivity were analyzed by MEP amplitude of the right FDI (Faber et al. 2017a).

4.2.1 $PAS_{SMA \rightarrow M1}$ has no effects on M1 excitability

Data of experiment 2 revealed that two blocks of $PAS_{SMA \rightarrow M1}$ + sham tDCS did not raise M1 excitability and effective SMA-M1 connectivity significantly. Findings

confirmed a previous study that showed that three blocks of $PAS_{SMA \rightarrow M1}$ did not alter M1 excitability significantly (Arai et al. 2011). By contrast, priming prior to bilateral near-synchronous primary motor cortex stimulation with $PAS_{SMA \rightarrow M1}$ significantly increased M1 excitability (Arai et al. 2011).

Physiologically, hand movements do not exclusively concern the SMA-M1 connection but involve a dynamic coupling of various motor areas (Grefkes et al. 2008a). Bilateral SMA activation precedes movement execution (Shibasaki and Hallett 2006). Coupling between active SMA, M1 and M1c changes during unimanual hand movements (Sarfeld et al. 2012). Compared to healthy controls, stroke patients show decreased effective connectivity between ipsilesional SMA and the primary motor cortex and increased inhibitory coupling from contralesional to ipsilesional primary motor cortex (Grefkes et al. 2008b). Normalization of augmented inhibitory coupling of contralesional primary motor cortex by rTMS increases motor abilities of stroke patients (Grefkes et al. 2010). After stroke reestablishing connectivity within the whole SMA-M1-M1c network correlates with hand motor recovery (Rehme et al. 2011). Those studies in healthy and diseased brain suggest that unimanual movements require changes in a wider network.

Furthermore, anatomical studies put in evidence dense connections between SMA, M1 and M1c and numerous callosal connecting fibers between left and right SMA proper (Liu et al. 2002). Arai et al. (2011) hypothesize that applying higher SMA-proper stimulation intensity in the absence of priming is insufficient in long-term associative plasticity. In our definition for AMT elicited MEPs needed to exceed a threshold of 200 μV in 5 out of 10 consecutive trials instead of 100 μV (Arai et al. 2011). As SMA stimulation intensity was defined as 140% AMT in both studies, intensity for SMA stimulation in the present experiment was higher than in Arai et al. (2011). Nevertheless, we did not observe a significant MEP increase after PAS. Therefore, our study further supports the existence of a subgroup of SMA neurons that are connected to both M1 and M1c (Arai et al. 2011, Liu et al. 2002).

Taken together, these findings suggest that for successful plasticity induction by $PAS_{SMA \rightarrow M1}$, modifications in a broader network involving SMA, M1c and M1 need to occur. Moreover, like in experiment 1, metaplastic alterations in this network seem to be crucial for plasticity induction with $PAS_{SMA \rightarrow M1}$ (Faber et al. 2017a).

4.2.2 PAS $_{SMA \rightarrow M1}$ does not alter effective SMA-M1 connectivity

Our results displayed that $PAS_{SMA \rightarrow M1}$ + sham tDCS did not increase effective SMA-M1 connectivity significantly (Figure 21). Compared to other studies (Arai et al. 2011, Civardi et al. 2001), we used a coil orientation for SMA stimulation with induced current direction from medial to lateral instead of anterior to posterior (see Section 2.1.1.7). This induced current direction is superior to any other current direction for a conditioning SMA stimulus (Arai et al. 2012). Bearing in mind the relevance M1c-M1 interactions even for contralateral unimanual movements (Sarfeld et al. 2012), induction of STDP-like plasticity of the SMA-M1 connection could demand connectivity modulations in the whole SMA-M1c-M1 network. From this viewpoint, even though less effective in probing effective SMA-M1 connectivity (Arai et al. 2012), stimulation of SMA with an induced current from anterior to posterior might be the preferred orientation for STDP-like plasticity induction in the SMA-M1-M1c.

4.2.3 TDCS in the novel montage is unable to change M1 excitability

The application of anodal, cathodal and sham tDCS alone in the novel montage did not induce significant changes in MEP amplitude. Why can we nevertheless assume that tDCS in the new montage alters neuronal membrane potentials?

Firstly, anodal and cathodal tDCS do not show opposing stimulation effects, when the electrode to stimulate the primary motor cortex is moved backwards (Nitsche and Paulus 2000). Both polarities tend to decrease MEP amplitude (Nitsche and Paulus 2000). Contrarily, five minutes of tDCS with equivalent stimulation parameters in the "classical" montage modulates M1 excitability bidirectionally (Nitsche and Paulus 2000). Anodal tDCS leads to an M1 excitability increase and cathodal tDCS decreases M1 excitability (cf. Figure 1 in (Nitsche and Paulus 2000)). Various other studies, which use the same electrode montage in comparable experimental conditions (short-duration (< 10 min) 1 mA tDCS stimulation) report the same polarity specific aftereffects (Liebetanz et al. 2002, Nitsche et al. 2007a, Nitsche and Paulus 2000, Nitsche et al. 2007b). This indicates, that the chosen experimental parameters for tDCS stimulation in the present experiment were not too weak to affect cortical structures.

Secondly, while the application of tDCS alone in the experimentally applied montage had no effects on M1 excitability, tDCS reversed or even suppressed plasticity-induction by $PAS_{SMA \rightarrow M1}$. Another study further supports the efficacy of a comparable,

more posterior electrode montage: application of anodal and cathodal tDCS with two small Ag/AgCl gelled electrodes placed 3.5 cm anterior and posterior to the M1 hand area bidirectionally modified plasticity induction of concurrent intermittent theta burst stimulation in a polarity-dependent manner (Tremblay et al. 2017).

Finally, according to computational modeling of the electric field, the strength of the tangential field in the area where SMA->M1 axon terminals reach M1 is sufficient to modify membrane potential (Salvador et al. 2015).

In summary, findings suggest that a more posterior placement of the motor cortex tDCS electrode modifies membrane potentials but does not alter motor cortex excitability. Since our tDCS stimulation parameters resembled previous studies, the induced electric field by our novel montage seems to be responsible for the observed stimulation alterations (Faber et al. 2017a).

4.2.4 Unidirectional effects of cooperative tDCS with $PAS_{SMA \rightarrow M1}$

Unlike our hypothesis, anodal and cathodal tDCS did not bidirectionally modify the induced changes by $PAS_{SMA \rightarrow M1}$ on M1 excitability and effective SMA-M1 connectivity. Contrarily, both anodal and cathodal tDCS suppressed or even reversed the $PAS_{SMA \rightarrow M1}$ effects on M1 excitability. Linear regression analysis displayed a significant correlation of the magnitude of these induced changes. Results on effective SMA-M1 connectivity additionally supported findings on unidirectional tDCS effects. In control experiment 2 anodal, cathodal and sham tDCS in our novel montage without $PAS_{SMA \rightarrow M1}$ had no influence on MEP amplitude. Findings suggested that tDCS influenced the SMA-M1 connection instead of modifying M1 excitability. Results thus permitted to reject our hypothesis of antithetic impact of anodal and cathodal tDCS on $PAS_{SMA \rightarrow M1}$ (Faber et al. 2017a).

4.2.4.1 Cortical geometry takes account for polarity-independent effects.

Our electrode montage caused inflowing (positive values) and outflowing (negative values) radial currents in M1 and the surrounding brain areas (see modeling data in Figure 9 (D) for anodal tDCS and Figure 9 (E) for cathodal tDCS).

The macroscopic cortical architecture can explain the observed polarity independent tDCS-effects. Direct current stimulation has opposing effects depending on the location of a specific neuron in the cortex (Creutzfeldt et al. 1962). Due to the gyral folding of

the motor cortex membranes of pyramidal neurons in opposing walls of one gyrus are polarized in opposite direction (Lafon et al. 2017, Opitz et al. 2015, Thielscher et al. 2011). The outward current of the radial component of the electric field shifts the membrane potential of one pyramidal neuron versus hyperpolarization (Lafon et al. 2017). Contrarily, the inward current on the opposite gyral wall causes pyramidal membrane depolarization (Lafon et al. 2017). At this stage gyral folding is thought to be a major determinant of the high interindividual variability and the on average small net effect of tDCS studies (Dyke et al. 2016, Horvath et al. 2015, Lopez-Alonso et al. 2014, Wiethoff et al. 2014).

SMA is located centrally, in the frontal region of the cortex (Lehericy et al. 2004b). The position of the hand area of M1 is more posterior and laterally (Lehericy et al. 2004b). In a simplistic model, we assumed that the connecting fibers between SMA and M1 run from anterior-medial to posterior-lateral in parallel to the cortical surface and the induced tangential field by our tDCS montage (see Sections 1.6.2.1 and 2.2.1). However, anatomical and imaging studies reveal that only a minority of connecting fibers (20-30%) is aligned parallel (Bracht et al. 2012, Vergani et al. 2014). The U-fibers run predominantly from superficial SMA to the basis of the precentral sulcus, then bending upward to reach its gyral crown (Bracht et al. 2012, Vergani et al. 2014). Thus, during tDCS the fibers connecting SMA and M1 are simultaneously de- and hyperpolarized. This results in an equivalent net effect during anodal and cathodal tDCS.

To conclude, during tDCS depolarization and hyperpolarization may concurrently occur in M1 pyramidal neurons and in the SMA-M1 connecting fibers. Those polarizing effects might cancel each other out and cause the polarity-independent net effect of tDCS on $PAS_{SMA \rightarrow M1}$ (Faber et al. 2017a).

4.2.4.2 Radial symmetry of cortical columns explains polarity-independent tDCS effects

Compared to the “classical” montage our montage differed as the stimulating electrode was placed 3 cm behind M1 (see Sections 1.6.2.1 and 2.2.1). We have chosen this montage, as it induced higher tangential electrical field strength in the region of SMA-M1 axon terminals than the “classical” montage (Miranda et al. 2013, Opitz et al. 2015, Rahman et al. 2013).

The dominating interneurons of the brain are organized in a horizontally oriented parallel “slice” system around the pyramidal cells (Szentagothai 1975). The apical dendritic arbor of pyramidal cell shows central symmetry (Marin-Padilla 2014). Ending axon collaterals form a compact disk around the apical dendritic arbor of the pyramidal cell parallel to the cortical surface (Gatter et al. 1978, Szentagothai 1975). Supposing axon terminals of the SMA-M1 connection have no preferential orientation, anodal and cathodal transcranial direct current stimulation would depolarize and hyperpolarize the same amount of axon collaterals, respectively. In a resting system the tDCS effects would cancel out and would not affect pyramidal cells. This is supported by the findings of control experiment 2 and another study, which reported no effects on cortical excitability, when placing the tDCS electrode posterior to the motor cortex (Nitsche and Paulus 2000) (Faber et al. 2017a).

4.2.4.3 $PAS_{SMA \rightarrow M1}$ and tDCS interact cooperatively

In reversing or even suppressing the effects of $PAS_{SMA \rightarrow M1,}$ both anodal and cathodal tDCS interacted with PAS cooperatively. Timing of tDCS in reference to PAS reverses the effects on M1 excitability (Nitsche et al. 2007b). Anodal tDCS in the “classical” montage immediately before PAS further increases the PAS-induced excitability enhancement (Nitsche et al. 2007b), while cathodal tDCS before PAS diminishes the induced excitability rise (Nitsche et al. 2007b, Stefan et al. 2000). The simultaneous application of tDCS and conventional PAS causes opposite effects: anodal tDCS reduces M1 excitability and cathodal tDCS rises M1 excitability (Nitsche et al. 2007b). This indicates that plasticity-inducing tDCS interacts with PAS and that timing between the protocols determines the effects of this interaction (Nitsche et al. 2007b, Stefan et al. 2000).

Moreover, our study is not the first to report on polarity-independent tDCS effects. Cerebellar tDCS, i.e. one electrode placed over the lateral cerebellum and the other over the ipsilateral buccinators muscle, bidirectionally modifies the inhibitory influence of the cerebellum to the primary motor cortex (Galea et al. 2009). Cerebellar tDCS abolishes the effects of peripheral PAS (Stefan et al. 2000) independent of polarity (Hamada et al. 2012). During motor learning the application of both anodal and cathodal tDCS reduces the training-induced directional change of thumb movements (Rosenkranz et al. 2000). TDCS has polarity-independent effects on generalization of

intrinsic patterns of motor learning (Orban de Xivry et al. 2011). During finger tapping, anodal tDCS reduces the blood-oxygenation-level dependent (BOLD) signal in SMA (Antal et al. 2011). Cathodal tDCS also shows a trend towards a reduction (Antal et al. 2011). The BOLD signal in fMRI studies is used as an indirect measure of changes in neuronal activity (Logothetis et al. 2001). fMRI reveals a trend towards a polarity-independent rise in the motor network strength after tDCS in the "classical" montage (Amadi et al. 2014). Both anodal and cathodal tDCS during a motor task decrease primary motor cortex excitability (Antal et al. 2007).

All in all these studies propose that during motor learning the concurrent or prior application of tDCS surpresses plasticity induction. This might not only be attributable to changes in the primary motor cortex but also to changes in premotor areas and their connections to the primary motor cortex (Di Lazzaro et al. 2008). According to modeling studies the highest electrical field strength is between the electrodes (Opitz et al. 2015). That is why changes in premotor areas and their dense neuronal connections to the primary motor cortex might contribute to the tDCS effects (Di Lazzaro et al. 2008). To further explore this, studies using i.e. TMS-EEG might give an insight into relevant co-activation of other brain areas (Premoli et al. 2014a, Premoli et al. 2014b) (Faber et al. 2017a).

4.2.4.4 Effects on effective SMA-M1 connectivity

We found polarity-independent tDCS effects on effective SMA-M1 connectivity (Figure 21). FMRI shows polarity-dependent effects on connectivity of tDCS in the "classical" montage (Amadi et al. 2014, Polania et al. 2012). After 10 min of 1 mA cathodal tDCS connectivity between both left and right SMA and the hand area of M1 and M1c increases (Amadi et al. 2014). This effect is absent after anodal or sham stimulation (Amadi et al. 2014). Both tDCS polarities significantly change connectivity within the motor system (Polania et al. 2012).

Modeling reveals the highest induced electric field strength on the edge of the tDCS electrode (Miranda et al. 2013, Opitz et al. 2015, Salvador et al. 2015). So tDCS in the "classical" montage might predominantly influence regions in front of M1. There fibers from SMA to M1 have a defined anisotropic orientation (Bracht et al. 2012, Vergani et al. 2014). This clear orientation could take account for the polarity-dependent tDCS effects in the "classical" montage (Nitsche and Paulus 2000). By contrast, our more

posterior electrode montage might induce polarity-independent effects due to isotropic fiber orientation in regions with maximum electric field strength (Gatter et al. 1978, Marin-Padilla 2014, Szentagothai 1975) (Faber et al. 2017a) (see Section 4.2.4.2).

4.2.4.5 Cooperative effects of tDCS with $PAS_{SMA \rightarrow M1}$ stabilize post-plasticity induction

Results indicated that cooperate tDCS effects on $PAS_{SMA \rightarrow M1}$ stabilized at later time points (Figure 18, Section 3.1.2.3). In neocortical pyramidal neurons, cooperative LTP-induction stabilizes after approximately 20 min of stimulation (Sjöström and Häusser 2006).

Statistical analysis on SMA-M1 effective connectivity revealed a significant TIME*CONDITION interaction. It was determined by differences at time points P30 and P60 of $PAS_{SMA \rightarrow M1}$ + sham tDCS and $PAS_{SMA \rightarrow M1}$ + cathodal tDCS. At P30 cathodal tDCS led to an increase in effective SMA-M1 connectivity, while anodal and sham tDCS led to a decrease (Figure 21). Additionally, contrarily to all other time points at P30, anodal tDCS and cathodal tDCS had contrasting effects on the M1 excitability change induced by $PAS_{SMA \rightarrow M1}$ (Figure 18). One could reason that manifold homeostatic mechanisms are especially active in the early phase post-plasticity induction to shield the brain from over-excitation (Karabanov et al. 2015, Müller-Dahlhaus and Ziemann 2015). Cortical reorganization might, therefore, gradually develop after cooperative stimulation.

This hypothesis is further supported by an identical time course of online effects of anodal, cathodal and sham tDCS in experiment 2 (Figure 17). Additionally, experiment 1 indicated growing differences between the experimental conditions within the first hour post-stimulation (Figure 16).

In the light of the current knowledge on effects of tDCS, $PAS_{SMA \rightarrow M1}$ and cortex anatomy, we concluded on polarity-independent aftereffects on effective SMA-M1 connectivity of cooperative tDCS with $PAS_{SMA \rightarrow M1}$ in the novel experimentally applied montage (Faber et al. 2017a).

4.2.5 Astrocytic Calcium surges accountable for polarity-independent tDCS effects

Ca^{2+} surges in astrocytes induced by tDCS could also take account for the observed polarity-independent tDCS effects (Monai et al. 2016). Astrocytes are thought to play a key role for brain homeostasis and participate in both plasticity and metaplasticity (Jones 2015, Singh and Abraham 2017). In mice, Ca^{2+} surges in astrocytes increase during tDCS, whereas neuronal activity remains the same as during spontaneous firing (Monai et al. 2016). Importantly, astrocytic Ca^{2+} surges are not limited to the stimulating sites but present anywhere in the brain (Monai et al. 2016). Plastic changes in neurons might, therefore, occur secondarily due to interactions between astrocytes and neurons (Monai et al. 2016).

Alzheimer's disease is characterized by disabled memory formation (Ballard et al. 2011). LTP induction is a model for memory formation (Cooke and Bliss 2006). In a mouse model of Alzheimer's disease astrocytic Ca^{2+} levels are globally elevated (Kuchibhotla et al. 2009). Astrocytic dysfunction is thought to also significantly contribute to the LTP impairment in Alzheimer's patients (Singh and Abraham 2017).

Based on those findings, one could reason that tDCS suppresses the $PAS_{SMA \rightarrow M1}$ effects by globally increasing Ca^{2+} levels in astrocytes and reducing the potential for LTP-induction. However, attention is needed, when transferring tDCS studies from animals to humans: firstly, modeling of the electric field distribution during tDCS indicates already a huge interindividual variability between humans (Datta et al. 2009a, Opitz et al. 2015). Secondly, in animal studies current intensity and density are chosen up to 100 times bigger than in humans (Brunoni et al. 2011, Jackson et al. 2016). Electrode size and position vary between mice and humans (Brunoni et al. 2011). Those factors influence the induced electric field (Miranda et al. 2013). Finally, tDCS in the "classical" montage in humans induces polarity-specific plasticity alterations, which make a polarity-independent global rise in astrocytic Ca^{2+} levels rather unlikely (Liebetanz et al. 2002, Nitsche and Paulus 2000, Nitsche and Paulus 2001). All in all, at this stage, it remains unclear whether the observed knowledge on increased astrocytic Ca^{2+} surges during tDCS in mice can be transferred to humans and at which portion it takes account for polarity-independent tDCS effects (Faber et al. 2017a).

