The role of network interactions in timing-dependent plasticity within the human motor cortex induced by paired associative stimulation

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List of Symbols

AMT  Active Motor Threshold
APB  Abductor Pollicis Brevis
ASL  Arterial Spin Labelling
BA   Brodmann Area
BDNF Brain-Derived Neurotrophic Factor
BOLD Blood-Oxygen-Level-Dependent
CS   Conditioning Stimulus
CSF  Cerebrospinal Fluid
DARTEL Diffeomorphic Anatomical Registration Through Exponentiated Lie algebra
EEG  Electroencephalography
EMG  Electromyography
ES   Electrical Stimulus
FDI  First Dorsal Interosseus
fMRI Functional Magnetic Resonance Imaging
FOV  Field of View
FWE  Family-Wise Error
GABA Gamma-aminobutyric acid
GM   Gray Matter
ICF  Intracortical Facilitation
IHF  Interhemispheric Facilitation
IHI  Interhemispheric Inhibition
ipsi-PAS Ipsilateral Paired Associative Stimulation
ipsi-SAI Ipsilateral Short-Latency Afferent Inhibition
ISI  Interstimulus Interval
LICI Long-Interval Intracortical Inhibition
LTD Long Term Depression
LTP Long Term Potentiation
M1  Primary motor cortex
MEP Motor Evoked Potential
MNI Montreal Neurological Institute
MNS Median Nerve Stimulation
MPRAGE Magnetization-Prepared Rapid Acquisition with Gradient Echo
MRI Magnetic Resonance Imaging
MSO Maximum Stimulator Output
MTG Medial Temporal Gyrus
NIBS Non-Invasive Brain Stimulation
NMDA-R N-methyl D-aspartate Receptor
PAS Paired Associative Stimulation
PASLTP LTP-like inducing Paired Associative Stimulation
PET Positron Emission Tomography
PMv Ventral Premotor Cortex
RMANOVA Repeated Measures Analysis of Variance
RMT Resting Motor Threshold
rTMS Repetitive Transcranial Magnetic Stimulation
S1 Primary Somatosensory Cortex
SAI Short-Latency Afferent Inhibition
SEP  Somatosensory Evoked Potential
SICI  Short-Interval Intracortical Inhibition
SM1  Primary Sensorimotor Cortex
SMA  Supplementary Motor Area
SPL  Superior Parietal Lobe
STDP  Spike Timing-Dependent Plasticity
STG  Superior Temporal Gyrus
TBS  Theta Burst Stimulation
TDP  Timing-Dependent Plasticity
TE  Echo Time
TMS  Transcranial Magnetic Stimulation
TR  Repetition Time
TS  Test Stimulus
VAS  Visual Analogue Scale
VBCT  Voxel-Based Cortical Thickness
VBM  Voxel-Based Morphometry
WM  White Matter
Bibliographic description

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Spike timing-dependent plasticity (STDP) has been suggested as one of the key mechanism underlying learning and memory. Due to its importance, timing-dependent plasticity studies have been approached in the living human brain by means of non-invasive brain stimulation (NIBS) protocols such as paired associative stimulation (PAS). However, contrary to STDP studies at a cellular level, functional plasticity induction in the human brain implies the interaction among target cortical networks and investigates plasticity mechanisms at a systems level.
This thesis comprises of two independent studies that aim at understanding the importance of considering broad cortical networks when predicting the outcome of timing-dependent associative plasticity induction in the human brain. In the first study we developed a new protocol (ipsilateral PAS (ipsiPAS)) that required timing- and regional-specific information transfer across hemispheres for the induction of timing-dependent plasticity within M1 (see chapter 3). Interestingly, ipsiPAS resulted in reversed timing-dependent plasticity as compared to a standard PAS protocol, where an asymmetric Hebbian-like plasticity mechanism has been previously described.

In the second study, we tested the influence of individual brain structure, as measured with voxel-based cortical thickness, on a standard PAS protocol (see chapter 4). We found that cortical thickness of both the primary somatosensory cortex (S1) and M1 influences the effectiveness of a LTP-like-inducing PAS protocol by almost 50%. More specifically, those participants showing a stronger LTP-like after-effect where those with higher cortical thickness values in SM1.

In summary, we observed that the near-synchronous associativity taking place within M1 is not the only determinant influencing the outcome of PAS protocols. Rather, the online interaction of the cortical networks integrating information during a PAS intervention determines the outcome of the pairing of inputs in M1.
Chapter 1

Introduction
1.1 Timing-dependent plasticity: from *in vitro* cellular studies to *in vivo* studies in humans

Synaptic plasticity has been considered by many in modern neuroscience as a basic cellular mechanism that underlie learning and memory formation. The mechanisms by which the strength of the connection between neurons gets altered due to experience seems to be broadly shared between different species and conserved along evolution. However, the concept of the brain as being subject to activity-dependent changes was not settled until the end of the 19th century when psychologist William James and neuropsychiatrist Ernesto Lugaro, among others during the same period, claimed that the central nervous system, in contrast to the Aristotle’s idea of *tabula rasa*, was suitable to plastic change and form memories via competition, intensity and repetition of events [1]. The concept of a plastic nervous system and the potential mechanisms behind such plasticity capabilities continued to develop until the psychologist Donald Hebb (and, simultaneously, the neurophysiologist Jerzy Konorski) elegantly summarized these ideas under the theory of "synaptic plasticity", usually known at present under the famous "neurons that fire together wire together" statement. The “synapses”, as portrayed by Ramón y Cajal, were now suggested to be the loci where plasticity at a cellular level was taking place. Synaptic plasticity implied that the firing activity between a presynaptic and a postsynaptic neuron could influence the strength of the synapse connecting both cells. This way, and as exposed by Hebb in his book “The organization of behavior”[2]:

“[...] when an axon of cell A is near enough to excite a cell B and repeatedly takes part in firing it, some [...] changes take place in one or both cells such that A’s efficiency, as one of the cells firing B, is increased. [...] When one cell repeatedly assists in firing another, the axon of the first cell develops synaptic knobs (or enlarges them if they already exist)”

In other words, what was later coined as “Hebbian plasticity” implied that strengthening of a synapse (i.e. the synaptic weight connecting a pair of neurons) would occur when there is presynaptic causality for the postsynaptic spiking [3]. The theoretical background proposed by Hebb was soon considered a potential key mechanism for plasticity at a cellular level and, thus, an ideal candidate to explain learning processes and the formation of memories. Soon thereafter, a set of new *in vitro* experiments (in acute brain slices or cellular cultures) aimed at exploring how firing causality between two cells affected their synaptic strength.

One of the first *in vitro* experiments that gave experimental support to the Hebbian theory was performed by Bliss and Lømo (1973 [4]) in hippocampal slices. The authors reported the presence of synaptic long-lasting potentiation (later termed long-
term potentiation (LTP)) induced by high-frequency trains of electrical stimuli (tetani) in the rabbit dentate gyrus, a potentiation that kept increasing beyond the synaptic stimulation [5, 1]. Further experiments, moreover, extended the idea of synaptic plasticity not only to the potential for an increase in synaptic strength (LTP) but to the bidirectional modification of synaptic strength with potential for both an increase and a decrease after stimulation. For example, it was found that the rate of stimulation could determine the outcome of the synaptic change, with higher rates inducing LTP and lower rates long-term depression (LTD) [1].

Additionally, the order and timing of events (pre and postsynaptic firing) was found to also influence plasticity. As Markram et al. (2012) posed it in a recent review, “it seems correct to assume that the repeated and persistent temporal orderings of events A & B actually means that event A somehow causes event B. [...] Time is important cause it could indicate causality” and, moreover, convey information [6]. This form of synaptic plasticity, thus, was termed spike timing-dependent plasticity (STDP). Regarding the timing-dependency of this form of plasticity, it was found that a critical window of tens of milliseconds resulted in effective synaptic changes, while no effective plasticity could be induced outside such window [7]. Furthermore, emphasis was placed on the order of firing within this time window: while presynaptic firing before postsynaptic firing resulted in a strengthening of the synapse (associative LTP), the reverse (post before pre) resulted in weakening of the synaptic weight (associative LTD) [8]. Interestingly, STDP was found to be tightly related to the activation of N-methyl D-aspartate receptors (NMDA-R). The NMDA-R has been considered a “coincidence detector” since it only opens to allow Ca\(^{2+}\) influx after both the release of glutamate by the presynaptic terminal and the depolarization of the postsynaptic membrane, but not after either of these events alone. The level of Ca\(^{2+}\) influx, in turn, is of utter importance to determine whether LTP or LTD is induced, since high and sharp levels of Ca\(^{2+}\) influx have been shown to result in LTP while low and steady levels of postsynaptic Ca\(^{2+}\) influx result in LTD [9, 1].

Associative LTP and LTD revealed then the importance of causality, as implied in Hebb’s theory, in determining the direction of the synaptic plasticity change [8]. However exciting was the finding of the importance of order and timing of neuronal events in plasticity induction in vitro, where the pairing of spikes occurs within a rather quiescent environment, the physiological relevance of STDP could not be determined unless tested in vivo. The main open question, thus, was whether timing at the millisecond scale was relevant or not in the intact brain considering the rate of spontaneous ongoing activity and the noisy nature of the system. The first study to successfully report induction of STDP in vivo was carried out by Poo and colleagues [10]. The authors used the retinotectal preparation of the Xenopus tadpole and found that cortical plasticity was dependent on the interval temporal order of visual and electrical cortical stimulation, which was consistent with STDP as studied in intracortical connections [10, 11]. Finally, the idea
that associative LTP might constitute a basic plasticity mechanism underlying learning
and memory gained weight when evidence of STDP-induction was found in an increasing
number of neural circuits including neocortical areas such as the primary sensory cortices
[8].

Among the properties that make STDP an ideal candidate to be considered a
highly relevant basic mechanism for plasticity are its role in network stability (driven
by the bidirectional synaptic modification relative to activity), synapse competition, se-
quence learning, prediction, information encoding and circuit plasticity [11, 3]. Especially
interesting is the growing evidence pointing to a role of STDP, at least in the somatosen-
sory cortex, in encoding information and, thus, in memory formation [12]. Naturally, the
stability and strength of associative LTP- and LTD-induction in vivo is much smaller than
in vitro, probably due to the effect of physiological spontaneous activity on the reversal of
artifically induced STDP. Moreover, the associative plasticity mechanisms found in vivo
constitute a form of artificially-induced plasticity that has not yet been shown to occur
as a natural phenomenon.

Both rate-dependent and timing-dependent plasticity have been recently reported
at the systems level of the human brain, leading to the idea that both spiking rate as
well as causality of neuronal events (consistent with classical and associative LTP and
LTD mechanisms) play a role in human plasticity induction. Based on the experimental
set ups used invasively in animals, non-invasive high/low frequency as well as associative
brain stimulation interventions were designed in order to test the potential for similar
mechanisms of plasticity acting at the human level. High and low frequency non-invasive
stimulation (i.e. repetitive transcranial magnetic stimulation [13]) of a target cortical area
was mainly based upon studies demonstrating that the discharge of the postsynaptic cell
alone at certain rates without the triggering coming from the presynaptic input could
in itself evoke LTP [14, 1]. Associativity, experimentally tested by Bruce McNaughton
in 1978 [15], indicates that two distinct pathways, which were not potentiated when
stimulated on their own, could cooperatively result in LTP when repeatedly paired. In
other words, this property ensures that weak tetanus, not capable by itself to successfully
induce potentiation, can result in LTP through association with a strong tetanus [16].
Paired associative stimulation, as developed by Joseph Claßen and colleagues [17], aimed
to test the role of associativity and timing- / order-dependency between afferent and direct
cortical events in the induction of plasticity in the living human motor cortex.
1.2 Non-invasive brain stimulation protocols to study plasticity mechanisms in the human brain

1.2.1 The origins and neurophysiology of Transcranial Magnetic Stimulation

Since Morton and Merton first applied non-invasive electrical stimulation through the scalp and skull of human subjects [18], brain stimulation techniques have been widely used both in the study of basic neurophysiology in humans and in the study of clinical populations with prospective therapeutical uses. The main drawback, however, of electrical stimulation is the associated adverse side-effects (such as pain), which reduces its application possibilities on healthy participants.