4.3 Limitations and outlook

4.3.1 MEPs as single output measure

We only evaluated MEP amplitude as output measure for induced excitability changes in M1 and motor learning (Muellbacher et al. 2001). MEP amplitude is influenced by a multitude of parameters like age (Müller-Dahlhaus et al. 2008), preceding motor cortex activation (Goldsworthy et al. 2014), attention (Stefan et al. 2002), time of day (Sale et al. 2008), menstrual cycle (Inghilleri et al. 2004, Smith et al. 1999) influence MEP amplitude. A recent review criticizes that MEP excitability seems to be the only cortical measure that is influenced by tDCS (Horvath et al. 2015). Researchers have asked for a stricter control of experimental condition to reduce the bias due to interindividual differences (Hamada et al. 2013, Lopez-Alonso et al. 2014).

Aware of those confounding factors we performed a highly controlled experiment: we strictly controlled experimental conditions (i.e. right-handers, neuronavigation, pseudo-randomized double blind study design, appropriate time intervals to exclude interactions between experimental conditions). Furthermore, we applied stimulation parameters with effects in previous studies (Arai et al. 2012, Arai et al. 2011, Civardi et al. 2001). Participants were asked to relax their FDI, which was monitored by surface EMG as muscle contraction during stimulation abolished stimulating effects (Arai et al. 2012, Civardi et al. 2001). Conditioning SMA stimulating intensity was at 140% AMT, an intensity that facilitates a test stimulus at M1 (Arai et al. 2012, Arai et al. 2011). We chose the most effective current orientation for conditioning facilitatory SMA stimulation (from medial to lateral) and for test stimulation M1 (from posterior-lateral to anterior-medial) (Arai et al. 2012, Di Lazzaro et al. 1998). The time interval between SMA stimulation and M1 stimulation was selected according to the physiological transmission time of a SMA stimulus to induce LTP-like PAS effects with subsequent M1 stimulation (Arai et al. 2011, Civardi et al. 2001, Müller-Dahlhaus et al. 2010, Stefan et al. 2000).

One could argue that our results are merely reflecting regression to the mean. This is highly unlikely as although anodal and cathodal tDCS influenced $PAS_{SMA\rightarrow M1}$ into the same direction in 13/15 subjects (87%, $p = 0.004$ for deviation from equality), in some participants tDCS further increased the $PAS_{SMA\rightarrow M1}$ induced excitability changes. We

suggest that future NIBS studies may include behavioral data and use bigger samples to minimize the probability for random errors.

4.3.2 Limitations of tDCS

To produce bigger and longer-lasting plasticity effects at the cortical level, we cooperatively applied anodal tDCS during $PAS_{SMA \rightarrow M1}$. Anodal stimulation of <10 min leads to a short lasting MEP increase (Liebetanz et al. 2002, Nitsche et al. 2007a, Nitsche and Paulus 2000, Nitsche and Paulus 2001, Nitsche et al. 2007b). Various approaches have displayed that augmenting tDCS effects is not easily possible. Longer duration of tDCS stimulation results in a longer-lasting excitability change (Nitsche et al. 2003, Nitsche and Paulus 2000) and decreases interindividual variability (Nitsche and Paulus 2001). However, maximum tDCS duration is limited by local heating and skin irritation (Datta et al. 2009b). To avoid this, tDCS protocols have been applied repetitively with varying inter-block intervals. In case of anodal tDCS in the "classical" montage, a second tDCS protocol after 20 min further increases LTP-like effects, while longer time intervals between the protocols turn LTP into a LTD-like excitability decrease (Monte-Silva et al. 2013). Increasing current intensity from 1 mA to 2 mA during 20 min of cathodal transcranial stimulation in the "classical" montage leads to a excitability shift from decrease to increase for at least two hours after stimulation (Batsikadze et al. 2013). This shows that it is not easily possible to predict the effects of consecutive tDCS protocols on cortical excitability. All in all, a systematic study of effects of various tDCS parameters is needed, as mechanisms underlying tDCS and interaction between different protocols are still poorly understood.

4.3.3 Participation of other cortical areas

Our modeling displayed, that polarizing effects of tDCS spread out over a multitude of cortical areas, which are highly interconnected. Beyond SMA and M1, the electric field during tDCS extends over premotor areas (Salvador et al. 2015). Especially dorsal and ventral premotor cortexes are potentially relevant as they are densely connected within the motor network and participate in movement execution (Grefkes et al. 2008a, Rehme et al. 2011) (see Section 1.5.5). Additionally they might contribute indirectly to the activation of M1 by TMS (Di Lazzaro et al. 2008). Even though our novel electrode montage induced smaller electric fields in those areas than the "classical" montage (see

Figures 9-10), we cannot rule out participation. Additionally, our more posterior placed electrode might have affected the primary somatosensory cortex. Since we did not obtain excitability data from the somatosensory cortex, we cannot exclude stimulation. As control experiment 2 did not show a significant alteration of M1 excitability we doubt on a significant stimulation of this brain area. The use of multiple electrodes in a ring arrangement to increase the focus of the tDCS stimulation (Datta et al. 2009a, Datta et al. 2009b) could limit uncertainty about participation of other brain areas in future experiments (Faber et al. 2017a).

4.3.4 Limitations of translational medical research with NIBS

The present experiment displayed no significant effects of $PAS_{SMA \rightarrow M1}$ + sham tDCS on MEP amplitude and effective SMA-M1 connectivity. This might be due to the high interindividual variability in response to various NIBS techniques (Wiethoff et al. 2014).

The same PAS protocol causes contrasting effects within a sample, resulting either in an LTP-like MEP increase or an LTD-like MEP decrease in approximately half of the subjects (Müller-Dahlhaus et al. 2008). Similar findings have been made for other stimulation techniques: 50% of the subjects showed no or only minor responses to tDCS (Wiethoff et al. 2014). Only 25% of subjects responded to both continuous theta burst stimulation and intermittent theta burst stimulation in the expected way (Hamada et al. 2013). Additionally, individuals show little test-retest reliability (Dyke et al. 2016).

Until now, several different individual factors like latency differences of MEPs between anterior-posterior and lateral-medial orientation of the TMS coil during stimulation (Hamada et al. 2013, Wiethoff et al. 2014), cortical thickness (List et al. 2013), SICI (Lopez-Alonso et al. 2014), RMT (Müller-Dahlhaus JF et al. 2008) have been discussed as possible predictors for NIBS response. So far no valid prognostic marker for NIBS protocols has been found. To further increase the intricacy, being responder to one NIBS protocol does not imply responsiveness to another (Hamada et al. 2013, Lopez-Alonso et al. 2014).

Aware of the influence of the previous state of the network (metaplasticity), interindividual and intraindividual differences, experiments stimulate based on the product of those variables – the actual brain state. EGG uncovers actual cortical activity

accompanying movement execution (Kornhuber et al. 1965). Data from intracortical electrodes of a tetraplegic patient can control an artificial limb (Boninger et al. 2013, Collinger et al. 2013). Applying non-invasive brain stimulation in a closed-loop-setting based on an individual's EEG signal might permit the use the right protocol for the right individual at the right time (Bergmann et al. 2016, Zrenner et al. 2016, Zrenner and Ziemann 2015). First results are auspicious: rTMS led to LTP-like excitability increase if triggered by high-excitability states in EEG, while triggered by low-excitability states the same protocol did not change corticospinal excitability (Zrenner et al. 2018).

In summary, medical translational research needs studies at both ends: On the one hand predictive factors for NIBS response need to be found and on the other hand appropriate protocols for the individual brain state need to be determined (Faber et al. 2017a).

4.4 Final conclusion

This thesis aimed to transfer the concept of cooperativity, a key characteristic of STDP at the cellular level, to the network level (Sjöström and Häusser 2006, Sjöström et al. 2001).

PAS of connected brain areas using noninvasive brain stimulation follows the principles of STDP as defined on the cellular level (Arai et al. 2011, Müller-Dahlhaus et al. 2010, Stefan et al. 2000, Wolters et al. 2003). However, whether cooperativity, a core principle of STDP, is mirrored on the cortical level was rather unclear. Results of our two studies pointed out that plasticity mechanisms on network resemble those at the cellular level. However, plasticity induction with NIBS has to take account for additional influencing factors.

Firstly, cooperative $PAS_{M1c \rightarrow M1}$ as well as anodal and cathodal tDCS during $PAS_{SMA \rightarrow M1}$ were not able to augment the possible excitability-rising effects $PAS_{SMA \rightarrow M1}$. At the cortical level, strong safeguard mechanisms shield the brain from excitotoxicity and damage (Abraham 2008, Hulme et al. 2013, Karabanov et al. 2015). Additionally, modifications of singular connections of the human brain might need to be embedded in broader network changes in line with the physiological situation where multiple motor areas interact during movement execution (Purves 2012).

Secondly, effects of anodal tDCS + $PAS_{SMA \rightarrow M1}$ and cathodal tDCS + $PAS_{SMA \rightarrow M1}$ were only present during concurrent $PAS_{SMA \rightarrow M1}$. Those findings indicated that cooperative mechanisms operate at the network level. Gyral folding and radial symmetry of cortical columns in M1 could be responsible for the observed polarity-independent effects of our novel tDCS montage (Lafon et al. 2017, Opitz et al. 2015, Szentagothai 1975, Thielscher et al. 2011).

Finally, our results displayed that medical translational research on NIBS faces high intra- and interindividual variability (Müller-Dahlhaus et al. 2008, Wiethoff et al. 2014). Modeling of the electric field during anodal and cathodal tDCS displayed the effect of individual gyral folding and electrode position in the healthy (Datta et al.

2009a, Opitz et al. 2015). Aware of those influencing factors, NIBS research is still searching for predictive factors for individual response to NIBS.

In conclusion, our outcomes support further systematic research on mechanisms underlying tDCS, behavioral effects in bigger samples and adaption of NIBS protocols on the current brain state. In the light of those future findings, it seems worthwhile to reassess the therapeutic value of cooperative stimulation for motor rehabilitation after stroke (Faber et al. 2017a).

5 Summary

Introduction: Neuronal connectivity can be up- or down-regulated according to the temporal sequence of in- and output potentials – a process called spike-timing plasticity (STDP) (Bi and Poo 2001, Hebb 1949, Markram et al. 2011). Paired associative stimulation (PAS) of the human cortex modifies brain connectivity in a STDP-like manner (Arai et al. 2011, Koch et al. 2013, Stefan et al. 2000). In stroke patients, the degree of motor recovery correlates with the grade of restored connectivity between the supplementary motor area (SMA) and the left primary motor cortex (M1) (Rehme et al. 2011). PAS of this connectivity ($PAS_{SMA \rightarrow M1}$) can modulate M1 excitability bidirectionally (Arai et al. 2011). As effects are short-lived, they are not yet beneficial for the patient.

This thesis aimed to overcome this obstacle in transferring the cellular principle of cooperativity to the network level. At the cellular level pairing a subthreshold presynaptic stimulus for long-term potentiation (LTP) with either (1) an extracellular stimulus or (2) postsynaptic depolarization rescues LTP (Sjöström and Häusser 2006, Sjöström et al. 2001). Experiment 1 examined, if cooperative PAS of the contralateral motor cortex (M1c) and M1 ($PAS_{M1c \rightarrow M1}$) with $PAS_{SMA \rightarrow M1}$ increases M1 excitability. Experiment 2 tested, whether cooperative anodal transcranial direct current stimulation (tDCS) with $PAS_{SMA \rightarrow M1,}$ in contrast to cathodal tDCS, enhances M1 excitability and effective SMA-M1 connectivity.

Material and Methods: Experiment 1 examined 11 healthy right-handed participants (8 males, 3 females). 15 healthy right-handed males participated in experiment 2. All subjects gave written informed consent. Both studies conformed to the latest version of the Declaration of Helsinki and were approved by the local Ethics Committee of the Medical Faculty of the Eberhard Karls University Tübingen, Germany. We developed two innovative stimulation techniques. In experiment 1 we used a triple coil technique for cooperative $PAS_{M1c \rightarrow M1}$ with $PAS_{SMA \rightarrow M1}$ in a double-blinded, pseudo-randomized crossover design. Effects on motor cortex excitability were analyzed up to one hour after the intervention. Experiment 2 tested the cooperative effects of the concurrent application of tDCS with $PAS_{SMA \rightarrow M1}$ in a double-blinded, pseudo-randomized crossover design. Based on electric field modulations, we applied a novel electrode

montage: the electrode for primary motor cortex stimulation was placed 3 cm posterior to the hand area of M1. We evaluated effects on M1 excitability and effective SMA-M1 connectivity on motor evoked potentials of a hand muscle up to two hours after the intervention and analyzed the data statistically.

Results: Cooperative $PAS_{M1c \rightarrow M1}$ with $PAS_{SMA \rightarrow M1}$ in experiment 1 caused no significant change in M1 excitability compared to a control condition. In experiment 2 cooperative anodal tDCS with $PAS_{SMA \rightarrow M1}$ did not increase M1 excitability or effective SMA-M1 connectivity significantly. However, post-hoc analysis showed a significant tDCS with $PAS_{SMA \rightarrow M1}$ interaction. Anodal and cathodal tDCS modulated the $PAS_{SMA \rightarrow M1}$ effect on M1 excitability in the majority of subjects in equivalent direction (87%, $p = 0.004$ for deviation from equality). The effect sizes of anodal and cathodal tDCS correlated ($r_S = 0.525$, $p = 0.044$). In a control experiment anodal or cathodal tDCS in our novel montage without $PAS_{SMA \rightarrow M1}$ did not change M1 excitability.

Conclusion: Cooperative application of two excitability-rising noninvasive brain stimulation protocols did not further increase M1 excitability. According to the concept of homeostatic metaplasticity, safeguard mechanisms operate at the network level to prevent over-excitation and runaway plasticity (Abraham 2008, Abraham and Bear 1996, Hulme et al. 2013, Karabanov et al. 2015). In contrast to our hypothesis, simultaneous, cooperative tDCS with $PAS_{SMA \rightarrow M1}$ suppressed or even reversed the $PAS_{SMA \rightarrow M1}$ effects. These findings can be explained by radial symmetry of cortical columns, gyral folding of the motor cortex and cooperativity of plasticity induction.

6 Bibliography / List of references

Abbott LF and Nelson SB (2000) Synaptic plasticity: taming the beast. Nat Neurosci 3: 1178-1183.

Abraham WC (2008) Metaplasticity: tuning synapses and networks for plasticity. Nat Rev Neurosci 9: 387-399.

Abraham WC and Bear MF (1996) Metaplasticity: the plasticity of synaptic plasticity. Trends Neurosci 19: 126-130.

Adeyemo BO, Simis M, Macea DD and Fregni F (2012) Systematic review of parameters of stimulation, clinical trial design characteristics, and motor outcomes in non-invasive brain stimulation in stroke. Front Psychiatry 3: 88.

Amadi U, Ilie A, Johansen-Berg H and Stagg CJ (2014) Polarity-specific effects of motor transcranial direct current stimulation on fMRI resting state networks. Neuroimage 88: 155-161.

Antal A, Polania R, Schmidt-Samoa C, Dechent P and Paulus W (2011) Transcranial direct current stimulation over the primary motor cortex during fMRI. Neuroimage 55: 590-596.

Antal A, Terney D, Poreisz C and Paulus W (2007) Towards unravelling task-related modulations of neuroplastic changes induced in the human motor cortex. Eur J Neurosci 26: 2687-2691.

Arai N, Lu MK, Ugawa Y and Ziemann U (2012) Effective connectivity between human supplementary motor area and primary motor cortex: a paired-coil TMS study. Exp Brain Res 220: 79-87.

Arai N, Müller-Dahlhaus F, Murakami T, Bliem B, Lu MK, Ugawa Y and Ziemann U (2011) State-dependent and timing-dependent bidirectional associative plasticity in the human SMA-M1 network. J Neurosci 31: 15376-15383.

Ardolino G, Bossi B, Barbieri S and Priori A (2005) Non-synaptic mechanisms underlie the after-effects of cathodal transcutaneous direct current stimulation of the human brain. J Physiol 568: 653-663.

Ballard C, Gauthier S, Corbett A, Brayne C, Aarsland D and Jones E (2011) Alzheimer's disease. The Lancet 377: 1019-1031.

Barker AT, Jalinous R and Freeston IL (1985) Non-invasive magnetic stimulation of human motor cortex. The Lancet 1: 1106-1107.

Batsikadze G, Moliadze V, Paulus W, Kuo MF and Nitsche MA (2013) Partially non-linear stimulation intensity-dependent effects of direct current stimulation on motor cortex excitability in humans. J Physiol 591: 1987-2000.

Bergmann TO, Karabanov A, Hartwigsen G, Thielscher A and Siebner HR (2016) Combining non-invasive transcranial brain stimulation with neuroimaging and electrophysiology: Current approaches and future perspectives. Neuroimage 140: 4-19.

Bi G and Poo M (2001) Synaptic modification by correlated activity: Hebb's postulate revisited. Annu Rev Neurosci 24: 139-166.

Bienenstock EL, Cooper LN and Munro PW (1982) Theory for the development of neuron selectivity: orientation specificity and binocular interaction in visual cortex. J Neurosci 2: 32-48.

Bikson M, Inoue M, Akiyama H, Deans JK, Fox JE, Miyakawa H and Jefferys JG (2004) Effects of uniform extracellular DC electric fields on excitability in rat hippocampal slices in vitro. J Physiol 557: 175-190.

Bindman LJ, Lippold OC and Redfearn JW (1962) Long-lasting changes in the level of the electrical activity of the cerebral cortex produced by polarizing currents. Nature 196: 584-585.

Bindman LJ, Lippold OC and Redfearn JW (1964) The action of brief polarizing currents on the cerebral cortex of the rat (1) during current flow and (2) in the production of long-lasting after-effects. J Physiol 172: 369-382.

Bliss TV and Lomo T (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. J Physiol 232: 331-356.