In 1985, Barker et al. developed the first stimulators that could, non-invasively and with minimum discomfort, depolarize neuronal circuits in the cortex [19]. Based on the electromagnetic induction principle, such stimulators contain a capacitor that discharges a fast electrical current through an isolated coil which, in accordance to Faraday’s law, generates a changing magnetic field of around 2 Tesla intensity and 100 $\mu$s duration that, in turn, induces an electric field and an associated electric current in a nearby conductive material [20, 21]. In the case of transcranial magnetic stimulation (TMS) with the inductor (coil) placed over the scalp, the conductive material is the scalp itself, the cerebrospinal fluid, grey matter, and white matter, which together can be considered as an homogeneous conductor.

Since the technique was developed, many studies have focused on the primary motor cortex due to the clear observable response (muscle contraction) that a high intensity TMS pulse induces, which in turn can be quantitatively measured with surface electrodes placed over the corresponding muscle. Since these muscle twitches increase or decrease in amplitude (mV) with stimulator output intensity, diverse quantifications of motor corticospinal excitability can be performed. For example, double-pulse TMS designs where a conditioning stimulus is applied before a test stimulus over the primary motor cortex (M1) at different interstimulus intervals (ISIs) have been used to indirectly examine neurotransmitter-mediated M1 intracortical mechanisms, such as glutamate-mediated intracortical facilitation (ICF, known to occur at ISIs between 10 and 15 ms) or GABA-mediated short-interval or long-interval intracortical inhibition (SICI (occurring at ISIs between 2 and 5 ms) and LICI (occurring at ISIs between 50 and 200 ms), respectively) [22, 23, 24]. Moreover, interhemispheric facilitatory and inhibitory M1 interactions have been addressed with bihemispheric TMS pulses, revealing a facilitatory interaction (interhemispheric facilitation (IHF)) taking place at short intervals (1-5 ms) and an inhibitory interaction (interhemispheric inhibition (IHI)) at slightly longer intervals (7-10 ms) [25, 26, 21]. Furthermore, mapping of motor function and topology (homunculus)
can be achieved because different muscles are engaged when stimulating different positions along the precentral sulcus, with a relative focality that allows for selective stimulation of distinct homunculus representations such as hand, upper arm or leg areas. Less focality can be achieved for specific muscles within an area (i.e. hand) due to the overlapping of their representations in the cortex [21].

Some technical details must be taken into account when performing TMS experiments, such as the shape of the coil (i.e. circular or figure-of-eight shape) which determines the size of the induced electric field and, thus, the focality of the stimulation, or the orientation of the coil over the scalp. For example, for motor activation, it has been shown that the largest MEPs are generated when the current is directed in the posterior-anterior direction in the brain (anterior-posterior in the coil) at an angle perpendicular to the central sulcus [20]. This is so because the neurons activated depend on the size, shape and orientation of the induced electric field, as well as on the simulation intensity and waveform (monophasic or biphasic) produced by the magnetic stimulator. The final coil position will most probably be the one that excites the neuronal population that projects to the target area [27].

TMS is thought to mostly activate intracortical fibers that travel “horizontally” with respect to the scalp surface [28], and eventually leads to the transsynaptic activation of pyramidal output cells [27]. When TMS is applied over M1, so called motor evoked potentials (MEPs) are generated. Invasive recordings from patients with implanted cervical epidural electrodes revealed that descending corticospinal volleys are generated when M1 is stimulated with TMS (see 1.1A; [29]). The higher the intensity of the stimulation, the higher the number of descending corticospinal volleys that can be recorded at the level of the cervical spinal cord. The corticospinal volleys are caused by highly synchronized action potentials in large-diameter axons that originate from corticospinal neurons (Fig 1.1C). Such corticospinal neurons make then direct monosynaptic connections with spinal motoneurons (1.1D). The descending excitatory drive will mostly depend on the intensity of the TMS pulse, in a way that the higher the intensity, the higher the excitatory drive to the spinal motoneurons and, thus, the bigger the amplitude of the motor evoked potential derived from the muscle response (1.1F). This relation between TMS intensity and MEP amplitude is, however, non-linear, as a plateau is reached after a certain percentage of maximum stimulator output (MSO) that varies among individuals [30].

The size of the MEP will also depend on the excitability of the cortex at the moment of stimulation and the excitability of the spinal motoneuron pool. Moreover, the MEP amplitude tends to vary from stimulus to stimulus probably due to intrinsic fluctuations of excitability in the corticomotor system [28, 20].

Another measure of corticospinal excitability is the motor threshold, which can
Figure 1.1: Schematic representation of the cortico-motor pathway including A) primary motor cortex (M1), B) ventral posterior nuclear complex of thalamus, C) corticospinal neurons, D) cortico-motoneuronal synapse, E) posterior root ganglion cells (spinal motoneurons) and F) peripheral motor axons. The black figure-of-eight represents a coil positioned over M1, while the red symbol represents the magnetic stimulus.
be measured both at rest (resting motor threshold (RMT)) or during voluntary muscle contraction (active motor threshold (AMT)). The RMT is usually defined as the lowest stimulation intensity that is capable of generating a MEP of at least 50 μV (peak-to-peak) in at least 5 out of 10 (or 3 out of 6) trials [30, 31]. The RMT is believed to represent the integrated excitability of a central core of neurons within the corticomotor projection (both at a cortical and spinal level). It has been suggested as a measure of membrane excitability because it increases with blockade of voltage-dependent calcium channels [32, 20].

1.2.2 Plasticity-inducing TMS protocols: the link to classical synaptic plasticity studies

The opportunity to non-invasively stimulate the intact human brain without inducing discomfort has opened the door to exciting new plasticity studies that in most cases are based upon in vitro and in vivo invasive stimulation paradigms in cultured cells and animals [33]. These protocols, thus, are aimed at investigating whether similar mechanisms of plasticity (such as LTP and LTD) as those found in invasive, more constraint and controlled studies apply at the systems level of the whole human brain in vivo [5, 16]. Neuronal plasticity here is understood as any functional change within the nervous system that outlasts an experimental manipulation, and is usually defined neurophysiologically by changes in the stimulus-response characteristics [27]. In order to address plasticity changes in the human primary motor cortex, the aforementioned measures of corticospinal excitability are used to investigate modulations in MEP amplitude or membrane excitability (as assessed with RMT) that occur after plasticity-inducing TMS protocols. Potential changes in MEP amplitudes after a TMS intervention could be due to effects occurring at all possible levels of the motor system (motor cortex, subcortical or spinal cord) independently or in combination.