Boninger M, Mitchell G, Tyler-Kabara E, Collinger J and Schwartz AB (2013) Neuroprosthetic control and tetraplegia--authors' reply. Lancet 381: 1900-1901.

Bracht T, Federspiel A, Schnell S, Horn H, Hofle O, Wiest R, Dierks T, Strik W, Muller TJ and Walther S (2012) Cortico-cortical white matter motor pathway microstructure is related to psychomotor retardation in major depressive disorder. PLoS One 7: e52238.

Brown DL, Boden-Albala B, Langa KM, Lisabeth LD, Fair M, Smith MA, Sacco RL and Morgenstern LB (2006) Projected costs of ischemic stroke in the United States. Neurology 67: 1390-1395.

Brunoni AR, Fregni F and Pagano RL (2011) Translational research in transcranial direct current stimulation (tDCS): a systematic review of studies in animals. Rev Neurosci 22: 471-481.

Buch ER, Johnen VM, Nelissen N, O'Shea J and Rushworth MF (2011) Noninvasive associative plasticity induction in a corticocortical pathway of the human brain. J Neurosci 31: 17669-17679.

Celnik P, Paik NJ, Vandermeeren Y, Dimyan M and Cohen LG (2009) Effects of combined peripheral nerve stimulation and brain polarization on performance of a motor sequence task after chronic stroke. Stroke 40: 1764-1771.

Chen SY and Winstein CJ (2009) A systematic review of voluntary arm recovery in hemiparetic stroke: critical predictors for meaningful outcomes using the international classification of functioning, disability, and health. J Neurol Phys Ther 33: 2-13.

Cincotta M, Borgheresi A, Jung P, Balestrieri F, Giovannelli F, Zaccara G and Ziemann U (2005) Physical interactions between induced electrical fields can have substantial effects on neuronal excitation during simultaneous TMS of two brain areas. Clin Neurophysiol 116: 1733-1742.

Civardi C, Cantello R, Asselman P and Rothwell JC (2001) Transcranial magnetic stimulation can be used to test connections to primary motor areas from frontal and medial cortex in humans. Neuroimage 14: 1444-1453.

Collinger JL, Wodlinger B, Downey JE, Wang W, Tyler-Kabara EC, Weber DJ, McMorland AJ, Velliste M, Boninger ML and Schwartz AB (2013) High-performance neuroprosthetic control by an individual with tetraplegia. Lancet 381: 557-564.

Cooke SF and Bliss TV (2006) Plasticity in the human central nervous system. Brain 129: 1659-1673.

Coupar F, Pollock A, Rowe P, Weir C and Langhorne P (2012) Predictors of upper limb recovery after stroke: a systematic review and meta-analysis. Clin Rehabil 26: 291-313.

Creutzfeldt OD, Fromm GH and Kapp H (1962) Influence of transcortical d-c currents on cortical neuronal activity. Exp Neurol 5: 436-452.

Cuypers K, Thijs H and Meesen RL (2014) Optimization of the transcranial magnetic stimulation protocol by defining a reliable estimate for corticospinal excitability. PLoS One 9: e86380.

Dan Y and Poo MM (2006) Spike timing-dependent plasticity: from synapse to perception. Physiol Rev 86: 1033-1048.

Datta A, Bansal V, Diaz J, Patel J, Reato D and Bikson M (2009a) Gyri-precise head model of transcranial direct current stimulation: improved spatial focality using a ring electrode versus conventional rectangular pad. Brain Stimul 2: 201-207.

Datta A, Elwassif M and Bikson M (2009b) Bio-heat transfer model of transcranial DC stimulation: comparison of conventional pad versus ring electrode. Conf Proc IEEE Eng Med Biol Soc 2009: 670-673.

Di Lazzaro V, Oliviero A, Pilato F, Saturno E, Dileone M, Mazzone P, Insola A, Tonali PA and Rothwell JC (2004) The physiological basis of transcranial motor cortex stimulation in conscious humans. Clin Neurophysiol 115: 255-266.

Di Lazzaro V, Oliviero A, Profice P, Saturno E, Pilato F, Insola A, Mazzone P, Tonali P and Rothwell JC (1998) Comparison of descending volleys evoked by transcranial magnetic and electric stimulation in conscious humans. Electroencephalogr Clin Neurophysiol 109: 397-401.

Di Lazzaro V, Ziemann U and Lemon RN (2008) State of the art: Physiology of transcranial motor cortex stimulation. Brain Stimul 1: 345-362.

Dick JP, Cantello R, Buruma O, Gioux M, Benecke R, Day BL, Rothwell JC, Thompson PD and Marsden CD (1987) The Bereitschaftspotential, L-DOPA and Parkinson's disease. Electroencephalogr Clin Neurophysiol 66: 263-274.

Dick JP, Rothwell JC, Day BL, Cantello R, Buruma O, Gioux M, Benecke R, Berardelli A, Thompson PD and Marsden CD (1989) The Bereitschaftspotential is abnormal in Parkinson's disease. Brain 112: 233-244.

Dimyan MA and Cohen LG (2011) Neuroplasticity in the context of motor rehabilitation after stroke. Nat Rev Neurol 7: 76-85.

Dyke K, Kim S, Jackson GM and Jackson SR (2016) Intra-Subject Consistency and Reliability of Response Following 2 mA Transcranial Direct Current Stimulation. Brain Stimul 9: 819-825.

Faber H, Opitz A, Müller-Dahlhaus F and Ziemann U (2017a) Polarity-independent effects of tDCS on paired associative stimulation-induced plasticity. Brain Stimulation 10: 1061-1069.

Faber H, Opitz A, Zipser CM, Müller-Dahlhaus F, Ziemann U (2017b) P 56 tDCS shows no effects on motor cortex excitability at rest. Clinical Neurophysiology 2017 (128): e356-e357.

Faber H, Zipser CM, Tünnerhoff J, Müller-Dahlhaus F, Ziemann U (2015) P149. Suppression of LTP-like associative plasticity in the human SMA–M1 network by simultaneous tDCS. Clinical Neurophysiology 126: e136.

Faber H, Zipser CM, Tünnerhoff J, Müller-Dahlhaus F, Ziemann U (2016) ID 394 – Polarity independent suppression of long-term associative plasticity in the human SMA–M1 network by simultaneous tDCS. Clinical Neurophysiology

127: e100.
Ferbert A, Priori A, Rothwell JC, Day BL, Colebatch JG and Marsden CD (1992) Interhemispheric inhibition of the human motor cortex. J Physiol 453: 525-546.
Fink GR, Frackowiak RS, Pietrzyk U and Passingham RE (1997) Multiple nonprimary motor areas in the human cortex. J Neurophysiol 77: 2164-2174.
Fritsch G and Hitzig E (1870) Electric excitability of the cerebrum (Über die elektrische Erregbarkeit des Grosshirns). Arch Anat Physiol Wissen 37: 300-332.
Fuhl A, Müller-Dahlhaus F, Lücke C, Toennes SW and Ziemann U (2015) Low Doses of Ethanol Enhance LTD-like Plasticity in Human Motor Cortex. Neuropsychopharmacology 40: 2969-2980.
Galea JM, Jayaram G, Ajagbe L and Celnik P (2009) Modulation of cerebellar excitability by polarity-specific noninvasive direct current stimulation. J Neurosci 29: 9115-9122.
Gandiga PC, Hummel FC and Cohen LG (2006) Transcranial DC stimulation (tDCS): a tool for double-blind sham-controlled clinical studies in brain stimulation. Clin Neurophysiol 117: 845-850.
Gartside IB (1968) Mechanisms of sustained increases of firing rate of neurons in the rat cerebral cortex after polarization: reverberating circuits or modification of synaptic conductance? Nature 220: 382-383.
Gatter KC, Sloper JJ and Powell TP (1978) The intrinsic connections of the cortex of area 4 of the monkey. Brain 101: 513-541.
Gerloff C, Corwell B, Chen R, Hallett M and Cohen LG (1997) Stimulation over the human supplementary motor area interferes with the organization of future elements in complex motor sequences. Brain 120: 1587-1602.
Ghosh S and Porter R (1988) Corticocortical synaptic influences on morphologically identified pyramidal neurones in the motor cortex of the monkey. J Physiol 400: 617-629.
Goldsworthy MR, Müller-Dahlhaus F, Ridding MC and Ziemann U (2014) Inter-subject variability of LTD-like plasticity in human motor cortex: a matter of preceding motor activation. Brain Stimul 7: 864-870.
Grefkes C, Eickhoff SB, Nowak DA, Dafotakis M and Fink GR (2008a) Dynamic intra- and interhemispheric interactions during unilateral and bilateral hand movements assessed with fMRI and DCM. Neuroimage 41: 1382-1394.
Grefkes C, Nowak DA, Eickhoff SB, Dafotakis M, Kust J, Karbe H and Fink GR (2008b) Cortical connectivity after subcortical stroke assessed with functional magnetic resonance imaging. Ann Neurol 63: 236-246.
Grefkes C, Nowak DA, Wang LE, Dafotakis M, Eickhoff SB and Fink GR (2010) Modulating cortical connectivity in stroke patients by rTMS assessed with fMRI and dynamic causal modeling. Neuroimage 50: 233-242.
Grillner S (2003) The motor infrastructure: from ion channels to neuronal networks. Nat Rev Neurosci 4: 573-586.
Groppa S, Oliviero A, Eisen A, Quartarone A, Cohen LG, Mall V, Kaelin-Lang A, Mima T, Rossi S, Thickbroom GW, Rossini PM, Ziemann U, Valls-Sole J and Siebner HR (2012) A practical guide to diagnostic transcranial magnetic stimulation: report of an IFCN committee. Clin Neurophysiol 123: 858-882.
Gross J, Kujala J, Hämäläinen M, Timmermann L, Schnitzler A and Salmelin R (2001) Dynamic imaging of coherent sources: Studying neural interactions in the

human brain. Proceedings of the National Academy of Sciences of the United States of America 98: 694-699.
Hallett M (2000) Transcranial magnetic stimulation and the human brain. Nature 406: 147-150.
Hallett M (2007) Transcranial magnetic stimulation: a primer. Neuron 55: 187-199.
Hamada M, Hanajima R, Terao Y, Arai N, Furubayashi T, Inomata-Terada S, Yugeta A, Matsumoto H, Shirota Y and Ugawa Y (2007) Quadro-pulse stimulation is more effective than paired-pulse stimulation for plasticity induction of the human motor cortex. Clin Neurophysiol 118: 2672-2682.
Hamada M, Murase N, Hasan A, Balaratnam M and Rothwell JC (2013) The role of interneuron networks in driving human motor cortical plasticity. Cereb Cortex 23: 1593-1605.
Hamada M, Strigaro G, Murase N, Sadnicka A, Galea JM, Edwards MJ and Rothwell JC (2012) Cerebellar modulation of human associative plasticity. J Physiol 590: 2365-2374.
Hamada M, Terao Y, Hanajima R, Shirota Y, Nakatani-Enomoto S, Furubayashi T, Matsumoto H and Ugawa Y (2008a) Bidirectional long-term motor cortical plasticity and metaplasticity induced by quadripulse transcranial magnetic stimulation. J Physiol 586: 3927-3947.
Hamada M, Ugawa Y and Tsuji S (2008b) High-frequency rTMS over the supplementary motor area for treatment of Parkinson's disease. Mov Disord 23: 1524-1531.
Hebb DO (1949) The organization of behavior. p. 62. John Wiley & Sons. New York.
Hendricks HT, Zwarts MJ, Plat EF and van Limbeek J (2002) Systematic review for the early prediction of motor and functional outcome after stroke by using motor-evoked potentials. Arch Phys Med Rehabil 83: 1303-1308.
Horvath JC, Forte JD and Carter O (2015) Evidence that transcranial direct current stimulation (tDCS) generates little-to-no reliable neurophysiologic effect beyond MEP amplitude modulation in healthy human subjects: A systematic review. Neuropsychologia 66: 213-236.
Hubbard JI and Willis WD (1962a) Hyperpolarization of mammalian motor nerve terminals. J Physiol 163: 115-137.
Hubbard JI and Willis WD (1962b) Mobilization of transmitter by hyperpolarization. Nature 193: 174-175.
Hubbard JI and Willis WD (1962c) Reduction of transmitter output by depolarization. Nature 193: 1294-1295.
Hulme SR, Jones OD and Abraham WC (2013) Emerging roles of metaplasticity in behaviour and disease. Trends Neurosci 36: 353-362.
Hummel F and Cohen L (2006) Non-invasive brain stimulation: a new strategy to improve neurorehabilitation after stroke? Lancet Neurol 5: 708-712.
Hummel FC and Gerloff C (2012) Transkranielle Hirnstimulation nach Schlaganfall. Der Nervenarzt 83: 957-965.
Inghilleri M, Conte A, Curra A, Frasca V, Lorenzano C and Berardelli A (2004) Ovarian hormones and cortical excitability. An rTMS study in humans. Clin Neurophysiol 115: 1063-1068.
Inman CS, James GA, Hamann S, Rajendra JK, Pagnoni G and Butler AJ (2012) Altered resting-state effective connectivity of fronto-parietal motor control

systems on the primary motor network following stroke. Neuroimage 59: 227-237.

Jackson MP, Rahman A, Lafon B, Kronberg G, Ling D, Parra LC and Bikson M (2016) Animal models of transcranial direct current stimulation: Methods and mechanisms. Clin Neurophysiol 127: 3425-3454.

Jones OD (2015) Astrocyte-mediated metaplasticity in the hippocampus: Help or hindrance? Neuroscience 309: 113-124.

Karabanov A, Ziemann U, Hamada M, George MS, Quartarone A, Classen J, Massimini M, Rothwell J and Siebner HR (2015) Consensus Paper: Probing Homeostatic Plasticity of Human Cortex With Non-invasive Transcranial Brain Stimulation. Brain Stimul 8: 993-1006.

Khedr EM, Ahmed MA, Fathy N and Rothwell JC (2005) Therapeutic trial of repetitive transcranial magnetic stimulation after acute ischemic stroke. Neurology 65: 466-468.

Koch G, Ponzo V, Di Lorenzo F, Caltagirone C and Veniero D (2013) Hebbian and anti-Hebbian spike-timing-dependent plasticity of human cortico-cortical connections. J Neurosci 33: 9725-9733.

Kornhuber H, Deecke L and (1965) Hirnpotentialänderungen bei Willkürbewegungen und passiven Bewegungen des Menschen: Bereitschaftspotential und reafferente Potentiale. Pflueger's Archiv für die gesamte Physiologie des Menschen und der Tiere 284: 1-17.

Kuchibhotla KV, Lattarulo CR, Hyman BT and Bacskai BJ (2009) Synchronous hyperactivity and intercellular calcium waves in astrocytes in Alzheimer mice. Science 323: 1211-1215.

Kujirai T, Caramia MD, Rothwell JC, Day BL, Thompson PD, Ferbert A, Wroe S, Asselman P and Marsden CD (1993) Corticocortical inhibition in human motor cortex. J Physiol 471: 501-519.

Lafleur LP, Tremblay S, Whittingstall K and Lepage JF (2016) Assessment of Effective Connectivity and Plasticity With Dual-Coil Transcranial Magnetic Stimulation. Brain Stimul 9: 347-355.

Lafon B, Rahman A, Bikson M and Parra LC (2017) Direct Current Stimulation Alters Neuronal Input/Output Function. Brain Stimul 10: 36-45.

Lange RK, Godde B and Braun C (2004) EEG correlates of coordinate processing during intermanual transfer. Exp Brain Res 159: 161-171.

Laplane D, Talairach J, Meininger V, Bancaud J and Orgogozo JM (1977) Clinical consequences of corticectomies involving the supplementary motor area in man. J Neurol Sci 34: 301-314.

Lefaucheur JP, Antal A, Ayache SS, Benninger DH, Brunelin J, Cogiamanian F, Cotelli M, De Ridder D, Ferrucci R, Langguth B, Marangolo P, Mylius V, Nitsche MA, Padberg F, Palm U, Poulet E, Priori A, Rossi S, Schecklmann M, Vanneste S, Ziemann U, Garcia-Larrea L and Paulus W (2017) Evidence-based guidelines on the therapeutic use of transcranial direct current stimulation (tDCS). Clin Neurophysiol 128: 56-92.

Lehericy S, Ducros M, Krainik A, Francois C, Van de Moortele PF, Ugurbil K and Kim DS (2004a) 3-D diffusion tensor axonal tracking shows distinct SMA and pre-SMA projections to the human striatum. Cereb Cortex 14: 1302-1309.

Lehericy S, Ducros M, Van de Moortele PF, Francois C, Thivard L, Poupon C, Swindale N, Ugurbil K and Kim DS (2004b) Diffusion tensor fiber tracking shows distinct corticostriatal circuits in humans. Ann Neurol 55: 522-529.

Liebetanz D, Nitsche MA, Tergau F and Paulus W (2002) Pharmacological approach to the mechanisms of transcranial DC-stimulation-induced after-effects of human motor cortex excitability. Brain 125: 2238-2247.

List J, Kubke JC, Lindenberg R, Kulzow N, Kerti L, Witte V and Floel A (2013) Relationship between excitability, plasticity and thickness of the motor cortex in older adults. Neuroimage 83: 809-816.

Liu J, Morel A, Wannier T and Rouiller EM (2002) Origins of callosal projections to the supplementary motor area (SMA): A direct comparison between pre-SMA and SMA-proper in macaque monkeys. The Journal of Comparative Neurology 443: 71-85.

Logothetis NK, Pauls J, Augath M, Trinath T and Oeltermann A (2001) Neurophysiological investigation of the basis of the fMRI signal. Nature 412: 150-157.

Lopez-Alonso V, Cheeran B, Rio-Rodriguez D and Fernandez-Del-Olmo M (2014) Inter-individual variability in response to non-invasive brain stimulation paradigms. Brain Stimul 7: 372-380.

Lu MK, Tsai CH and Ziemann U (2012) Cerebellum to motor cortex paired associative stimulation induces bidirectional STDP-like plasticity in human motor cortex. Front Hum Neurosci 6: 260.

Luppino G, Matelli M, Camarda R and Rizzolatti G (1994) Corticospinal projections from mesial frontal and cingulate areas in the monkey. Neuroreport 5: 2545-2548.

Mackay J, Mensah G, Mendis S and Greenland K (2004) Atlas of Heart Disease and Stroke. pp. 50-51. Mackay J, Mensah G, Mendis S, Greenland K, World Health Organisation and Departement of Management of Noncommunicalbe Diseases. World Health Organization. Geneva.