One of the first protocols that was developed in order to induce a plastic change within the underlying stimulated cortical area was termed repetitive TMS (rTMS, [13]), which consists on a set of stimuli applied repeatedly at high (5 Hz) or low (1 Hz) frequencies and is based upon the tetanic stimulation initially used in experiments in vitro ([4], see 1.1). In most studies, even though subject to a high inter-individual variability in the after-intervention effects, high frequency rTMS leads to an increase in the MEP amplitude, while low frequency stimulation results in a decrease in MEP amplitude, which has been related to LTP-like and LTD-like plasticity mechanisms taking place in the human brain [33]. Generally, rTMS after-effects depend on the frequency, intensity and length of time of the stimulation. Another form of plasticity-inducing repetitive TMS that is based on the same principle but uses trains of stimuli delivered at theta-frequency is called theta burst stimulation (TBS, [34]). TBS induces LTP-like or LTD-like effects depending on the
pattern of stimuli applied (continuous or intermittent), and its after-effects can last up to 1 hour approximately [21]. Even though the underlying mechanisms behind the observed rTMS after-effects are not completely understood, a recent experiment investigating the effect of magnetic stimulation on slice cultures in vitro revealed coordinated functional and structural plasticity of excitatory postsynapses after a 10 Hz magnetic stimulation intervention. The authors suggested that these results are consistent with a long-term potentiation of synaptic transmission after repetitive magnetic stimulation [35].

1.2.3 Timing-dependent plasticity-inducing TMS protocols: interventional paired associative stimulation

In addition to classical synaptic plasticity-like protocols, specific non-invasive brain stimulation protocols such as paired associative stimulation (PAS) have been developed to investigate spike timing-dependent plasticity (STDP)-like effects in humans. The investigation of STDP-like mechanisms in the human brain is relevant to understand how timing of events shapes the induction of plasticity at a systems level (and, thus, how relevant the factor timing and causality of inputs is when applied to the whole intact human brain) when compared to quiescent in vitro networks or animal experiments in a translational fashion. Moreover, it directly tests the role of cortical associativity in the formation of plasticity at the neuronal population level. PAS, first described by Stefan et al. [17], consists of a low frequency afferent stimulation of the median nerve (median nerve stimulation, MNS) followed by transcranial magnetic stimulation (TMS) over the contralateral primary motor cortex (M1) (for an schematic representation of a PAS intervention see Figure 1.2).

PAS in humans has been considered as a model of STDP-induction since (a) it takes into account local interaction between two inputs reaching a target cortical area and (b) the after-intervention effects are critically dependent on the timing of arrival of these two inputs, resembling a comparable time-window as the one seen in STDP cellular studies [33, 16]. For instance, near-synchronous arrival of MNS and TMS inputs to M1 (with an assumed margin of around 10 ms with respect to exact synchronicity) elicits an increase in M1 corticospinal excitability that outlasts the intervention period by at least 30 minutes [17]. In contrast, when M1 is activated by TMS before the peripheral signal (MNS) arrives, a long-lasting decrease in corticospinal excitability has been shown [36]. Those results have been linked to Hebbian-like rules for the induction of plasticity (long term potentiation (LTP)-like or depression (LTD)-like) in humans. Moreover, the PAS-induced plasticity resembles features of STDP such as NMDA-R activation dependence in the case of LTP-like and both NMDA-R and voltage-dependent calcium channel activation in the case of LTD-like effects, as well as topographic specificity (for a detailed review see [37] and [38]). Interestingly, GABAergic inhibitory mechanisms also seem to play an important role.
Figure 1.2: Schematic overview of an average PAS experimental set up. The figure of the brain and shoulders (Pre / Post 1 / Post 2) represents corticospinal excitability measurements (1 mV MEP / RMT) before and after the PAS intervention (Post 1 = immediately after intervention). The figure were hands are shown represents the PAS intervention (pairing of TMS pulses applied directly over M1 and MNS applied over the contralateral wrist (target hand colored in red)).

in PAS-induced excitability changes since the application of a GABA_B receptor agonist has been shown to abolish PAS-induced after-effects [39]. Additionally, PAS_LTP seems to change somatosensory evoked potential (SEP) components in the ipsilateral S1 as well [40], inducing a form of network plasticity that seems to go beyond the target area where direct associativity of inputs takes place. Finally, the effects of PAS do not only seem to depend on the timing of inputs but also on the activity of neuromodulators such as norepinephrine, dopamine and acetylcholine ([41]).

All these plasticity-inducing protocols rely on the measurement of MEP size changes when applied over the motor cortex, and it is considered the indirect measure of plasticity mechanisms taking place in the human brain par excellence. However, there are certain limitations when considering the MEP size as indicative for plasticity, since the MEP size usually vary from one stimulus to the next (see 1.2.1) and highly vary from one subject to another (see 1.3). In order to achieve a normalized response across subjects (i.e. as a measure of corticospinal excitability baseline before any intervention is applied), a certain MEP amplitude is set as target size (commonly between 0.5 and 1 mV peak-to-peak) and the intensity of the simulation is adjusted in each subject until the target MEP amplitude is reached. Moreover, there are at least three possible physiological mechanisms that influence the size of MEPs, such as the number of motor neurons that are recruited in the spinal cord, the number of motor neurons that discharge more than one time to the same magnetic stimulus and the synchronization of the motor neuron discharges induced by TMS [27, 42]. When considering a change in the MEP amplitude due to a prior plasticity-inducing intervention, an underlying change in cortical motor output responsible for the generation of MEPs is presumed. These variations in cortical
motor output could be due to a change in synaptic efficacy, changes in the threshold for the generation of action potentials and / or changes in intracortical inhibitory networks [5].

1.3 Peculiarities of artificially-induced plasticity in the human brain

As seen in 1.2, non-invasive brain stimulation techniques have been used to study activity-dependent changes in cortical motor maps or to influence behaviour by up- or down-regulating cortical excitability [38, 33]. Even though artificially inducing plasticity in the human brain presents itself as a highly promising way of studying both basic plasticity mechanisms and pathological brain states at a systems level, TMS protocols seem to alter corticospinal excitability with a high degree of intersubject variability [43, 44, 45, 33], rendering the reliability of the technique (specially in clinical studies) rather weak. Such variability, however, is to be expected in in vivo studies due to the high level of spontaneous ongoing activity underlying the stimulation-induced response (see 1.1), and specially in humans where other components such as cognitive or genetic determinants might play a role in the state of the system during intervention [46]. These interindividual outcome differences have raised the important question about possible underlying determinants influencing the effectiveness of plasticity-inducing interventions.

A commonly used TMS protocol is PAS (see 1.2.3), which consists of median nerve stimulation followed by TMS applied over contralateral M1. Although PAS has received quite some attention due to its relevance in investigating plasticity in vivo in the human brain, as well as for its potential in clinical research, the after-effects of PAS show an important intersubject variability that can reach 30% of the total sample in most studies [33]. The variability in PAS interventions as well as in other plasticity-inducing TMS protocols [46, 43] has been addressed by focusing on different potentially relevant external (suitable for manipulation) or internal (not suitable for manipulation) factors, such as attentional state of the subject during intervention, genetic profile (particularly BDNF), exercise, gender or time of the day when the intervention is performed. Among these factors, age seems to play an important role [47]. For example, PAS-induced after effects have been shown to be more pronounced in healthy young and middle-aged subjects as compared to elderly subjects, suggesting an age-dependent reduction of cortical plasticity. Furthermore, it has been shown that the outcome of PAS depends on the respective time of the day when the intervention is applied [44]. According to the results of Sale and colleagues, the outcome of a PAS intervention seems to be more effective in the afternoon as compared to an intervention applied in the morning.

Genetic factors seem to largely influence functional plasticity [46], and the genetic
factors (amongst others) that have been shown to significantly influence the effect of non-invasive brain stimulation protocols are polymorphisms of the brain-derived neurotrophic factor (BDNF). A common polymorphism in the BDNF gene (val66met) has been shown to affect the learning of simple motor tasks [48] and is related to intersubject variability in plasticity-inducing TMS protocols such as PAS [49]. Additionally, in a recent study by Missitzi et al. (2011 [50]), the authors applied PAS\textsubscript{LTP} in a set of either genetically identical (monozygotic) or genetically different (dizygotic) twins, revealing significantly larger intra-pair variability for the dizygotic group and thus emphasizing the importance of genetic factors in TMS-induced plasticity.

Finally, another potentially relevant external factor affecting the outcome of PAS is the attentional state of the subjects during intervention, resulting in an increased effect when subjects actively pay attention to the stimulation and, for example, count the number of applied stimuli along the intervention and in a decreased or no effect when subjects actively pay attention to an unrelated demanding task such as arithmetic problems [51].

In addition to these already known factors, other peculiar aspects of plasticity induction at the systems level of the human brain entail the ongoing whole-brain cortico-cortical and cortico-subcortical interactions during intervention, the applicability of Hebbian properties on the plasticity induced at the neuronal population level or the emergence of new mechanisms of plasticity derived from the sum of the single units that conform human cortical networks (see for example [52]).

1.4 Plasticity at the network level

Clearly, timing-dependent plasticity at the systems level of the human brain requires the interaction among functionally related networks such as the primary sensorimotor cortex (SM1), which is involved in information integration during PAS intervention, and represents a more \textit{in vivo} approach at a systems level as compared to the cellular mechanisms of plasticity studied in STDP experiments. Such difference underpins the potential for PAS and timing-dependent plasticity in humans to induce a form of plasticity that could better be termed \textit{network plasticity}. As recently argued by Schulz and colleagues [6]: “it seems probable that synaptic plasticity in the intact brain is governed by rules that are more complex than classical STDP [...] The outcome of plasticity-inducing interventions will not only depend on precise timing but also on converging inhibitory and neuromodulatory inputs”. It is known that, for PAS, neuromodulatory influence is a key factor for the development of timing-dependent plasticity (see for example [53, 54, 41]).

Moreover, the Hebbian-like nature of the seen PAS after-effects represent a higher-order example of the Hebbian theory since, as explained by Hopfield in 1982: “\textit{the Hebbian}
property need not reside in single synapses; small groups of cells which produce such a net effect will suffice” [52]. Thus, the neurophysiological measures of neuronal populations in the human brain via TMS after a plasticity-inducing intervention are to be considered not only as an indirect measure of STDP-like mechanisms. Rather, timing of events can be considered as an important factor modulating plasticity from the synaptic to the network level. Timing-dependent network plasticity as a form of plasticity in itself may thus go beyond the sum of STDP occurring at single synapses, alternatively representing an emergent mechanism taking place at the network level. Hopfield discusses that “the bridge between simple circuits and the complex computational properties of higher nervous systems may be the [...] spontaneous emergence of new computational capabilities from the collective behavior of large number of simple processing elements” [52]. Moreover, authors like Chiappalone and colleagues ([55]) have already used terms such as “long-term network potentiation” to refer to the potentiation of a stimulus-response interaction of a neuronal network, reinforcing the idea that to better understand learning and memory it is necessary to investigate, beyond the synapse, how plasticity develops at a network level.

Following this line, novel paired associative stimulation interventions have addressed the potential for timing- and order-dependent plasticity to be induced in known interconnected cortical networks, such as the premotor-to-motor network or the inhibitory interhemispheric motor-to-motor interaction. For example, in a study performed by Buch and colleagues [56], a series of paired stimuli applied over the ventral premotor cortex (PMv) and M1 at specific interstimulus intervals (known to induce an inhibitory effect from PMv to M1) resulted in a stronger PMv - M1 functional connectivity (measured as increased inhibitory drive from PMv to M1) that depended on both the time between stimuli and on the order of corticocortical stimulation. Using a similar paradigm, Rizzo and colleagues [57] performed bihemispheric M1 stimulation at interstimulus intervals known to induce IHI from left-to-right M1. The authors, surprisingly, found a reduced interhemispheric inhibitory effect after paired stimulation which was then interpreted as a Hebbian-like mechanism due to its timing-dependency. Moreover, in a similar study performed by Koganemaru and colleagues [58], the authors performed paired bihemispheric stimulation between M1 (right-to-left) cortices at an IHI interval and addressed corticospinal excitability within the left M1 (receiving repeated inhibitory input) after the intervention. In line with Rizzo et al., a time-dependent increase in left M1 corticospinal excitability was found after the paired bihemispheric stimulation intervention, indicating a similar Hebbian-like mechanism taking place. Furthermore, other PAS paradigms including distant cortico-cortical interactions have been recently designed, such as cerebellar-M1 [59] or supplementary motor area-M1 [60] associative plasticity induction.