Marin-Padilla M (2014) The mammalian neocortex new pyramidal neuron: a new conception. Front Neuroanat 7: 51.

Markram H, Gerstner W and Sjostrom PJ (2011) A history of spike-timing-dependent plasticity. Front Synaptic Neurosci 3: 4.

Mauguiere F, Allison T, Babiloni C, Buchner H, Eisen A, Goodin D, Jones S, Kakigi R, Matsuoka S and Nuwer M (1999) Somatosensory evoked potentials. Electroencephalogr Clin Neurophysiol Suppl 52: 79-90.

McDonnell MN, Orekhov Y and Ziemann U (2007) Suppression of LTP-like plasticity in human motor cortex by the GABAB receptor agonist baclofen. Exp Brain Res 180: 181-186.

Merton PA and Morton HB (1980) Stimulation of the cerebral cortex in the intact human subject. Nature 285: 227.

Miranda PC, Mekonnen A, Salvador R and Ruffini G (2013) The electric field in the cortex during transcranial current stimulation. Neuroimage 70: 48-58.

Monai H, Ohkura M, Tanaka M, Oe Y, Konno A, Hirai H, Mikoshiba K, Itohara S, Nakai J, Iwai Y and Hirase H (2016) Calcium imaging reveals glial involvement in transcranial direct current stimulation-induced plasticity in mouse brain. Nat Commun 7: 11100.

Monte-Silva K, Kuo MF, Hessenthaler S, Fresnoza S, Liebetanz D, Paulus W and Nitsche MA (2013) Induction of late LTP-like plasticity in the human motor cortex by repeated non-invasive brain stimulation. Brain Stimul 6: 424-432.

Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, Das SR, de Ferranti S, Despres JP, Fullerton HJ, Howard VJ, Huffman MD, Isasi CR, Jimenez MC, Judd SE, Kissela BM, Lichtman JH, Lisabeth LD, Liu S, Mackey RH, Magid DJ, McGuire DK, Mohler ER, 3rd, Moy CS, Muntner P, Mussolino ME, Nasir K, Neumar RW, Nichol G, Palaniappan L, Pandey DK, Reeves MJ, Rodriguez CJ, Rosamond W, Sorlie PD, Stein J, Towfighi A, Turan TN, Virani SS, Woo D, Yeh RW and Turner MB (2016) Heart Disease and Stroke Statistics-2016 Update: A Report From the American Heart Association. Circulation 133: e38-360.

Muellbacher W, Ziemann U, Boroojerdi B, Cohen L and Hallett M (2001) Role of the human motor cortex in rapid motor learning. Exp Brain Res 136: 431-438.

Muellbacher W, Ziemann U, Wissel J, Dang N, Kofler M, Facchini S, Boroojerdi B, Poewe W and Hallett M (2002) Early consolidation in human primary motor cortex. Nature 415: 640-644.

Müller F and Ziemann U (2007) Bestimmung der zentralmotorischen Leitungszeit. pp. 71-78. Siebner HR and Ziemann U. Springer Berlin Heidelberg.

Müller-Dahlhaus F, Lücke C, Lu MK, Arai N, Fuhl A, Herrmann E and Ziemann U (2015) Augmenting LTP-Like Plasticity in Human Motor Cortex by Spaced Paired Associative Stimulation. PLoS One 10: e0131020.

Müller-Dahlhaus F and Ziemann U (2015) Metaplasticity in human cortex. Neuroscientist 21: 185-202.

Müller-Dahlhaus F, Ziemann U and Classen J (2010) Plasticity resembling spike-timing dependent synaptic plasticity: the evidence in human cortex. Front Synaptic Neurosci 2: 34.

Müller-Dahlhaus JF, Orekhov Y, Liu Y and Ziemann U (2008) Interindividual variability and age-dependency of motor cortical plasticity induced by paired associative stimulation. Exp Brain Res 187: 467-475.

Murray CJ, Atkinson C, Bhalla K, Birbeck G, Burstein R, Chou D, Dellavalle R, Danaei G, Ezzati M, Fahimi A, Flaxman D, Foreman, Gabriel S, Gakidou E, Kassebaum N, Khatibzadeh S, Lim S, Lipshultz SE, London S, Lopez, MacIntyre MF, Mokdad AH, Moran A, Moran AE, Mozaffarian D, Murphy T, Naghavi M, Pope C, Roberts T, Salomon J, Schwebel DC, Shahraz S, Sleet DA, Murray, Abraham J, Ali MK, Atkinson C, Bartels DH, Bhalla K, Birbeck G, Burstein R, Chen H, Criqui MH, Dahodwala, Jarlais, Ding EL, Dorsey ER, Ebel BE, Ezzati M, Fahami, Flaxman S, Flaxman AD, Gonzalez-Medina D, Grant B, Hagan H, Hoffman H, Kassebaum N, Khatibzadeh S, Leasher JL, Lin J, Lipshultz SE, Lozano R, Lu Y, Mallinger L, McDermott MM, Micha R, Miller TR, Mokdad AA, Mokdad AH, Mozaffarian D, Naghavi M, Narayan KM, Omer SB, Pelizzari PM, Phillips D, Ranganathan D, Rivara FP, Roberts T, Sampson U, Sanman E, Sapkota A, Schwebel DC, Sharaz S, Shivakoti R, Singh GM, Singh D, Tavakkoli M, Towbin JA, Wilkinson JD, Zabetian A, Murray, Abraham J, Ali MK, Alvardo M, Atkinson C, Baddour LM, Benjamin EJ, Bhalla K, Birbeck G, Bolliger I, Burstein R, Carnahan E, Chou D, Chugh SS, Cohen A, Colson KE, Cooper LT, Couser W, Criqui MH, Dabhadkar KC, Dellavalle RP, Jarlais, Dicker D, Dorsey ER, Duber H, Ebel BE, Engell RE, Ezzati M, Felson DT,

Finucane MM, Flaxman S, Flaxman AD, Fleming T, Foreman, Forouzanfar MH, Freedman G, Freeman MK, Gakidou E, Gillum RF, Gonzalez-Medina D, Gosselin R, Gutierrez HR, Hagan H, Havmoeller R, Hoffman H, Jacobsen KH, James SL, Jasrasaria R, Jayarman S, Johns N, Kassebaum N, Khatibzadeh S, Lan Q, Leasher JL, Lim S, Lipshultz SE, London S, Lopez, Lozano R, Lu Y, Mallinger L, Meltzer M, Mensah GA, Michaud C, Miller TR, Mock C, Moffitt TE, Mokdad AA, Mokdad AH, Moran A, Naghavi M, Narayan KM, Nelson RG, Olives C, Omer SB, Ortblad K, Ostro B, Pelizzari PM, Phillips D, Raju M, Razavi H, Ritz B, Roberts T, Sacco RL, Salomon J, Sampson U, Schwebel DC, Shahraz S, Shibuya K, Silberberg D, Singh JA, Steenland K, Taylor JA, Thurston GD, Vavilala MS, Vos T, Wagner GR, Weinstock MA, Weisskopf MG, Wulf S and Murray (2013) The state of US health, 1990-2010: burden of diseases, injuries, and risk factors. Jama 310: 591-608.

Nachev P, Kennard C and Husain M (2008) Functional role of the supplementary and pre-supplementary motor areas. Nat Rev Neurosci 9: 856-869.

Nakamura K, Sakai K and Hikosaka O (1999) Effects of local inactivation of monkey medial frontal cortex in learning of sequential procedures. J Neurophysiol 82: 1063-1068.

Nitsche MA, Doemkes S, Karakose T, Antal A, Liebetanz D, Lang N, Tergau F and Paulus W (2007a) Shaping the effects of transcranial direct current stimulation of the human motor cortex. J Neurophysiol 97: 3109-3117.

Nitsche MA, Nitsche MS, Klein CC, Tergau F, Rothwell JC and Paulus W (2003) Level of action of cathodal DC polarisation induced inhibition of the human motor cortex. Clin Neurophysiol 114: 600-604.

Nitsche MA and Paulus W (2000) Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. J Physiol 527: 633-639.

Nitsche MA and Paulus W (2001) Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. Neurology 57: 1899-1901.

Nitsche MA, Roth A, Kuo MF, Fischer AK, Liebetanz D, Lang N, Tergau F and Paulus W (2007b) Timing-dependent modulation of associative plasticity by general network excitability in the human motor cortex. J Neurosci 27: 3807-3812.

Oldfield RC (1971) The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 9: 97-113.

Opitz A, Paulus W, Will S, Antunes A and Thielscher A (2015) Determinants of the electric field during transcranial direct current stimulation. Neuroimage 109: 140-150.

Opitz A, Windhoff M, Heidemann RM, Turner R and Thielscher A (2011) How the brain tissue shapes the electric field induced by transcranial magnetic stimulation. Neuroimage 58: 849-859.

Orban de Xivry JJ, Marko MK, Pekny SE, Pastor D, Izawa J, Celnik P and Shadmehr R (2011) Stimulation of the human motor cortex alters generalization patterns of motor learning. J Neurosci 31: 7102-7110.

Penfield W (1950) The supplementary motor area in the cerebral cortex of man. Arch Psychiatr Nervenkr Z Gesamte Neurol Psychiatr 185: 670-674.

Picard N and Strick PL (1996) Motor areas of the medial wall: a review of their location and functional activation. Cereb Cortex 6: 342-353.

Picard N and Strick PL (2001) Imaging the premotor areas. Curr Opin Neurobiol 11: 663-672.

Polania R, Paulus W and Nitsche MA (2012) Reorganizing the intrinsic functional architecture of the human primary motor cortex during rest with non-invasive cortical stimulation. PLoS One 7: e30971.

Premoli I, Castellanos N, Rivolta D, Belardinelli P, Bajo R, Zipser C, Espenhahn S, Heidegger T, Müller-Dahlhaus F and Ziemann U (2014a) TMS-EEG signatures of GABAergic neurotransmission in the human cortex. J Neurosci 34: 5603-5612.

Premoli I, Rivolta D, Espenhahn S, Castellanos N, Belardinelli P, Ziemann U and Müller-Dahlhaus F (2014b) Characterization of GABAB-receptor mediated neurotransmission in the human cortex by paired-pulse TMS-EEG. Neuroimage 103: 152-162.

Purves DA, George J.; Fitzpatick, David; Hall, William C.; LaMantia, Anthony-Samuel; White, Leonard E. (2012) Unit III Movement and its central control. 351-474. Purves DA, George J.; Fitzpatick, David; Hall, William C.; LaMantia, Anthony-Samuel; White, Leonard E. Sinauer.Sunderland, Mass.

Rahman A, Reato D, Arlotti M, Gasca F, Datta A, Parra LC and Bikson M (2013) Cellular effects of acute direct current stimulation: somatic and synaptic terminal effects. J Physiol 591: 2563-2578.

Rehme AK, Eickhoff SB, Wang LE, Fink GR and Grefkes C (2011) Dynamic causal modeling of cortical activity from the acute to the chronic stage after stroke. Neuroimage 55: 1147-1158.

Ridding MC, Taylor JL and Rothwell JC (1995) The effect of voluntary contraction on cortico-cortical inhibition in human motor cortex. J Physiol 487 (Pt 2): 541-548.

Rioult-Pedotti MS, Friedman D and Donoghue JP (2000) Learning-induced LTP in neocortex. Science 290: 533-536.

Rivera-Urbina GN, Batsikadze G, Molero-Chamizo A, Paulus W, Kuo MF and Nitsche MA (2015) Parietal transcranial direct current stimulation modulates primary motor cortex excitability. Eur J Neurosci 41: 845-855.

Rizzo V, Bove M, Naro A, Tacchino A, Mastroeni C, Avanzino L, Crupi D, Morgante F, Siebner HR and Quartarone A (2011) Associative cortico-cortical plasticity may affect ipsilateral finger opposition movements. Behav Brain Res 216: 433-439.

Rizzo V, Siebner HS, Morgante F, Mastroeni C, Girlanda P and Quartarone A (2009) Paired associative stimulation of left and right human motor cortex shapes interhemispheric motor inhibition based on a Hebbian mechanism. Cereb Cortex 19: 907-915.

Rosenkranz K, Nitsche MA, Tergau F and Paulus W (2000) Diminution of training-induced transient motor cortex plasticity by weak transcranial direct current stimulation in the human. Neurosci Lett 296: 61-63.

Rossi S, Hallett M, Rossini PM and Pascual-Leone A (2011) Screening questionnaire before TMS: an update. Clin Neurophysiol 122: 1686.

Rossini PM, Barker AT, Berardelli A, Caramia MD, Caruso G, Cracco RQ, Dimitrijevic MR, Hallett M, Katayama Y, Lucking CH and et al. (1994) Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. Electroencephalogr Clin Neurophysiol 91: 79-92.

Rossini PM, Burke D, Chen R, Cohen LG, Daskalakis Z, Di Iorio R, Di Lazzaro V, Ferreri F, Fitzgerald PB, George MS, Hallett M, Lefaucheur JP, Langguth B,

Matsumoto H, Miniussi C, Nitsche MA, Pascual-Leone A, Paulus W, Rossi S, Rothwell JC, Siebner HR, Ugawa Y, Walsh V and Ziemann U (2015) Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: Basic principles and procedures for routine clinical and research application. An updated report from an I.F.C.N. Committee. Clin Neurophysiol 126: 1071-1107.

Sale MV, Ridding MC and Nordstrom MA (2008) Cortisol inhibits neuroplasticity induction in human motor cortex. J Neurosci 28: 8285-8293.

Salvador R, Wenger C and Miranda PC (2015) Investigating the cortical regions involved in MEP modulation in tDCS. Front Cell Neurosci 9: 405.

Sarfeld AS, Diekhoff S, Wang LE, Liuzzi G, Uludag K, Eickhoff SB, Fink GR and Grefkes C (2012) Convergence of human brain mapping tools: neuronavigated TMS parameters and fMRI activity in the hand motor area. Hum Brain Mapp 33: 1107-1123.

Schaefer N, Rotermund C, Blumrich EM, Lourenco MV, Joshi P, Hegemann RU, Jamwal S, Ali N, Garcia Romero EM, Sharma S, Ghosh S, Sinha JK, Loke H, Jain V, Lepeta K, Salamian A, Sharma M, Golpich M, Nawrotek K, Paidi RK, Shahidzadeh SM, Piermartiri T, Amini E, Pastor V, Wilson Y, Adeniyi PA, Datusalia AK, Vafadari B, Saini V, Suarez-Pozos E, Kushwah N, Fontanet P and Turner AJ (2017) The malleable brain: plasticity of neural circuits and behavior - a review from students to students. J Neurochem 142: 790-811.

Shibasaki H and Hallett M (2006) What is the Bereitschaftspotential? Clin Neurophysiol 117: 2341-2356.

Siebner HR and Ziemann U (2007) Hirnstimulation - Physiologische Grundlagen. pp. 27-45. Siebner HR and Ziemann U. Springer Berlin Heidelberg.

Singh A and Abraham WC (2017) Astrocytes and synaptic plasticity in health and disease. Exp Brain Res 235: 1645-1655.

Sjöström PJ and Häusser M (2006) A cooperative switch determines the sign of synaptic plasticity in distal dendrites of neocortical pyramidal neurons. Neuron 51: 227-238.

Sjöström PJ, Turrigiano GG and Nelson SB (2001) Rate, timing, and cooperativity jointly determine cortical synaptic plasticity. Neuron 32: 1149-1164.

Sloper JJ (1973) An electron microscope study of the termination of afferent connections to the primate motor cortex. J Neurocytol 2: 361-368.

Smith MJ, Adams LF, Schmidt PJ, Rubinow DR and Wassermann EM (2002) Effects of ovarian hormones on human cortical excitability. Ann Neurol 51: 599-603.

Smith MJ, Keel JC, Greenberg BD, Adams LF, Schmidt PJ, Rubinow DA and Wassermann EM (1999) Menstrual cycle effects on cortical excitability. Neurology 53: 2069-2072.

Stefan K, Kunesch E, Benecke R, Cohen LG and Classen J (2002) Mechanisms of enhancement of human motor cortex excitability induced by interventional paired associative stimulation. J Physiol 543: 699-708.

Stefan K, Kunesch E, Cohen LG, Benecke R and Classen J (2000) Induction of plasticity in the human motor cortex by paired associative stimulation. Brain 123: 572-584.

Stinear C (2010) Prediction of recovery of motor function after stroke. Lancet Neurol 9: 1228-1232.

Stinear CM and Byblow WD (2003) Role of intracortical inhibition in selective hand muscle activation. J Neurophysiol 89: 2014-2020.
Stinear CM and Byblow WD (2004) Impaired modulation of intracortical inhibition in focal hand dystonia. Cereb Cortex 14: 555-561.
Sumner P, Nachev P, Morris P, Peters AM, Jackson SR, Kennard C and Husain M (2007) Human medial frontal cortex mediates unconscious inhibition of voluntary action. Neuron 54: 697-711.
Szentagothai J (1975) The 'module-concept' in cerebral cortex architecture. Brain Res 95: 475-496.
Takeuchi N, Tada T, Toshima M, Matsuo Y and Ikoma K (2009) Repetitive transcranial magnetic stimulation over bilateral hemispheres enhances motor function and training effect of paretic hand in patients after stroke. J Rehabil Med 41: 1049-1054.
Tanji J, Kurata K and Okano K (1985) The effect of cooling of the supplementary motor cortex and adjacent cortical areas. Exp Brain Res 60: 423-426.
Tanji J and Shima K (1994) Role for supplementary motor area cells in planning several movements ahead. Nature 371: 413-416.
Thielscher A, Antunes A and Saturnino GB (2015) Field modeling for transcranial magnetic stimulation: A useful tool to understand the physiological effects of TMS? Conf Proc IEEE Eng Med Biol Soc 2015: 222-225.
Thielscher A, Opitz A and Windhoff M (2011) Impact of the gyral geometry on the electric field induced by transcranial magnetic stimulation. Neuroimage 54: 234-243.
Tremblay S, Hannah R, Rawji V and Rothwell JC (2017) Modulation of iTBS after-effects via concurrent directional TDCS: A proof of principle study. Brain Stimulation: Basic, Translational, and Clinical Research in Neuromodulation 10: 744-747.
Turrigiano GG and Nelson SB (2000) Hebb and homeostasis in neuronal plasticity. Curr Opin Neurobiol 10: 358-364.
Veniero D, Ponzo V and Koch G (2013) Paired associative stimulation enforces the communication between interconnected areas. J Neurosci 33: 13773-13783.
Vergani F, Lacerda L, Martino J, Attems J, Morris C, Mitchell P, Thiebaut de Schotten M and Dell'Acqua F (2014) White matter connections of the supplementary motor area in humans. J Neurol Neurosurg Psychiatry 85: 1377-1385.
Vorobiev V, Govoni P, Rizzolatti G, Matelli M and Luppino G (1998) Parcellation of human mesial area 6: cytoarchitectonic evidence for three separate areas. Eur J Neurosci 10: 2199-2203.
Wiethoff S, Hamada M and Rothwell JC (2014) Variability in response to transcranial direct current stimulation of the motor cortex. Brain Stimul 7: 468-475.
Windhoff M, Opitz A and Thielscher A (2013) Electric field calculations in brain stimulation based on finite elements: an optimized processing pipeline for the generation and usage of accurate individual head models. Hum Brain Mapp 34: 923-935.
Wolters A, Sandbrink F, Schlottmann A, Kunesch E, Stefan K, Cohen LG, Benecke R and Classen J (2003) A temporally asymmetric Hebbian rule governing plasticity in the human motor cortex. J Neurophysiol 89: 2339-2345.
Yazawa S, Ikeda A, Kunieda T, Ohara S, Mima T, Nagamine T, Taki W, Kimura J, Hori T and Shibasaki H (2000) Human presupplementary motor area is active

before voluntary movement: subdural recording of Bereitschaftspotential from medial frontal cortex. Exp Brain Res 131: 165-177.
Zhang S, Ide JS and Li CS (2012) Resting-state functional connectivity of the medial superior frontal cortex. Cereb Cortex 22: 99-111.
Ziemann U, Ilic TV, Pauli C, Meintzschel F and Ruge D (2004) Learning modifies subsequent induction of long-term potentiation-like and long-term depression-like plasticity in human motor cortex. J Neurosci 24: 1666-1672.
Ziemann U, Rothwell JC and Ridding MC (1996) Interaction between intracortical inhibition and facilitation in human motor cortex. J Physiol 496: 873-881.
Zrenner C, Belardinelli P, Müller-Dahlhaus F and Ziemann U (2016) Closed-Loop Neuroscience and Non-Invasive Brain Stimulation: A Tale of Two Loops. Front Cell Neurosci 10: 92.
Zrenner C, Desideri D, Belardinelli P and Ziemann U (2018) Real-time EEG-defined excitability states determine efficacy of TMS-induced plasticity in human motor cortex. Brain Stimul 11: 374-389.
Zrenner C and Ziemann U (2015) Therapeutischer Einsatz von Closed-loop Hirnstimulation. Erfolge und Erwartungen. Nervenarzt 86: 1523-1527.

7 German summary

Kooperative nichtinvasive Hirnstimulation zur Induktion von Langzeit-Potenzierung im motorischen System

Einleitung und Fragestellung: Die Verbindungsstärke zwischen Neuronen kann abhängig von der zeitlichen Abfolge prä- und postsynaptischer Potentiale reduziert oder gesteigert werden (Bi and Poo 2001). Dieser Prozess ist als *spike-timing dependent plasticity* (STDP) bekannt (Bi and Poo 2001, Hebb 1949, Markram et al. 2011). Gepaarte assoziative Stimulation (PAS) zeigt vergleichbare Effekte auf die Verbindungsstärke zwischen Hirnarealen (Arai et al. 2011, Koch et al. 2013, Stefan et al. 2000). Nach einem Apoplex korreliert der Wiedererwerb der Handfunktionen mit der Normalisierung der Verbindungsstärke zwischen dem supplementär motorischem Areal (SMA) und dem linken primär motorischen Cortex (M1) (Rehme et al. 2011). PAS dieser Hirnregionen ($PAS_{SMA \rightarrow M1}$) modifiziert die M1 Erregbarkeit bidirektional (Arai et al. 2011). Aufgrund der kurzen Effektdauer ist dies bisher ohne klinischen Nutzen für den Patienten. Die vorliegende Arbeit versuchte diese Lücke durch den Transfer des zellulären Prinzips der Kooperativität auf die Netzwerkebene zu schließen: Ein unterschwelliger präsynaptischer Stimulus kann durch Kombination mit (1) einem extrazellulären Stimulus, oder (2) postsynaptischer Depolarisation Langzeitpotenzierung (LTP) erzielen (Sjöström and Häusser 2006, Sjöström et al. 2001). Experiment 1 untersuchte durch kooperative PAS des kontralateralen Motorkortex (M1c) und M1 ($PAS_{M1c \rightarrow M1}$) mit $PAS_{SMA \rightarrow M1}$ die Erregbarkeit von M1 zu erhöhen. Experiment 2 prüfte, ob anodale transkranielle Gleichstromstimulation (tDCS), im Gegensatz zu kathodaler tDCS, mit $PAS_{SMA \rightarrow M1}$, M1 Erregbarkeit und effektive SMA-M1 Konnektivität steigert.

Material und Methoden: Experiment 1 umfasste 11 gesunde Rechtshänder (8 Männer und 3 Frauen). Experiment 2 schloss 15 gesunde, rechtshändige Männer ein. Alle Probanden gaben nach ausführlicher Aufklärung ihre schriftliche Einwilligung zur Studienteilnahme. Beide Studien entsprachen der aktuellen Version der Deklaration von Helsinki und wurden von der Ethikkommission der Medizinischen Fakultät der Eberhard Karls Universität Tübingen, Deutschland, genehmigt. Unter Anwendung von Neuronavigation wurden zwei innovative Stimulationstechniken entwickelt. In

Experiment 1 benutzten wir eine neue Tripelstimulationstechnik zur kooperativen $PAS_{M1c \rightarrow M1}$ mit $PAS_{SMA \rightarrow M1}$ und analysierten die Effekte bis zu einer Stunde nach der Intervention. Experiment 2 prüfte die kooperativen Effekte von tDCS mit $PAS_{SMA \rightarrow M1}$. Basierend auf Modellierungsarbeiten des elektrischen Feldes unter tDCS wurde eine neue Elektrodenmontage getestet. Die Stimulationselektrode wurde dabei 3 cm hinter dem M1 Handareal platziert. Effekte auf die M1 Erregbarkeit sowie die effektive SMA-M1 Konnektivität wurden durch motorisch evozierten Potentiale eines Handmuskels bis zu zwei Stunden nach Intervention evaluiert und statistisch ausgewertet. Alle Experimente wurden im doppelblinden, randomisierten Crossover Design durchgeführt.

Ergebnisse: Die kooperative Anwendung von $PAS_{SMA \rightarrow M1}$ und $PAS_{M1c \rightarrow M1}$ führte zu keiner signifikanten Änderung der M1 Erregbarkeit im Vergleich zu einer Kontrollkondition. Kooperative tDCS während $PAS_{SMA \rightarrow M1}$ zeigte keine signifikante Wirkung auf M1 Erregbarkeit und effektive SMA-M1 Konnektivität. In einer post-hoc Analyse zeigte sich eine signifikante Interaktion zwischen tDCS und $PAS_{SMA \rightarrow M1}$. Anodale und kathodale tDCS modulierten die $PAS_{SMA \rightarrow M1}$ Effekte auf M1 Erregbarkeit in der Mehrzahl der Probanden (13/15 = 87%) in identische Richtung. Außerdem korrelierten die Effektgrößen von anodaler und kathodaler tDCS auf $PAS_{SMA \rightarrow M1}$ ($r_S = 0.525$, $p = 0.044$). Die alleinige Anwendung von tDCS in der neuen Montage ohne $PAS_{SMA \rightarrow M1}$ beeinflusste die M1 Erregbarkeit nicht.

Schlussfolgerung: Die kooperative Anwendung zweier Erregbarkeits-steigernden Stimulationsprotokolle erzeugte keine zusätzliche Erregbarkeitssteigerung in M1. Gemäß dem Konzept der homöostatischen Metaplastizität limitieren kortikale Schutzmechanismen die Effekte von Hirnstimulation um das Gehirn vor Übererregung und unkontrollierte Plastizität zu schützen (Abbott and Nelson 2000, Abraham 2008, Hulme et al. 2013). Im Gegensatz zur ursprünglichen Hypothese führte die kooperative tDCS während PAS_{SMA-M1} zu einer Auslöschung beziehungsweise Umkehr der PAS_{SMA-M1} Effekte. Dieses Phänomen könnten Radialsymmetrie kortikaler Kolumnen, Gyrusgeometrie des Motorcortex sowie Kooperativität der Plastizitätsinduktion erklären.

8 Publications

Parts of this thesis have been published in form of the following scientific articles:

Faber H, Opitz A, Müller-Dahlhaus F, Ziemann U.
Polarity-independent effects of tDCS on paired associative stimulation-induced plasticity
Brain Stimulation 2017 (6): 1061-1069

Faber H, Opitz A, Zipser CM, Müller-Dahlhaus F, Ziemann U
P 56 tDCS shows no effects on motor cortex excitability at rest
Clinical Neurophysiology 2017 (128): e356-e357

Faber H, Zipser CM, Tünnerhoff J, Müller-Dahlhaus F, Ziemann U
P149. Suppression of LTP-like associative plasticity in the human SMA–M1 network by simultaneous tDCS
Clinical Neurophysiology 2015 (126): e136

Faber H, Zipser CM, Tünnerhoff J, Müller-Dahlhaus F, Ziemann U
ID 394 – Polarity independent suppression of long-term associative plasticity in the human SMA–M1 network by simultaneous tDCS
Clinical Neurophysiology 2016 (127): e100

9 Declaration of candidates own contribution

This work was performed in the Center of Neurology, Hertie Institute for Clinical Brain Research, Department Neurology and Stroke, University Hospital Tübingen under the supervision of Prof. Dr. Ulf Ziemann.

Prof. Dr. Ulf Ziemann designed the project in assistance with Hanna Faber, Dr. Florian Müller-Dahlhaus and Prof. Dr. Alexander Opitz.. PhD Ľubomíra Anderková helped with the experimental set-up for experiment 2. PhD Isabella Premoli and PhD Svenja Espenhahn performed the pilot experiment 1. I performed all other experiments after training by Dr. Florian Müller-Dahlhaus, PhD Isabella Premoli, PhD Svenja Espenhahn and Prof Dr. Ziemann. During the intervention of experiment 1, Dr. Florian Müller-Dahlhaus, PhD Isabella Premoli, PhD Svenja Espenhahn, Dr. Lena Zeltner and Dr. Johannes Tünnerhoff held the 25 mm-figure-of-eight coil for SMA stimulation. MRI data was acquired by Dr. Friedemann Bender, Edyta Charyasz, Dr. Andreas Bungert, Dr. Susanne Dietrich, Dr. Stephanie Kullmann, Dr. Thomas Pfeffer, Dr. Maren Praß and Dr. Maartje Spetter.

Data was analyzed by Hanna Faber and Prof. Dr. Ulf Ziemann. Hanna Faber performed the statistical evaluation under supervision of Prof. Dr. Ulf Ziemann, and Prof. Dr. Peter Martus and PhD Lisa Wang from the Institute for Clinical Epidemiology and Applied Biometry of the University of Tübingen, Germany. Hanna Faber, Dr. Florian Müller-Dahlhaus, Prof. Dr. Alexander Opitz and Prof. Dr. Ulf Ziemann discussed and interpreted the data.

Collaborating groups:

Prof. Dr. Alexander Opitz (Departement of Biomedical Engineering, University of Minnesota, Minneapolis, Minesota, USA) modeled the electric field during tDCS (Figures 9 and 10).

I, Hanna Faber, herewith declare, that I wrote this PhD thesis on my own under the supervision of Prof. Dr. Ulf Ziemann and did not use any unnamed sources or aid.

Tübingen, 25.06.2018

10 Appendix

10.1 Study information and informed consent for experiment 1

PROBANDENAUFKLÄRUNG

Titel der Studie:

Modulation of effective connectivity and enhancement of motor performance by timing- and site-specific cooperative multi-coil associative transcranial magnetic stimulation of the distributed human motor cortical network

Deutscher Titel der Studie:

Modulation effektiver Konnektivität und Verbesserung motorischer Leistungen durch zeit- und ortsspezifische kooperative assoziative transkranielle Magnetstimulation des distribuierten motorkortikalen Netzwerkes.

Protokoll-Nr.: 2012-11

Sponsor der Studie:

Prof. Dr. med. Ulf Ziemann
Ärztlicher Direktor
Abteilung Neurologie mit Schwerpunkt neurovaskuläre Erkrankungen
Hertie-Institut für Klinische Hirnforschung
Universitätsklinik der Eberhard-Karls Universität Tübingen
Hoppe-Seyler-Str. 3, D-72076 Tübingen

Leiter der klinischen Prüfung (Ihr Ansprechpartner):

Prof. Dr. med. Ulf Ziemann
Ärztlicher Direktor
Abteilung Neurologie mit Schwerpunkt neurovaskuläre Erkrankungen
Hertie-Institut für Klinische Hirnforschung
Universitätsklinik der Eberhard-Karls Universität Tübingen
Hoppe-Seyler-Str. 3, D-72076 Tübingen
Tel. 07071 2982049
E-Mail: ulf.ziemann@med.uni-tuebingen.de

Sehr geehrter Proband,

Ihr Studienarzt bietet Ihnen an, an einer klinischen Studie teilzunehmen. Sie erhalten mit dieser Probandenaufklärung Informationen zur Studie. Nehmen Sie sich ausreichend Zeit, um diese sorgfältig durchzulesen und Ihrem Studienarzt bzw. seinen Mitarbeitern eventuelle Fragen zu stellen. Entscheiden Sie anschließend, ob Sie an der Studie teilnehmen möchten oder nicht.

Warum wird diese Studie durchgeführt?

Das Ziel dieser Studie ist es, neue wissenschaftliche Erkenntnisse über die Funktionsweise des menschlichen Gehirns und Nervensystems zu gewinnen. Untersucht wird dabei die Veränderlichkeit von Verbindungen zwischen verschiedenen Gehirnregionen durch nicht-invasive Hirnstimulation. Die Ergebnisse dieser Studie könnten in der Zukunft z.B. dazu beitragen, den Erfolg rehabilitativer Maßnahmen bei Schlaganfallpatienten zu steigern.

Informationen zu dieser Studie:

Die hier beschriebene Studie untersucht die Wirkung von nichtinvasiver Hirnstimulation auf Netzwerke innerhalb unseres Gehirns. Dabei wird versucht, durch hochfokale Reizspulen, d.h. Spulen, die räumlich sehr genau Hirnregionen stimulieren können, diese Verbindungen zu modulieren.

Es sind Gründe denkbar, die gegen Ihre Teilnahme an dieser Studie sprechen. Der Studienarzt wird diese Gründe mit Ihnen besprechen. Es werden insgesamt ca. 12 Personen an der Studie teilnehmen. Ihre Teilnahme an der Studie wird je nach Teilstudie 2 bis maximal 5 Studienvisiten umfassen, die jeweils ca. 4 Stunden dauern werden. Gegebenenfalls kommt in einigen Fällen ein zusätzlicher Besuch vor Beginn der Studie hinzu, an dem Ihre Eignung für die Studie untersucht wird.

Im Folgenden werden die wesentlichen Untersuchungsverfahren der Studie erläutert. Sie werden diese Erklärungen benötigen, um den Studienablauf zu verstehen.

Magnetresonanztomographie (MRT), Transkranielle Magnetstimulation (TMS) und

Magnetenzephalographie (MEG)

Bei der Magnetresonanztomographie (MRT) handelt es sich um ein bildgebendes Verfahren, das in der Medizin zur Darstellung von Organen und Geweben im Körper eingesetzt wird. In diesem Experiment wird es benutzt, um den exakten Stimulationsort auf der individuellen Hirnoberfläche für die transkranielle Magnetstimulation (TMS) herauszufinden.

Bei der TMS handelt es sich um eine nichtinvasive (d.h. Sie werden dabei nicht gestochen oder in einer anderen Weise verletzt) Technologie, bei der mit Hilfe eines Magnetfelds Bereiche des Gehirns mit Stromstößen stimuliert werden. Hierfür werden Oberflächenelektroden auf die Haut über der Hautmuskulatur geklebt, um zu messen, welche Reaktion auf solche schwachen Stromstöße von den Muskeln generiert wird. Auf diese Weise können verschiedene Reaktionen gemessen werden, zum Beispiel die "motorische Schwelle" (= der kleinstmögliche Reiz, der noch an die für Bewegung zuständigen Teile des Gehirns weitergeleitet wird). Dabei wird die TMS in dem Experiment auf zwei Arten eingesetzt, einerseits um Gehirnaktivität zu modulieren, anderseits um Effekte dieser Modulation zu messen.

Die Magnetenzephalographie (MEG) ist ein Verfahren zur Messung der magnetischen Aktivität des Gehirns durch äußere Sensoren. Die Magnetfelder unseres Gehirns induzieren in Spulen oder Spulensystemen Stromflüsse, die dann mit einer sehr guten räumlichen und zeitlichen Auflösung registriert werden können. Es stellt ein Verfahren dar, mit dem man sehr gut die Gesamtaktivität des Gehirns beurteilen kann. Da Zellen in unserem Gehirn verschiedene Zustände einnehmen, je nachdem ob sie aktiv sind oder nicht, werden sie gebeten werden im Verlauf des Experiments verschiedene motorische Tests mit ihren Händen auszuüben.

Wie ist der Ablauf der Studie und was müssen Sie bei Teilnahme beachten?

Zu Beginn der ersten Visite wird Sie der Studienarzt zu Ihrer Krankengeschichte befragen, um zu prüfen, ob Sie für die Teilnahme an der Studie geeignet sind. Zwischen den je nach Teilstudie geplanten 2-5 Visiten liegt jeweils mindestens eine Woche. Die einzelnen Visiten dauern ca. 4 Stunden.

Was geschieht während Ihrer Besuche beim Studienarzt?

Der Studienarzt oder seine Mitarbeiter werden nacheinander die folgenden Maßnahmen durchführen:

1. Befragung zur Krankengeschichte und den Studieneinschlusskriterien (nur 1. Visite)
2. Befragung zu Nebenwirkungen nach der letzten Visite (alle Visiten)
3. Körperliche und neurologische Untersuchung (zu Beginn und am Ende jeder Visite)
4. Urinprobe zur Untersuchung auf Drogen
5. MRT (nur 1.Visite)
6. TMS und/oder MEG

Welchen persönlichen Nutzen haben sie von der Teilnahme an der Studie?