Altogether, these results point to the potential for timing-dependent plasticity to be induced within distant interconnected networks in the human brain. However, it
still remains elusive whether the intrinsic network interactions (intracortical and cortico-cortical facilitation and inhibition) taking place during pairing of inputs play a role in the outcome of timing-dependent network plasticity interventions. For STDP at a synaptic level, it is known that the timing-dependent curve for LTP and LTD can substantially vary depending on the type of synapse and can even be reversed in an anti-Hebbian fashion, especially when considering inhibitory synapses ([3, 8, 11, 12]). Moreover, STDP expression \textit{in vivo} has been shown to dramatically depend on the state of the neuronal network (i.e. overall inhibition), its ongoing activity and neuromodulatory influence [6]. So far, only standard STDP-like timing-dependent windows have been reported in the human brain, independent of the nature of the network interactions taking place during intervention.
Chapter 2

Aims
As presented in the introduction, timing is an important factor in neuroplasticity and has been studied in humans as a model of STDP at the systems level. Furthermore, the idea behind PAS in the sensorimotor areas is that the afferent information is received in the primary motor cortex via somatosensory input that occurs at short latency and with high topographical specificity [16], implying that the cortico-cortical interactions between the areas integrating the inputs during standard PAS is essential for the seen effects. The general aim of the studies conforming this dissertation is to investigate the role that the interaction between cortical networks has over the timing-dependent network plasticity induced with paired associative stimulation protocols. More specifically, with our first study (see chapter 3) we wanted to keep the basic design of PAS (in opposition to the cortico-cortical PAS interventions described in 1.4), consisting of paired afferent and cortical stimulation as done in vivo in animal studies ([11] for example) but with an extended target network that included bihemispheric sensorimotor cortices as well as interhemispheric interactions. This new PAS protocol implies:

1. a new assumed pathway for the afferent stimulation to reach the target cortical area,
2. a new timing to consider near-synchronous arrival of inputs to the target cortical area and
3. a set of specific inhibitory and facilitatory (cortico-cortical and interhemispheric) interactions for the new target cortical network.

Our main assumption was that, due to the potential of PAS as a protocol to induce plasticity at the network level (see 1.4), a broader interaction among distant cortical (and most probably subcortical and spinal, although not directly measured in the present study) areas would still result in an effective associativity and therefore in a significant corticospinal plastic change (as measured with MEPs). Furthermore, we hypothesized that the outcome of plasticity-inducing protocols would ultimately depend on the intrinsic interactions between the active networks integrating the information during the paired associative intervention.

Moreover, considering the known interindividual variability of PAS after-effects (see section 1.3), we aimed to investigate the influence that brain structure (derived from cortical thickness whole-brain measures) has on the seen variability (see chapter 4). Our assumption was that the structure of functionally relevant cortical interconnected areas and, most probably, of those cortical areas involved in the information integration during PAS, determined the degree of timing-dependent plasticity induction.
Chapter 3

First Study
The role of inhibitory network interactions in the induction of timing-dependent plasticity in humans


3.1 Abstract

Spike timing-dependent plasticity (STDP) has been proposed as one of the key mechanisms underlying learning and memory. Repetitive median nerve stimulation (MNS) followed by transcranial magnetic stimulation (TMS) of the contralateral primary motor cortex (M1), defined as paired associative stimulation (PAS), has been used as an in vivo model of STDP in humans. PAS-induced excitability changes in M1 have been repeatedly shown to be time-dependent in an STDP-like fashion since synchronous arrival of inputs within M1 induces long term potentiation (LTP)-like effects while an asynchronous arrival induces long term depression (LTD)-like effects. Here we show that interhemispheric inhibition of the sensorimotor network during PAS with the peripheral stimulation over the hand ipsilateral to the motor cortex receiving TMS (ipsi-PAS) results in an LTD-like effect, as opposed to the standard STDP-like effect seen for contralateral PAS. Furthermore, we could show that this reversed associative plasticity critically depends on the timing interval between afferent and cortical stimulation. These results indicate that the outcome of associative stimulation in the human brain depends on functional network interactions (inhibition or facilitation) at a systems level and can either follow standard or reversed STDP-like plasticity.

3.2 Introduction

Timing-dependent plasticity at the systems level of the human brain differs from STDP in that it requires the interaction among functionally related networks such as the primary sensorimotor cortex (SM1), which are involved in information integration during PAS intervention, and represents a more in vivo systems level approach as compared to the cellular mechanisms of plasticity studied in STDP experiments. In addition to PAS studies, several other experiments have been performed to study intra- and interhemispheric sensorimotor function in healthy volunteers. For example, in a study performed by Hlushchuk and Hari, tactile stimulation over one hand, apart from activating the con-
tralateral primary somatosensory cortex (S1), deactivated the ipsilateral S1 regardless of hemispheric dominance [61]. In similar imaging experiments performing unilateral hand stimulation, a “contralateral sensorimotor activation – ipsilateral sensorimotor deactivation” has been consistently reported [62, 63, 64, 65, 66, 67, 68, 69]. This inhibitory S1-S1 communication has been further investigated in a recently published study by Ragert et al. [70], in which the authors identified a critical time window where activation of S1 leads to deactivation in the opposite S1 that, additionally, matched the S1-S1 transcallosal conduction time found in prior experiments [71]. Hence, the aim of the present study was to investigate the influence of interhemispheric information processing on PAS-induced LTP-like and LTD-like plastic changes, taking into account the “contralateral activation / ipsilateral deactivation” pattern that takes place between sensorimotor cortices after unilateral afferent stimulation at the wrist. Here we introduce a novel PAS paradigm where in contrast to the standard PAS protocol [17, 37] a TMS pulse was applied over M1 ipsilateral to the wrist receiving afferent stimulation (ipsiPAS). We hypothesized that ipsiPAS would result in a significant LTD-like decrease in M1 corticospinal excitability mediated by inhibitory S1-S1 communication, due to repeated associative stimulation over a down-regulated SM1 network.

### 3.3 Materials and Methods

The study comprised of three different single-blinded experiments consisting of a total of 7 different interventions in a cross-sectional design and a fourth experiment with an additional control group. In the first experiment, a total of 11 subjects were randomly allocated in two experimental target conditions (PAS25 and ipsiPAS45) in order to directly compare the outcome of standard PAS (PAS25, LTP-like) with a PAS protocol consisting of MNS applied over the left forearm ipsilateral to the TMS site (left M1; ipsiPAS45). Subsequently, we performed a series of either PAS or ipsiPAS control experiments (ipsiPAS25 (n = 11), ipsiPAS35 (n = 10), ipsiPAS60 (n = 10), ipsiPAS75 (n = 8) and PAS45 (n = 11), for details see below) to disentangle the timing specificity for the induction of either LTP- or LTD-like plasticity. Finally, 6 of the 11 participants underwent an additional experiment where we tested for GABA_A- and GABA_B-mediated inhibition as well as for glutamate-mediated facilitation in left M1 at baseline before an ipsiPAS45 intervention, in order to test for potential influencing neuromodulators associated with ipsiPAS45 after-effects. The additional control group (n = 13) underwent a modified version of the short-latency afferent inhibition (SAI) protocol first described by Tokimura et al. [23]. Here, we paired MNS (left wrist) with TMS (left-ipsilateral M1) at 3 different ISIs (42, 45 and 47 ms) and compared the effects to TMS pulses applied over left M1 alone (ipsi-SAI, see below for a detailed description of this protocol). Each experimental condition was separated by at least one week in order to avoid any carry-over effect. PAS sessions only differed in the
Figure 3.1: Experimental setup and design. Prior to each intervention, resting motor thresholds (RMT) as well as 1 mV MEPs were measured over left M1. Subsequently either PAS or ipsiPAS was applied over left M1 with different interstimulus intervals for a total duration of 10 minutes. The red shaded hand represents the different location of median nerve stimulation for PAS (right hand) and ipsiPAS (left hand) interventions. The term ISI indicates different intervals between MNS and TMS pulses where Bold numbers indicate assumed synchronous target interventions. After termination of the respective PAS / ipsiPAS protocol, RMT and 1mV MEP recordings were repeated 0-5 min (post1) and 15-20 minutes (post2) after intervention and were compared to baseline (pre).

interstimulus interval (ISI; 25 ms, 35 ms, 45 ms, 60 ms or 75 ms) between MNS and TMS and site of median nerve stimulation (MNS; left (ipsiPAS) or right hand (PAS)). TMS was always applied over the left abductor pollicis brevis (APB) hotspot within M1 and M1 was considered “ipsilateral” or “contralateral” depending on the MNS site (MNS over right wrist was contralateral to left M1 and MNS over left wrist was ipsilateral to left M1). The order of PAS sessions for each subject was counterbalanced. For a schematic view of the experimental design see Figure 3.1.

3.3.1 Subjects

Eleven right-handed healthy subjects (mean age: 24.90 ± 4.07 years old, 5 female) participated in the study. Prior to participation, all subjects underwent a comprehensive neurological examination. Handedness was assessed by the Edinburgh inventory scale
Subjects were neither TMS nor PAS naive and were selected according to effective facilitatory response to a standard PAS protocol (PAS$_{25}$) in a prior experiment [73]. Each subject fulfilled the inclusion criteria in agreement with the safety guidelines approved by TMS consensus [74] and gave written informed consent to participate in the experiment according to the declaration of Helsinki. The study was approved by the local ethics committee of Leipzig. None of the subjects were using drugs acting in the central nervous system by the time of the study. Levels of attention, fatigue and discomfort were assessed for each subject before (pre) and after (post) each intervention in order to account for differences in internal individual factors between experiments. Since we used a cross-sectional experimental design, all participants were supposed to participate in all experiments. However, the reduced number of participants in some of the applied protocols (10 out of 11 in ipsiPAS$_{35}$ and ipsiPAS$_{60}$, 8 out of 11 in ipsiPAS$_{75}$ and 6 out of 11 in SICI/ICF and LICI measurements, for details see below) was due to some of those participants being no longer available for experimental testing.

3.3.2 Transcranial Magnetic Stimulation

For TMS, two Magstim Stimulators 200 connected by a BiStim module (Magstim Co., Whitland, Dyfed, UK) were used. We used a standard figure-of-eight coil with 70 mm outer diameter. EMG responses were recorded with Ag-AgCl surface cup electrodes positioned in a tendon-belly configuration and stored on a personal computer. The signal was amplified using an EMG device (D360 8-channel amplifier, Digitimer Ltd, Welwyn Garden City, Hertfordshire, UK) with band pass filtering between 50 and 2000 Hz. The signal was digitized at a frequency of 5000 Hz (CED Power 1401. Cambridge Electronic Design, Cambridge, UK) and fed off-line to a data acquisition system (Signal Version 4.02 for Windows, Cambridge Electronic Design, Cambridge, UK and NuCursor software, J. Rothwell, Institute of Neurology, University College of London, UK). Trials with background EMG activity were discarded from further analysis.

Subjects were seated in a comfortable chair in which the height was individually adjusted to place each subject feet and arms in the most relaxed way. In order to ensure optimal hand and elbow relaxation while sitting, a pillow was placed underneath each subject’s arm. The APB hotspot was identified at the optimal site for eliciting maximal and most consistent thumb abduction-like responses at minimum stimulator output, with the coil held tangentially to the scalp approximately 45° away from midline and then adjusted for each subject in order to reach the best individual coil orientation for the induction of an anterior-posterior (AP) current in the underlying cortical tissue. The APB hotspot was marked and tracked with a neuronavigation system (Brainsight 2, Rogue Research Inc., Montreal Quebec, Canada) to ensure same site of stimulation over the experiment. The tracked APB hotspot for each subject in a given session was then used
as reference hotspot for the following session and adjusted (if needed) to account for potential differences in landmark settings. The resting motor threshold (RMT) over the APB hotspot, defined as the minimum magnetic stimulator output intensity that can elicit a MEP of at least 50 µV in 5 out of 10 trials [75, 31], was measured along with 10 motor evoked potentials (MEPs) of approximately 0.5 to 1 mV peak-to-peak as cortical excitability baseline (pre) in APB.