Sie werden durch die Teilnahme an dieser Studie außer einer ärztlichen Untersuchung voraussichtlich keinen persönlichen Gesundheitsnutzen haben. Die Ergebnisse der Studie können aber möglicherweise anderen Menschen zukünftig helfen.

Welche anderen Möglichkeiten haben Sie, wenn Sie nicht an der Studie teilnehmen?

Da die Studie lediglich zu Forschungszwecken durchgeführt wird, ist die einzige Alternative ein Verzicht auf die Teilnahme an der Studie.

Welche Risiken sind mit der Teilnahme an der Studie verbunden?

Die angewandten Untersuchungsmethoden können Unwohlsein verursachen oder Risiken mit sich bringen. Die verwendeten Verfahren, haben folgende UAW:

- **MRT**: Bislang sind keine schädigenden Wirkungen von Magnetfeldern und Radiofrequenzstrahlen auf den menschlichen Körper bekannt. Vereinzelt kann

es durch Make-up und Tattoos (wenn diese ferromagnetische Materialien enthalten) zu leichten Hautreizungen kommen.

- **TMS**: Die elektromagnetischen Impulse, die für dieses Verfahren verwendet werden, sind harmlos und in der Regel nicht unangenehm oder schmerzhaft. Während oder nach den TMS - Messungen können leichte Kopfschmerzen auftreten, die in der Regel ohne weitere Behandlung zurückgehen.

- **MEG**: Da es ein passives Instrument ist, das nur das natürliche Magnetfeld des Gehirns aufzeichnet sind mit der MEG Messung keine Risiken verbunden.

Bitte teilen Sie den Mitarbeitern der Prüfstelle alle Beschwerden, Erkrankungen oder Verletzungen mit, die im Verlauf der klinischen Prüfung auftreten. Falls diese schwerwiegend sind, teilen Sie den Mitarbeitern der Prüfstelle diese bitte umgehend mit, ggf. telefonisch.

Wie wird mit Zufallsbefunden im Rahmen der klinischen Prüfung umgegangen?

Vergleichen Sie bitte die Angaben zu Zufallsbefunden auf Seite 8f.

Wer darf an dieser klinischen Prüfung nicht teilnehmen?

Sie können an dieser klinischen Prüfung nur teilnehmen, wenn Sie gesund sind und sich nicht gleichzeitig für andere klinische Prüfungen oder andere klinische Forschungsprojekte zur Verfügung stellen oder bis vor kurzem teilgenommen haben

Schwangere Frauen dürfen an dieser klinischen Prüfung nicht teilnehmen.

Zu Beginn der klinischen Prüfung müssen sich deshalb alle Frauen einem Schwangerschaftstest unterziehen. Davon ausgenommen sind Frauen nach den Wechseljahren oder solche, die operativ sterilisiert wurden. Durch einen Schwangerschaftstest kann jedoch eine Schwangerschaft erst einige Tage nach der Empfängnis verlässlich nachgewiesen werden. Im Falle Ihrer Teilnahme an dieser klinischen Prüfung müssen Sie eine hormonelle Maßnahme zur Schwangerschaftsverhütung anwenden. Diese sind: Pille, Hormonspritze, Nuvaring. Der

Grund dafür ist, dass hormonelle Schwankungen die Messergebnisse der Prüfung beeinflussen können. Sollten Sie während der klinischen Prüfung schwanger werden oder den Verdacht haben, dass Sie schwanger geworden sind, müssen Sie umgehend den Prüfarzt informieren.

Entstehen für Sie Kosten durch die Teilnahme an der klinischen Prüfung? Erhalten Sie eine Aufwandsentschädigung?

Durch Ihre Teilnahme an dieser klinischen Prüfung entstehen für Sie keine Kosten. Für Ihre Teilnahme an dieser Studie werden Sie wie folgt bezahlt: Sie erhalten 10 EUR / Stunde. Falls ihr Prüfarzt Sie aus der laufenden Studie ausschließt, z.B. aus medizinischen Gründen, werden nur die bereits stattgefundenen Visiten bezahlt. Für die für die Studie erforderlichen Untersuchungen und Leistungen dürfen die Krankenkassen nicht belastet werden.

Werden Ihnen neue Erkenntnisse während der klinischen Prüfung mitgeteilt?

Sie werden über neue Erkenntnisse, die in Bezug auf diese klinische Prüfung bekannt werden und die für Ihre Bereitschaft zur weiteren Teilnahme wesentlich sein können, informiert. Auf dieser Basis können Sie dann Ihre Entscheidung zur weiteren Teilnahme an dieser klinischen Prüfung überdenken.

Kann Ihre Teilnahme an der klinischen Prüfung vorzeitig beendet werden?

Ihre Studienteilnahme erfolgt freiwillig. Sie haben die Möglichkeit, die Beteiligung an der Studie ohne Angabe von Gründen abzulehnen bzw. Ihre Teilnahme zu jedem Zeitpunkt zu beenden. Daraus entstehen Ihnen keine Nachteile und Sie verlieren dadurch auch keinen Anspruch auf Leistungen, auf die Sie ansonsten einen Anspruch hätten.

Darüber hinaus kann Ihre Beteiligung auch durch den Sponsor oder den Studienarzt unabhängig von Ihrer Einwilligung beendet werden (z.B. falls bei Ihnen eine andere Behandlung erforderlich wird, Sie den Studienplan nicht einhalten, eine studienbedingte Gesundheitsschädigung auftritt oder ein anderer wichtiger Grund vorliegt).

Sollte Ihre Teilnahme an der Studie vorzeitig beendet werden, so werden Ihre bis dahin verwendeten Daten in der Art und Weise, wie unter „Datenschutz" beschrieben, weiterverwendet, sofern dies zur Auswertung der Studie oder zur Wahrung Ihrer schutzwürdigen Interessen erforderlich ist.

Wie werden Ihre persönlichen Daten geschützt?

Vergleichen Sie hierzu bitte die Angaben zum Datenschutz auf Seite 10.

An wen wenden Sie sich bei weiteren Fragen?

Sie haben stets die Gelegenheit zu weiteren Beratungsgesprächen mit dem auf Seite 1 genannten oder einem anderen Prüfarzt.

Prüfstelle: Klinik für Neurologie, Eberhard Karls Universität Tübingen

Leiter der klinischen Prüfung: Prof. Dr. Ulf Ziemann

Modulation of effective connectivity and enhancement of motor performance by timing- and site-specific cooperative multi-coil associative transcranial magnetic stimulation of the distributed human motor cortical network

Protokoll-Nr.: 2012-11

Einwilligungserklärung

....
..

Name des Probanden in Druckbuchstaben

geb. am ....Teilnehmer-Nr. ....

Ich bin in einem persönlichen Gespräch durch den Prüfarzt

....
..

Name der Ärztin/des Arztes

ausführlich und verständlich über Wesen, Bedeutung, Risiken und Tragweite der klinischen Prüfung aufgeklärt worden. Ich habe darüber hinaus den Text der Probandeninformation sowie die hier nachfolgend abgedruckte Datenschutzerklärung gelesen und verstanden. Ich hatte die Gelegenheit, mit dem Prüfarzt über die Durchführung der klinischen Prüfung zu sprechen.

Möglichkeit zur Dokumentation zusätzlicher Fragen seitens des Probanden oder sonstiger Aspekte des Aufklärungsgesprächs:

Ich hatte ausreichend Zeit, mich zu entscheiden. Insbesondere wurde ich über folgende Punkte informiert:

- Ziele, Dauer, Ablauf, Nutzen sowie Risiken und Nebenwirkungen der Studienteilnahme
- Umgang mit den erhobenen Daten

Mir ist bekannt, dass ich jederzeit und ohne Angabe von Gründen meine Einwilligung zur Teilnahme an der Prüfung zurückziehen kann (mündlich oder schriftlich), ohne dass mir daraus Nachteile entstehen.

______________________	____________________	_________________________________
Datum	Unterschrift	Name des Probanden in Blockschrift

Zufallsfunde im Rahmen einer Magnetresonanz-Untersuchung

Im Rahmen dieser MRT-Untersuchung wird kein Arzt-Patient-Verhältnis begründet und es
wird keine klinische Individualdiagnostik durchgeführt. Daher werden tatsächlich vorhandene klinisch relevante Normabweichungen möglicherweise nicht entdeckt.

Allerdings besteht die Möglichkeit, dass bei der wissenschaftlichen Analyse der Daten Signalauffälligkeiten entdeckt werden; in diesem Falle veranlasst der Studienarzt eine neuroradiologische Expertise ohne erneute Rücksprache mit Ihnen, um festzustellen, ob ein abklärungsbedürftiger Befund vorliegt.

Die Wahrscheinlichkeit dafür, dass ein abklärungsbedürftiger Befund auftritt, ist nach unseren Erfahrungen sehr gering (ca. 0,5 %). Trotzdem können aus solchen Befunden medizinische sowie berufliche und andere soziale Konsequenzen entstehen. Sollte nach eingehenderer neurologischer Diagnostik ein pathologischer Befund erhoben werden, besteht bspw. eine Mitteilungspflicht beim Abschluss privater Krankenversicherungen oder Lebensversicherungen. Ferner ist mit psychischen Belastungen zu rechnen, die durch die Kenntnis eines Zufallsfundes und gegebenenfalls eines anschließend diagnostisch spezifizierten hirnpathologischen Befundes entstehen können.

Einwilligungserklärung

Hiermit willige ich ein, dass im Rahmen der Studie „Modulation of effective connectivity and enhancement of motor performance by timing- and site-specific cooperative multi-coil associative transcranial magnetic stimulation of the distributed human motor cortical network“

(1) der Studienarzt im Falle eines Zufallsbefundes ohne erneute Rücksprache mit mir einen neuroradiologischen Experten hinzuzieht, dem die Bilddaten unter Beachtung der Bestimmungen des Datenschutzes zugänglich gemacht werden und dass (2) mir ein abklärungsbedürftiger Zufallsbefund mitgeteilt wird, andererseits dass (3) mir nicht abklärungsbedürftige Zufallsbefunde nicht mitgeteilt werden.

Ich besitze einen Krankenversicherungsschutz.

O ja

O nein

______________________	____________________	_________________________________
Datum	Unterschrift	Name des Probanden in Blockschrift

Information zum Datenschutz

Ihre im Rahmen der wissenschaftlichen Untersuchung erhobenen Daten werden vertraulich behandelt und ausschließlich in verschlüsselter Form weitergegeben. Die für die wissenschaftliche Untersuchung wichtigen Daten werden in verschlüsselter (pseudonymisiert, ohne Namensnennung) Form in einen gesonderten Dokumentationsbogen eingetragen. Die Zuordnung der verschlüsselten Daten zu Ihrer Person ist nur anhand einer Probandenliste möglich, die in einem verschlossenen Schrank, getrennt von den Studienunterlagen aufbewahrt wird und nur dem Studienleiter und dem Ärztlichen Direktor der Abteilung zugänglich ist. Die Daten werden für die Dauer min. 10 Jahren aufbewahrt und anschließend vernichtet. Sollten Sie von der Studie zurücktreten, können Sie entscheiden, ob die bereits vorliegenden Daten vernichtet werden müssen oder weiterverwendet werden dürfen.

Einwilligungserklärung

Ich erkläre mich der Verwendung der im Rahmen der Studie „Modulation of effective connectivity and enhancement of motor performance by timing- and site-specific cooperative multi-coil associative transcranial magnetic stimulation of the distributed human motor cortical network" erhobenen Daten in der oben beschriebenen Weise einverstanden. Ich kann jederzeit meine Daten beim Studienleiter einsehen.

________________	________________	________________
Datum	Unterschrift	Name des Probanden in Blockschrift

Ich erkläre mich bereit,

an der oben genannten klinischen Prüfung

freiwillig teilzunehmen.

Ein Exemplar der Probanden-Information und -Einwilligung habe ich erhalten. Ein Exemplar verbleibt im Prüfzentrum.

....

Studienepisode (vom Prüfarzt auszufüllen)

....

Name des Probanden in Druckbuchstaben

....

Datum Unterschrift des **Probanden**

Ich habe das Aufklärungsgespräch geführt und die Einwilligung des Probanden eingeholt.

....

Name des Prüfarztes/der Prüfärztin in Druckbuchstaben

....
....
......

Datum Unterschrift des aufklärenden **Prüfarztes/der Prüfärztin**

10.2 Study information and informed consent for experiment 2

Neurologische Klinik · Hoppe-Seyler-Str. 3· 72076 Tübingen

Universitätsklinikum Tübingen

Neurologische Klinik

Abt. Neurologie mit
Schwerpunkt Vaskuläre Neurologie

Ärztl. Direktor:
Prof. Dr. med. U. Ziemann

Hoppe-Seyler-Straße 3
72076 Tübingen

Telefon:	07071/29-82141
Sekretariat:	07071/29-82049
Poliklinik:	07071/29-82051
Fax:	07071/29 - 5260

28.02.2014

Probandenaufklärung

Titel der Studie:

Strengthening the SMA-M1 connection of human motor cortex by a novel non-invasive brain stimulation protocol to enhance motor performance and learning

Deutscher Titel der Studie:

Verstärkung der SMA-M1 Verbindung des menschlichen Motorkortex durch neuartige nicht-invasive Hirnstimulation zur Verbesserung motorischer Leistungen und Lernprozesse

Protokoll-Nr.: 2014-02-ZI

Studienleiter:

Prof. Dr. med. Ulf Ziemann
Ärztlicher Direktor
Abteilung Neurologie mit Schwerpunkt neurovaskuläre Erkrankungen
Hertie-Institut für Klinische Hirnforschung
Universitätsklinik der Eberhard-Karls Universität Tübingen
Hoppe-Seyler-Str. 3, D-72076 Tübingen

Sehr geehrte Probandin, sehr geehrter Proband,

Wir bieten Ihnen an, an einer Studie teilzunehmen. Sie erhalten mit dieser Probandenaufklärung Informationen zur Studie. Nehmen Sie sich ausreichend Zeit, um diese sorgfältig durchzulesen und dem Studienarzt eventuelle Fragen zu stellen. Entscheiden Sie anschließend, ob Sie an der Studie teilnehmen möchten oder nicht.

Warum wird diese Studie durchgeführt?

Das Ziel dieser Studie ist es, neue wissenschaftliche Erkenntnisse über die Funktionsweise des menschlichen Gehirns zu gewinnen. Dabei soll in 12 gesunden Probanden die Veränderlichkeit von Verbindungen zwischen verschiedenen Gehirnregionen durch transkranielle Magnetstimulation in Kombination mit transkranieller Gleichstromstimulation untersucht werden (transkraniell bedeutet, dass die Stimulation des Gehirns durch eine auf die Kopfhaut aufgelegte Reizspule durch den intakten Schädelknochen erfolgt). Insbesondere sollen zwei Regionen der motorischen Hirnrinde gereizt werden, die für die Durchführung von Bewegungen (Motorik) verantwortlich sind. Hierbei werden fokale Reizspulen aufgesetzt und Einzelreize in einer bestimmten zeitlichen Beziehung wiederholt abgegeben. Frühere Studien unserer Arbeitsgruppe haben gezeigt, dass hierdurch die Stärke der signalübertragenden Verbindungen zwischen den stimulierten Regionen erhöht oder vermindert werden kann. Diese Effekte halten allenfalls wenige Stunden an, liefern aber ein wichtiges Modell für Plastizität (Änderung von Verbindungen zwischen Nervenzellen des Gehirns), das in späteren Studien für die gezielte Verbesserung von Motorik, z.B. bei Patienten nach Schlaganfall im Rahmen der Neurorehabilitation beitragen kann.

Informationen zu dieser Studie

Die hier beschriebene Studie untersucht die Wirkung von transkranieller Hirnstimulation auf Netzwerke innerhalb unseres Gehirns (transkraniell bedeutet: durch den intakten Schädelknochen). Dabei wird untersucht inwieweit diese Netzwerke durch die Kombination von transkranieller Magnetstimulation (TMS, Erklärung s.u.) und transkranieller Gleichstromstimulation (tDCS, Erklärung s.u.) moduliert werden können. Sowohl TMS als auch tDCS sind schmerzfreie, sichere Methoden, die weltweit eingesetzt werden und für Sie (bei Beachtung der Kontraindikationen, s.u.) keine Risiken bergen. Sie werden von der Stimulation selbst und ihren Effekten kaum etwas bemerken. Die Wirkung der Stimulation auf Netzwerke des Gehirns lässt sich allerdings mittels Magnetencephalographie (MEG, Erklärung s.u.), einem nichtinvasiven Verfahren zur Messung der elektrischen Aktivität des Gehirnes, und Elektroenzephalographie (EEG, Erklärung s.u.) abbilden, wovon wir in der Studie Gebrauch machen werden.

Es sind Gründe denkbar, die gegen Ihre Teilnahme an dieser Studie sprechen. Der Studienarzt wird diese mit Ihnen besprechen. Die Studie besteht aus mindestens 2 Versuchssitzungen, die jeweils ca. 4 Stunden dauern werden. Zwischen diesen liegt jeweils ein Abstand von mindestens sieben Tagen. Vor Beginn Ihrer Teilnahme an der Studie wird Ihre Eignung für die Studie durch den Studienarzt untersucht.

An den einzelnen Versuchstagen werden mehrere Messungen und Stimulationsprotokolle in unterschiedlicher Reihenfolge durchgeführt. Diese sind im nächsten Abschnitt erläutert. Sie werden diese Erklärungen benötigen, um den Studienablauf zu verstehen.

Magnetresonanztomographie (MRT)

Bei der Magnetresonanztomographie (MRT) handelt es sich um ein bildgebendes Verfahren, das in der Medizin zur Darstellung von Organen und Geweben im Körper eingesetzt wird. Dabei werden die Signale des Wassers im Hirngewebe detektiert. In dem vorliegenden Experiment wird sie benutzt um den exakten Stimulationsort auf der individuellen Kopfoberfläche für die TMS festzustellen.

Transkranielle Magnetstimulation (TMS)

Bei der TMS handelt es sich um eine transkranielle (s.o.) Reizmethode. Hierbei induziert ein kurzer Stromfluss durch eine auf die Kopfhaut aufgelegte Reizspule ein Magnetfeld, das wiederum im Gehirn unter der Reizspule einen schwachen Stromstoß auslöst und Nervenzellen aktiviert. Über der motorischen Hirnrinde kann die TMS eine leichte Zuckung z.B. von Handmuskeln auslösen. Diese werden wir mit Oberflächenelektroden, die auf die Haut über der Hautmuskulatur geklebt werden, messen. Zusätzlich setzen wir die TMS in dieser Studie auch ein, um Erregbarkeitsänderungen der stimulierten motorischen Hirnregionen auszulösen. Diese Änderungen sind vorübergehend und bilden sich innerhalb von 1-2 Stunden zurück. Sie bemerken hiervon nichts.
Die elektromagnetischen Impulse, die für dieses Verfahren verwendet werden, sind harmlos und in der Regel nicht unangenehm oder schmerzhaft. Während oder nach den TMS - Messungen können leichte Kopfschmerzen auftreten, die in der Regel ohne weitere Behandlung zurückgehen. Die hier verwendeten Einzelpulse und die gepaarte assoziative Stimulation (hierbei werden zwei oder drei Reizspulen auf der Kopfhaut platziert und in festgelegten Abständen Reize abgegeben) gelten bei Gesunden hinsichtlich der Auslösung von epileptischen Anfällen als sicher, allerdings kann aufgrund der neuartigen Versuchsanordnung ein Restrisiko nicht vollständig ausgeschlossen werden. Sollte es unerwartet zu einem Notfall kommen, sind alle notwendigen medizinischen Maßnahmen durch eine entsprechende Laborausstattung ohne Verzögerung sofort möglich. Schließlich entstehen bei der Reizung laute Klickgeräusche. Wir werden Ihre Ohrfunktion auch während der TMS-Untersuchungen mittels Ohrstöpseln schützen.

Wie wird mit MRT Zufallsbefunden im Rahmen der Studie umgegangen?

Im Rahmen der MRT-Untersuchung wird kein Arzt-Patient-Verhältnis begründet und es wird keine klinische Individualdiagnostik durchgeführt. Daher werden tatsächlich vorhandene klinisch relevante Normabweichungen möglicherweise nicht entdeckt.

Allerdings besteht die Möglichkeit, dass bei der wissenschaftlichen Analyse der Daten Signalauffälligkeiten entdeckt werden; in diesem Falle holt der Studienarzt eine neuroradiologische Expertise ein ohne erneute Rücksprache mit Ihnen, um festzustellen, ob ein abklärungsbedürftiger Befund vorliegt.

Die Wahrscheinlichkeit dafür, dass ein abklärungsbedürftiger Befund auftritt, ist nach unseren Erfahrungen gering (ca. 0,5 %). Mit der Einwilligungserklärung willigen Sie ein, dass im Rahmen dieser Studie (1) der Studienarzt im Falle eines Zufallsbefundes ohne erneute Rücksprache mit Ihnen einen neuroradiologischen Experten hinzuzieht, dem die Bilddaten unter Beachtung der Bestimmungen des Datenschutzes zugänglich gemacht werden und dass (2) Ihnen ein abklärungsbedürftiger Zufallsbefund mitgeteilt wird, andererseits dass (3) Ihnen nicht abklärungsbedürftige Zufallsbefunde nicht mitgeteilt werden.

Transkranielle Gleichstromstimulation (tDCS)

Die transkraniellen Gleichstromstimulation (tDCS) ist gleichfalls ein schmerzfreies Verfahren das mit schwachem Gleichstrom (1 Milliampere) das Gehirns polarisiert. Dazu werden zwei Elektroden auf die Kopfhaut angebracht, durch die der Strom fließt. Dieser Strom ist für Sie nicht oder allenfalls geringfügig wahrnehmbar.

Magnetenzephalographie (MEG)

Die Magnetenzephalographie (MEG) ist ein nichtinvasives Verfahren zur Messung der magnetischen Aktivität des Gehirns durch äußere Sensoren. Die Magnetfelder unseres Gehirns induzieren in Spulen oder Spulensystemen Stromflüsse, die dann mit einer sehr guten räumlichen und zeitlichen Auflösung registriert werden können. Es

stellt ein Verfahren dar, mit dem man sehr gut die Gesamtaktivität des Gehirns beurteilen kann. Da Zellen in unserem Gehirn verschiedene Zustände einnehmen, je nachdem ob sie aktiv sind oder nicht, werden sie gebeten werden im Verlauf des Experiments verschiedene motorische Tests mit ihren Händen auszuüben.

Elektroenzephalographie (EEG)

Die Elektroenzephalografie (EEG) ist eine nichtinvasive Methode zur Messung der elektrischen Aktivität des Gehirns. Dies geschieht mit Hilfe eine EEG-Kappe, einer Stoffkappe mit Öffnungen, in die Gel eingebracht wird und an denen anschließend Elektroden befestigt werden. Mittels jener Elektroden können Spannungsschwankungen des Gehirns aufgezeichnet werden.

Motorisches Lernen

Bei zwei Teilexperimenten werden Sie gebeten, zwei motorische Lernaufgaben durchzuführen. Bei einer werden Ihnen sog. Akzelerometer (ähnlich den o.g. Oberflächenelektroden) am Daumen der rechten Hand befestigt. Diese messen die Beschleunigung Ihres Zeigefingers bei Bewegung. Sie werden in 7 zweiminütigen Blöcken dreißigmal eine schnellst mögliche Bewegung Ihres Zeigefingers in eine bestimmte Richtung durchführen (akustischer Reiz, 1 Bewegung pro 2 Sekunden, 0.5 Hz). Dabei erhalten Sie über einen Computerbildschirm Feedback, wie schnell Sie den Finger bewegt/beschleunigt haben. Dadurch werden Sie über den Trainingszeitraum in der Regel bzgl. der trainierten Bewegung schneller werden, was ihr motorisches Lernen abbildet.
Bei der anderen motorischen Lernaufgabe werden Sie eine Fingerbewegungssequenz auf einem Keyboard durchführen. Die Abfolge der Fingerbewegungen wird extern durch ein Metronom auf 2 Hz getriggert (2 Bewegungen pro Sekunde). Um motorisches Lernen zu evaluieren wird eine Fingerbewegungssequenz in 7 zweiminütigen Blöcken (15 Sequenzen) über einen Zeitraum von 20 min geübt.

Wie ist der Ablauf der Studie, und was müssen Sie bei Teilnahme beachten?

Zu Beginn der ersten Visite wird Sie der Studienarzt zu Ihrer Krankengeschichte befragen, um zu prüfen, ob Sie für die Teilnahme an der Studie geeignet sind. Bei jeder Visite wird der Studienarzt Sie nach Ihrem aktuellen Befinden befragen und Sie neurologisch untersuchen, um zu entscheiden, ob Sie die jeweilige Visite durchführen können (siehe auch unten).

Alle Probanden, die an der Studie teilnehmen, werden gebeten mindestens 2 Studienvisiten wahrzunehmen. Zwischen diesen Visiten liegen jeweils mindestens 7 Tage. Die einzelnen Visiten dauern jeweils ca. 4 Stunden. Bei jeder Visite werden mehrere TMS-Messungen und –Stimulationen sowie tDCS-Stimulationen durchgeführt. Abhängig vom Teilexperiment werden eventuell zusätzlich EEG/MEG-Messungen oder Messungen zur Evaluation motorischen Lernens durchgeführt.

Wenn Sie an der Studie teilnehmen, wird im Detail Folgendes von Ihnen erwartet:

- Die Menge an koffeinhaltigen Getränken und Alkohol, die Sie während der Studie trinken dürfen, ist begrenzt. Ihr Studienarzt oder seine Mitarbeiter werden Sie genauer informieren.

Was geschieht während Ihrer Besuche beim Studienarzt?

Der Studienarzt wird nacheinander die folgenden Maßnahmen durchführen:

- Befragung zur Krankengeschichte und den Studienein- und Auschlusskriterien (nur 1. Visite)
- Körperliche und neurologische Untersuchung am Anfang und Ende jeder Visite
- Befragung zu Nebenwirkungen am Ende jeder Visite
- MRT (nur 1. Visite)
- TMS-Erregbarkeitsmessung
- TMS- und tDCS-Stimulation
- Abhängig vom Teilexperiment werden nach 0, 30, 60 und 120 min nach der Stimulation unterschiedliche Messungen durchgeführt:

  <u>Experiment 1A und 2A:</u>

  TMS-Erregbarkeitsmessungen

Experiment 1B und 2B:

Randomisierte, d.h. in zufälliger Reihenfolge, Durchführung einer der folgenden Visiten

- Visite 1: Zunächst folgt eine Erregbarkeitsmessung mittels TMS dann die Durchführung einer komplexen Sequenz von 16 aufeinanderfolgenden Fingerbewegungen auf einem Keyboard.
- Visite 2: Zunächst folgt eine Erregbarkeitsmessung mittels TMS danach wird die schnellstmögliche Zeigefingerbewegung in eine bestimmte Richtung gemessen.

Experiment 1C und 2C

Randomisierte Durchführung einer der folgenden Visiten

Direkt nach der Stimulation

- Visite 1: Zunächst folgt eine Erregbarkeitsmessung mittels TMS dann das Erlernen einer komplexen aus 16 aufeinanderfolgenden Fingerbewegungen bestehenden Sequenz auf einem Keyboard.
- Visite 2: Zunächst folgt eine Erregbarkeitsmessung mittels TMS danach wird die schnellstmögliche Zeigefingerbewegung in eine bestimmte Richtung über 20 min trainiert mit dem Ziel diese Bewegung zu beschleunigen.

Nach jeweils 30, 60 und 120 min:

Test der erlernten motorischen Aufgabe (Fingersequenz (Visite 1) oder schnellstmögliche Zeigefingerbewegung (Visite 2)).

Bei diesem Experiment (Experiment 1C und 2C) ist es nötig, dass Sie am Folgetag (24h später) erneut erscheinen. Am Folgetag werden erneut Erregbarkeitsmessungen mittels TMS sowie ein Test der erlernten motorischen Aufgabe (Fingersequenz oder schnellstmögliche Zeigefingerbewegung) durchgeführt.

Experiment 1D und 2D:

MEG/EEG Messungen zunächst 10 min in Ruhe, gefolgt von 5 min Fingerklopfen des rechten Zeigefingers, gefolgt von 5 min einer komplexen Fingerbewegung (Sie müssen mit den übrigen Fingern der Hand abwechselnd den Daumen in einer festgelegten Reihenfolge berühren)

Jede Studienvisite dauert ungefähr 4 Stunden.

Welchen persönlichen Nutzen haben Sie von der Teilnahme an der Studie?

Im Rahmen der Studie werden Sie klinisch-neurologisch untersucht und es werden elektrophysiologische sowie bildgebende Daten Ihres Gehirns aufgenommen. Einen unmittelbaren Gesundheitsnutzen können Sie hieraus nicht ziehen. Vielmehr helfen Sie mit Ihrer freiwilligen Teilnahme an dieser Studie der Erforschung von Mechanismen der Plastizität des Gehirns, wie sie etwa bei der motorischen Rehabilitation von Schlaganfallpatienten eine Rolle spielen. Durch die gewonnenen Erkenntnisse können ggfs. zukünftig derartige Therapien verbessert werden.

Welche anderen Möglichkeiten haben Sie, wenn Sie nicht an der Studie teilnehmen?

Da die Studie lediglich zu Forschungszwecken durchgeführt wird, ist die einzige Alternative ein Verzicht auf die Teilnahme an der Studie.

Welche Risiken sind mit der Teilnahme an der Studie verbunden?

Es gibt Personengruppen, die an den genannten Untersuchungen (TMS, tDCS, EEG, MEG, MRT) nicht teilnehmen dürfen. Um herauszufinden, ob Sie hierzu gehören, wird der Studienarzt vor Beginn der Studie jeweils einen separaten Fragebogen (sog. Check-Liste) mit Ihnen durchgehen.

Alle Methoden sind schmerzfrei. Bei fehlenden Ausschlusskriterien bestehen keine relevanten gesundheitlichen Risiken. Bei der TMS tritt bei Entladung der Reizspule allerdings ein Klickgeräusch auf, vor dem Sie sich durch von uns bereit gestellte Ohrstöpsel schützen sollten, wenn mit hoher Intensität (i.d.R. ≥ 80% der maximalen

Stimulatorleistung) stimuliert wird. Während oder nach der Messung können leichte Kopfschmerzen auftreten, die in der Regel ohne weitere Behandlung zurückgehen. Bei der MRT bekommen Sie in jedem Fall einen Ohrenschutz, da es bei der Untersuchung technisch bedingt zu lauten Klopfgeräuschen im MRT-Scanner kommt. Bisher sind keine schädigenden Wirkungen von Magnetfeldern und Radiofrequenzstrahlen auf den menschlichen Körper bekannt. Vereinzelt kann es durch Make-up und Tattoos sofern diese ferromagnetische Materialien enthalten zu Hautreizungen kommen.
Bei tDCS können selten und lediglich nach kontinuierlicher Gleichstromapplikation über Stunden leichte Kopfschmerzen und Hautreizungen im Bereich der Elektroden auf, die relativ schnell (üblicherweise binnen 30-60min) vollständig reversibel waren. Bei den EEG Messungen kann es durch das Auftragen der Elektrodenpaste zu leichten Reizungen der Kopfhaut kommen, die in der Regel rasch rückläufig sind. MEG-Messungen zeigten bisher keine Nebenwirkungen.

Die Untersuchungen haben keine Auswirkungen auf Ihre Lebensführung. Bitte teilen Sie den Mitarbeitern der Prüfstelle alle Beschwerden, Erkrankungen oder Verletzungen mit, die im Verlauf der Studie auftreten. Falls diese schwerwiegend sind, teilen Sie den Mitarbeitern der Prüfstelle diese bitte umgehend mit, ggf. telefonisch.

Gibt es eine Nachbeobachtung bzw. Nachuntersuchung?

Nach Abschluss der Messungen werden Sie am Ende jedes Studientages zu Nebenwirkungen befragt. Sollten dabei Auffälligkeiten festgestellt werden oder sollten Sie sich in irgendeiner Weise unwohl oder beeinträchtigt fühlen, wird Ihr Studienarzt Sie bitten, solange im Untersuchungsraum unter Beobachtung zu bleiben, bis Sie sich wieder gut fühlen bzw. bis die festgestellten Auffälligkeiten abgeklungen sind. Sollte medizinische Hilfe nötig sein, ist diese jederzeit verfügbar.

Wer darf an dieser Studie nicht teilnehmen?

Sie können an dieser Studie nur teilnehmen, wenn Sie gesund sind und sich nicht gleichzeitig für andere Studien oder andere klinische Forschungsprojekte zur Verfügung stellen oder bis vor kurzem an solchen teilgenommen haben

Entstehen für Sie Kosten durch die Teilnahme an der Studie? Erhalten Sie eine Aufwandsentschädigung?

Durch Ihre Teilnahme an dieser Studie entstehen für Sie keine Kosten.
Für Ihre Teilnahme an dieser Studie werden Sie wie folgt bezahlt: Sie erhalten 10.- Euro pro abgeleisteter Stunde. Falls Ihr Prüfarzt Sie aus der laufenden Studie ausschließt, z.B. aus medizinischen Gründen, werden nur die bereits stattgefundenen Visiten bezahlt.
Für die für die Studie erforderlichen Untersuchungen und Leistungen dürfen die Krankenkassen nicht belastet werden.

Werden Ihnen neue Erkenntnisse während der Studie mitgeteilt?

Sie werden über neue Erkenntnisse, die in Bezug auf diese Studie bekannt werden und die für Ihre Bereitschaft zur weiteren Teilnahme wesentlich sein können, informiert. Auf dieser Basis können Sie dann Ihre Entscheidung zur weiteren Teilnahme an dieser Studie überdenken.

Kann Ihre Teilnahme an der Studie vorzeitig beendet werden?

Ihre Studienteilnahme erfolgt freiwillig. Sie haben die Möglichkeit, die Beteiligung an der Studie ohne Angabe von Gründen abzulehnen bzw. Ihre Teilnahme zu jedem Zeitpunkt zu beenden. Daraus entstehen Ihnen keine Nachteile. Darüber hinaus kann

Ihre Beteiligung auch durch den Studienarzt unabhängig von Ihrer Einwilligung beendet werden (z.B. falls bei Ihnen eine andere Behandlung erforderlich wird, Sie den Studienplan nicht einhalten, eine studienbedingte Gesundheitsschädigung auftritt oder ein anderer wichtiger Grund vorliegt).

Wie werden Ihre persönlichen Daten geschützt?

Ihre im Rahmen der wissenschaftlichen Untersuchung erhobenen Daten werden vertraulich behandelt und ausschließlich in verschlüsselter Form weitergegeben. Die für die wissenschaftliche Untersuchung wichtigen Daten werden in verschlüsselter (pseudonymisiert, ohne Namensnennung) Form in einen gesonderten Dokumentationsbogen eingetragen. Die Zuordnung der verschlüsselten Daten zu Ihrer Person ist nur anhand einer Probandenliste möglich, die in einem verschlossenen Schrank, getrennt von den Studienunterlagen aufbewahrt wird und nur dem Studienleiter und dem Ärztlichen Direktor der Abteilung zugänglich ist. Die Daten werden für die Dauer von 10 Jahren aufbewahrt und anschließend vernichtet. Im Rahmen wissenschaftlicher Veröffentlichungen (z.B. Publikationen, Vorträge) werden Ihre Daten ausschließlich in anonymisierter Form dargestellt, die eine Rückverfolgung auf ihren Ursprung ausschließt. Sollten Sie von der Studie zurücktreten, können Sie entscheiden, ob die bereits vorliegenden Daten vernichtet werden müssen oder weiterverwendet werden dürfen.

An wen wenden Sie sich bei weiteren Fragen?

Sie haben stets die Gelegenheit zu weiteren Beratungsgesprächen mit den auf Seite 1 und 2 genannten Prüfärzten.

Aufklärungsbogen für die Teilnahme an Studien mit der Magnetresonanz-Tomographie (MRT)

Es sind Gründe denkbar, die gegen Ihre Teilnahme an dieser Teiluntersuchung sprechen. Der technische Assistent wird diese und andere Gründe, wird dies mit Ihnen besprechen. Dieser Teil der Studie wird etwa 90 Minuten dauern, von denen Sie etwa eine Stunde im Magnettomographen verbringen werden. Die MRT Messung wird aus einer Reihe verschiedener Einzelmessungen mit einer Dauer von je zwischen 5 und 15 Minuten bestehen.

1. Magnetresonanztomographie (MRT)

Bei MRT werden Radiowellen in einem Magnetfeld verwendet, die im Gegensatz zu Röntgenstrahlung keine ionisierende Wirkung haben. Bei den hier verwendeten Magnetfeldstärken von 3 Tesla und bei ordnungsgemäßer Durchführung der MRT Untersuchung sind bislang keine schädigenden Wirkungen aufgetreten oder bekannt. Vorsorglich ist jedoch eine maximale Versuchsdauer von 2 Stunden pro Tag vorgeschrieben. Die Untersuchung wird in einem speziellen Raum (MR-Raum) durchgeführt. Sie werden auf einer beweglichen Liege in den Tomographen, eine kurze Röhre, gefahren. Die Röhre ist vorne und hinten offen. Dennoch kann es bei bestimmten Personen zu klaustrophobischen Zuständen (Engegefühle) kommen. Deshalb erhalten Sie einen Gummiball, mit dem Sie im Überwachungsraum ein Signal auslösen können. Darüber hinaus besteht ein laufender Gesprächskontakt über ein eingebautes Mikrofon. Bei Bedarf wird die Untersuchung sofort abgebrochen und wir holen Sie umgehend aus dem MRT Gerät. Während der Untersuchung hören Sie verschiedene laute Klopfgeräusche, die von uns über einen Lautsprecher angekündigt werden. Da diese Klopfgeräusche teilweise sehr laut werden können (bis max. 140 dB) ist das Tragen von Ohrenstöpsel Pflicht.

2. Wie ist der Ablauf der Studie und was müssen Sie bei Teilnahme beachten?

Zu Beginn werden wir Sie befragen, um etwaige Risiken einer MRT-Untersuchung auszuschließen. Zusätzlich ist ein Fragebogen mit sicherheitsrelevanten Fragen auszufüllen. Dann werden wir Sie bitten, sämtliche metallischen Gegenstände abzulegen.
Bitte kommen Sie ungeschminkt, da Schminke metallische Stoffe enthalten kann. Gegebenenfalls würden wir Sie bitten die Schminke vor der Untersuchung zu entfernen.
Die eigentliche Untersuchung wird etwa eine Stunde dauern.

3. Einschränkungen für die MRT

Sie können an dieser Studie nur teilnehmen, wenn Sie gesund sind und sich nicht gleichzeitig für andere Studien oder andere Forschungsprojekte zur Verfügung stellen oder bis vor kurzem teilgenommen haben.

Es gibt eine Reihe von Ausschlusskriterien für die Teilnahme an dieser Untersuchung, die sich insbesondere auf Metallische Implantate und mögliche Metallteile im Körper beziehen. Bitte lesen den Fragebogen diesbezüglich gründlich durch und beantworten alle Fragen.

Schwangere Frauen dürfen an dieser Studie nicht teilnehmen. In dieser Studie werden in einer Gruppe junger Erwachsener keine Frauen eingeschlossen, und in einer Gruppe älterer Erwachsener nur solche in der Post-Menopause (nach der letzten Regelblutung).

4. Mögliche Belastungen und Komplikationen einer MRT Untersuchung.
Im Rahmen eines MRTs kann es zu Erhitzungen/Überwärmungen, Muskelzuckungen durch
Nervenreizungen, insbesondere beim Einfahren in den Magneten, Kopfschmerzen und Tinnitus kommen, die möglicherweise auch länger anhaltend sind. Sollten Sie eine dieser

Empfindungen während der Messung bei sich bemerken können Sie die Untersuchung jederzeit durch einen Notalarm abbrechen.

5. Wichtiger Hinweis

Die verwendeten Methoden dienen zur Darstellung von verschiedenen Strukturen und Vorgängen in Ihrem Gehirn. Weder wird die Hirnleistungsfähigkeit untersucht noch wird diese Untersuchung diagnostisch genutzt. Eine korrekte medizinische Diagnose kann nur von einem fachkundigen Arzt durchgeführt werden und bedarf unter Anderem abweichender Untersuchungsparameter. Es ist jedoch möglich, dass wir Unregelmäßigkeiten bei Ihren Magnetresonanz-Aufnahmen beobachten.
Mit der Einwilligungserklärung willigen Sie ein, dass im Rahmen dieser Studie (1) der Studienarzt im Falle eines Zufallsbefundes ohne erneute Rücksprache mit Ihnen einen neuroradiologischen Experten hinzuzieht, dem die Bilddaten unter Beachtung der Bestimmungen des Datenschutzes zugänglich gemacht werden und dass (2) Ihnen ein abklärungsbedürftiger Zufallsbefund mitgeteilt wird, andererseits dass (3) Ihnen nicht abklärungsbedürftige Zufallsbefunde nicht mitgeteilt werden.

5. **Fragen vor Durchführung der Untersuchung:**

a) Ist durch einen Unfall oder eine Verletzung Metall in Ihren Körper gekommen?
- durch einen Berufsunfall (Metallsplitter, Metallstaub)
- durch Schussverletzungen oder Granatsplitter
- bei Verletzungen im Gesicht vor allem am Auge

☐ ja ☐ nein

b) Befinden sich seit einer Operation Metallteile bzw. andere Implantate/Prothesen in Ihrem Körper? Wie z.B. …
- Herzschrittmacher oder andere Geräte Implantate an Herz oder Blutgefäßen, z.B.
  künstliche Herzklappe, Shunt oder Port, Clips, Coils, Filter, Katheder, etc.
- bei Frauen: Hormonspirale
- orthopädische oder chirurgische Metallteile (Clips, Platten, Nägel, Drähte,
  Klammern, Nähte, etc.

- irgendwelche Prothesen (Einfache Zahnplomben sind ohne Belang)?
- andere Implantate, z.B. Gelenkimplantate, Intrauterinpessar, etc.
- metallhaltige Teile anderer Art (z.B. abgebrochene Biopsienadeln)

☐ ja ☐ Nein

c) Einige Erkrankungen und besondere Umstände sollten bei der Untersuchung besonders berücksichtigt werden. Trifft einer der folgenden Punkte bei Ihnen zu?
   - Epilepsie Schlaganfall oder Zustand nach einem Schädel-Hirn-Trauma oder der Operation von Hirntumoren
   - Zuckerkrankheit,
   - Bluthochdruck,
   - Lungen- oder Herzerkrankung,
   - Blutarmut,
   - neurologische Erkrankungen wie Epilepsie,
   - Schlaganfall, etc.
   - sonstige Erkrankungen, die eine regelmäßige ärztliche Behandlung bzw. eine regelmäßige Einnahme von Medikamenten erfordern
   - Abhängigkeit (auch frühere) von Alkohol, Drogen oder Medikamenten
   - bei Frauen: mögliche Schwangerschaft
   - Klaustrophobie (Raumangst)

☐ ja ☐ nein

d) Weil Tätowierungen Metall enthalten, kann es zu einer Erwärmung bzw. auch zu Verbrennungen während den Experimenten kommen. Tragen Sie eine Tätowierung?

☐ ja ☐ nein

Vor der Untersuchung ist zu beachten:

Legen Sie bitte alle der im Folgenden aufgelisteten Gegenstände ab, bevor Sie den MR-Raum betreten, damit diese nicht beschädigt werden bzw. keine Unfälle verursachen können.

Vorher abzulegende Gegenstände:

- Hörgerät
- Armbanduhr
- Haarklammern, -nadeln, Sicherheitsnadeln
- Schmuck (Piercings, Ohrringe, Ringe, Halsketten, etc.)
- Brieftasche, Geldbeutel incl. Kleingeld in den Taschen !
- Stifte
- Schlüssel
- Taschenmesser
- Kreditkarten u. a. Karten mit Magnetstreifen
- Gürtel
- Make-up (insbesondere Lidschatten)
- evtl. vorhandene Prothesen/Gebisse soweit möglich
- alle weiteren Gegenstände aus Metall, die Sie bei sich tragen
- Brille und Schuhe

Bitte durchsuchen Sie alle Ihre Taschen und vergewissern Sie sich, dass Sie sicher keine Gegenstände mehr bei sich haben, die Metall enthalten könnten, bevor Sie den MR- Raum betreten.

Während der Untersuchung ist es notwendig und sehr wichtig, dass Sie ruhig liegen bleiben und den Kopf nicht bewegen!

Ich habe das Aufklärungsgespräch geführt und die Einwilligung des Probanden eingeholt.

....

Name des Prüfarztes/der Prüfärztin in Druckbuchstaben

....

Datum Unterschrift des aufklärenden Prüfarztes/der Prüfärztin

Prüfstelle: Klinik für Neurologie, Eberhard Karls Universität Tübingen

Studienleiter: Prof. Dr. Ulf Ziemann

Strengthening the SMA-M1 connection of human motor cortex by a novel non-invasive brain stimulation protocol to enhance motor performance and learning

Protokoll-Nr.: 2014-01

Einwilligungserklärung

…………………………………………………………………………………………

Name des Probanden in Druckbuchstaben

geb. am ………………………………… Teilnehmer-Nr. …………………………………

Ich bin in einem persönlichen Gespräch durch den Prüfarzt

…………………………………………………………………………………………

Name der Ärztin/des Arztes

ausführlich und verständlich über Wesen, Bedeutung, Risiken und Tragweite der klinischen Prüfung aufgeklärt worden. Ich habe darüber hinaus den Text der Probandeninformation sowie die hier nachfolgend abgedruckte Datenschutzerklärung gelesen und verstanden. Ich hatte die Gelegenheit, mit dem Prüfarzt über die Durchführung der klinischen Prüfung zu sprechen.

Möglichkeit zur Dokumentation zusätzlicher Fragen seitens des Probanden oder sonstiger Aspekte des Aufklärungsgesprächs:

Ich hatte ausreichend Zeit, mich zu entscheiden. Insbesondere wurde ich über folgende Punkte informiert:

- Ziele, Dauer, Ablauf, Nutzen sowie Risiken und Nebenwirkungen der Studienteilnahme
- Umgang mit den erhobenen Daten

Mir ist bekannt, dass ich jederzeit und ohne Angabe von Gründen meine Einwilligung zur Teilnahme an der Prüfung zurückziehen kann (mündlich oder schriftlich), ohne dass mir daraus Nachteile entstehen

Datum	Unterschrift	Name des Probanden in Blockschrift

Information zum Datenschutz

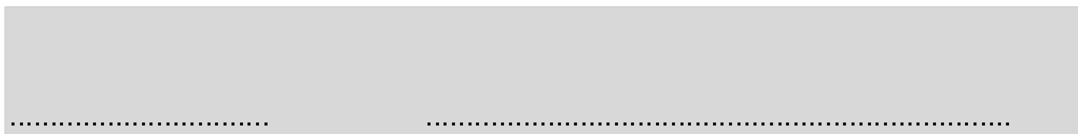
Ihre im Rahmen der wissenschaftlichen Untersuchung erhobenen Daten werden vertraulich behandelt und ausschließlich in verschlüsselter Form weitergegeben. Die für die wissenschaftliche Untersuchung wichtigen Daten werden in verschlüsselter (pseudonymisiert, ohne Namensnennung) Form in einen gesonderten Dokumentationsbogen eingetragen. Die Zuordnung der verschlüsselten Daten zu Ihrer Person ist nur anhand einer Probandenliste möglich, die in einem verschlossenen Schrank, getrennt von den Studienunterlagen aufbewahrt wird und nur dem Studienleiter und dem Ärztlichen Direktor der Abteilung zugänglich ist. Die Daten werden für die Dauer von 10 Jahren aufbewahrt und anschließend vernichtet. Sollten Sie von der Studie zurücktreten, können Sie entscheiden, ob die bereits vorliegenden Daten vernichtet werden müssen oder weiterverwendet werden dürfen.

Einwilligungserklärung

Ich erkläre mich der Verwendung der im Rahmen der Studie „Strengthening the SMA-M1 connection of human motor cortex by a novel non-invasive brain stimulation protocol to enhance motor performance and learning“ erhobenen Daten in der oben beschriebenen Weise einverstanden. Ich kann jederzeit meine Daten beim Studienleiter einsehen.

....

Name des Probanden in Druckbuchstaben


....

Datum Unterschrift des Probanden

Ich erkläre mich bereit,
an der oben genannten klinischen Prüfung

Strengthening the SMA-M1 connection of human motor cortex by a novel non-invasive brain stimulation protocol to enhance motor performance and learning

freiwillig teilzunehmen.

Ein Exemplar der Probanden-Information und -Einwilligung habe ich erhalten. Ein Exemplar verbleibt im Prüfzentrum.

....

Studienepisode (vom Prüfarzt auszufüllen)

....

Name des Probanden in Druckbuchstaben

....

Datum Unterschrift des Probanden

Ich habe das Aufklärungsgespräch geführt und die Einwilligung des Probanden eingeholt.

....

Name des Prüfarztes/der Prüfärztin in Druckbuchstaben

....

Datum Unterschrift des aufklärenden Prüfarztes/der Prüfärztin

10.3 Permission for the transfer of MRI-images

Hiermit erkläre ich, ________________________________,

geb. am ________________________

mich zur Weitergabe meiner MRT-Bilder von ________________________ an Hanna Faber zur Teilnahme an der Studie

„Strengthening the SMA-M1 connection of human motor cortex by a novel non-invasive brain stimulation protocol to enhance motor performance and learning"

bereit.

Tübingen, den ____________

____

Unterschrift

10.4 Detection of handedness

Edinburgh Handedness Inventory

Bitte kreuzen Sie an, welche Hand Sie bei folgenden Aktivitäten benutzen. Nur wenn Sie beide Hände etwa gleich häufig benutzen, wählen Sie bitte "**Beide**". Wenn Sie für eine bestimmte Tätigkeit niemals die andere Hand benutzen, wählen Sie bitte "**Nein**".

Wenn Sie...	**Welche Hand benutzen Sie?**		
	Links	**Rechts**	**Beide**
...schreiben:			
...malen:			
...etwas werfen:			
...eine Schere benutzen:			
...sich die Zähne putzen:			
...ein Messer benutzen (ohne Gabel):			
...einen Löffel benutzen:			
...einen Besen benutzen (obere Hand am Stiel):			
...ein Streichholz anzünden:			
...eine Schachtel öffnen (Hand am Deckel):			

____

Datum, Unterschrift Prüfarzt

after (Oldfield 1971)

10.5 TMS Screening Form

TMS - Eignungsfragebogen für Erwachsene

Haben Sie jemals

1.) ...eine TMS-Anwendung bekommen?

2.) ...Nebenwirkungen durch eine TMS-Anwendung verspürt?

3.) ...einen Krampfanfall erlitten?

4.) ...einen Schlaganfall erlitten?

5.) ...eine Kopfverletzung oder eine Kopf-/Gehirnoperation gehabt?

6.) Tragen Sie Metall, z.B. in Form von Clips oder Splittern, irgendwo im Kopfbereich?

7.) Tragen Sie implantierte Geräte wie Herzschrittmacher, Insulin- oder Schmerzpumpen?

8.) Leiden Sie unter häufigen und/oder schweren Kopfschmerzen?

9.) Hatten Sie jemals eine Erkrankung des Hirns oder der Hirnhäute?

10.) Hatten Sie jemals eine andere Erkrankung, die zu einer begleitenden Erkrankung oder Verletzung des Gehirns führte?

11.) Nehmen Sie Medikamente ein?

12.) Für Frauen im gebärfähigen Alter: verwenden Sie eine sichere Verhütungsmethode?

13.) Gibt es in Ihrer Familie Fälle von Epilepsie/Krampfleiden?

14.) Haben Sie noch Fragen zur Transkraniellen Magnetstimulation?

Sollten Sie eine oder mehrere der Fragen mit „ja“ beantwortet haben, so bedeutet dies nicht automatisch, dass Sie an der Studie nicht teilnehmen können. Der Studienarzt wird diese Fragen nochmals ausführlich mit Ihnen besprechen und danach entscheiden, ob Sie in die Studie eingeschlossen werden können.

____

Unterschrift Studienarzt Ort, Datum

(Rossi et al. 2011)

10.6 Expense allowance for study participants

Verwaltung
Finanzen und Rechnungswesen

Zentrum für Neurologie
Hertie-Institut für klinische Hirnforschung
Abt. Allgemeine Neurologie

Tel.82141

SOLL	
HABEN	
Kostenstelle	
Projekt	

Klinik / Institut / Abteilung / Telefon

Tübingen, den _____

mit _____ Unterlage(n)

Auszahlungs-Anordnung

Die Kasse des Universitätsklinikums Tübingen wird hierdurch angewiesen,

den Betrag von _____ € _____ Cent

in Buchstaben _____ Euro _____ Cent

an
Bankverbindung

Kontonummer
Bankleitzahl
IBAN
BIC

auszuzahlen und wie oben angegeben zu buchen.

Begründung:

Rechnerisch richtig

(Unterschrift)

Sachlich richtig

(Unterschrift)

(Anordnungsbefugte/-r)

10.7 Case report form

Case report form

Study: Strengthening the SMA-M1 connection of human motor cortex by a novel non-invasive brain stimulation protocol to enhance motor performance and learning

Subject Nr:
Datum:
Visite:

Körperliche Untersuchung

Neurologische Untersuchung

Kann der Proband die Studienvisite durchführen?
Sind nach der letzten Studienvisite Nebenwirkungen aufgetreten?

11 Acknowledgments

The use of travelling is to regulate imagination by reality, and instead of thinking how things may be, to see them as they are.

Samuel Johnson

I am thankful for the chance to perform my PhD with Prof. Dr. Ziemann and for my PhD Board members, Prof. Dr. Christoph Braun and PD Dr. Marc Himmelbach, as well as Prof. Dr. Peter Martus and Dr. Inka Montero. Thank you for your time, wise advice and encouragements. Thanks to my colleagues of the BNP lab and especially to Mrs. Riegraf.

I thank all my participants – your participation made this work possible.

My special thanks go to my good friends and amazing scientists Alexander Opitz, Stephanie Kullmann and Lisa Wang. I admire all of you and thank you for your personal as well as scientific advice. It’s a privilege having you in my life.

I thank the Evangelisches Studienwerk Villigst, especially my mentor Prof. Dr. Uwe Pietrzyk and Dr. Michael Weichbrodt who both have been an amazing support.

Last but not least, I like to thank my friends and my family for supporting me regardless all scientific work. Thank you for bike tours, dinners, parcels, postcards, phone calls - without you I would never have made it.

When I started this project – I never thought, it would shape me this way.

Thank you.