### 3.3.3 Paired Associative Stimulation protocols

Each PAS protocol consisted of 120 peripheral stimuli applied over the median nerve (right or left wrist) followed by single-pulse TMS over the left APB hotspot at a frequency of 0.20 Hz for a total duration of 10 minutes. An electrical stimulator (Digitimer DS7, Digitimer Ltd., Welwyn Garden City, Hertfordshire, UK) was used for median nerve stimulation (MNS, cathode proximal). The electrical stimulus (ES) had a duration of 200 µs and a maximum voltage of 400 V. The stimulation intensity was adjusted in each subject to elicit a slight thumb abduction of approximately 1 mV peak-to-peak, which was near three times the perceptual threshold [76]. EMG co-registration of the APB and FDI muscles (innervated by median and ulnar nerve, respectively) was performed in order to control for the topographic specificity of each PAS intervention and to ensure the correct placement of the electrical stimulation electrode over the median nerve. MEP responses elicited by both MNS and TMS were recorded for off-line analysis. Two interstimulus intervals (ISIs) between MNS and TMS were tested in all subjects (n = 11): 25 ms (PAS\(_{25}\) and ipsiPAS\(_{25}\)) and 45 ms (PAS\(_{45}\) and ipsiPAS\(_{45}\)), while ISIs of 35 ms and 60 ms were tested in 10 out of the 11 subjects (ipsiPAS\(_{35}\) and ipsiPAS\(_{60}\)) and 75 ms in 8 out of the 11 subjects (ipsiPAS\(_{75}\)). PAS\(_{25}\) corresponded to the settings first described by Stefan et al. [17, 77], considering an average latency of 20 ms for the peripheral signal to reach the contralateral S1 and an average of 5 ms to reach the M1 on the same hemisphere through S1-M1 cortico-cortical connections [78]. For a schematic view of simplified pathways and effective neurophysiological timings see Fig 3.2.

The rationale for using a 45 ms ISI for ipsiPAS\(_{45}\) was motivated by a previous study from our group [70] where we showed that a peripheral conditioning stimulus reaching the right S1 after approximately 20 ms attenuates the early cortical N20 response in the contralateral S1 20 to 25 ms later. Since S1 and M1 within one hemisphere are interconnected via direct cortico-cortical pathways, it might take another 2 to 5 ms [78] for the signal from S1 to reach M1. Based on these findings, we hypothesized that a critical time window ranging around 45 ms might result in LTD-like plasticity in the left M1 due to a significant down-regulation of the ipsilateral S1, which in turn might lead to a down-regulation of the left M1 during intervention. In summary, the only difference with respect to normal PAS\(_{25}\) was the ISI and the MNS site (left hand instead of right hand,
The effects of each PAS intervention on corticospinal excitability within left M1 were studied using 1 mV MEP and RMT recordings at two different time points: immediately after (0-5 min; post1) and 15-20 minutes (post2) after intervention and were compared to baseline (pre) (see Fig. 1.2 for an schematic view of an average PAS intervention). During each PAS intervention, subjects were asked to focus attention on the stimulated hand while counting the number of electrical stimuli applied to the median nerve \[51\]. In order to ensure attention maintenance, we asked the subjects in three random time points about the amount of stimuli received along the intervention.

### 3.3.4 Control PAS protocols

Five additional control PAS interventions were performed in order to disentangle the time window of effectiveness of LTP- and LTD-like PAS and ipsiPAS after-effects: PAS\textsubscript{45}, ipsiPAS\textsubscript{25}, ipsiPAS\textsubscript{35}, ipsiPAS\textsubscript{60} and ipsiPAS\textsubscript{75}. In the case of PAS\textsubscript{45}, the MNS was applied to the right hand 45 ms before TMS was applied over left M1 (arrival asynchrony of around +20 ms). In accordance with previous studies \[36\], we hypothesized that this intervention would not result in any significant changes in corticospinal excitability within left M1. The same would apply for ipsiPAS\textsubscript{25}, since the ISI of 25 ms between the MNS applied to the left hand and the TMS pulse over ipsilateral M1 would be, according to Ragert et al. \[70\],

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**Figure 3.2:** Schematic pathways (arrows) and timing intervals for PAS\textsubscript{25}. Red circles indicate activation of the respective brain area. The hand receiving MNS is depicted in grey. The TMS pulse is represented by the red thunder symbol. Please note that schematic pathways are not intended to represent exact putative pathways but rather effective timing-constrained interactions. M1: primary motor cortex, S1: primary somatosensory cortex. LH: left hand, RH: right hand.
too short for interhemispheric inhibition between S1 cortices (arrival asynchrony of -20 ms). Furthermore, we wanted to test the possibility of an afferent signal transfer directly through interhemispheric M1-M1 inhibitory interaction, which has been shown to occur with a latency of around 10 ms between bihemispheric TMS pulses [25]. The assumed pathway for this protocol would result in a timing of 25 ms for the afferent stimulus to reach contralateral M1 plus 10 ms to reach ipsilateral M1 through interhemispheric interactions (ipsiPAS\textsubscript{45}, synchronous when considering M1-M1 interhemispheric pathway, -10 ms asynchronous when considering S1-S1 interhemispheric pathway, see Fig. 3.4). Due to the bidirectional potential for plasticity induction in standard PAS protocols (near-synchronous PAS\textsubscript{25} leads to LTP-like effects, asynchronous PAS\textsubscript{10} leads to LTD-like effects [36]), we wanted to test the possibility of inducing a significant corticospinal change in M1 with our new ipsiPAS protocol within the assumption of an asynchronous arrival of signals to our target cortical area (left APB motor hotspot). Since the timing of our near-synchronous ipsiPAS intervention (ipsiPAS\textsubscript{45}) was chosen in agreement with the standard PAS timing-dependency (near-synchronous arrival of signals to M1), we selected a target asynchronous timing that fitted the time-window described for standard PAS (15 ms difference PAS\textsubscript{25} and PAS\textsubscript{10}; ipsiPAS\textsubscript{45} (near-synchronous) + 15 ms: 60 ms (asynchronous ipsiPAS\textsubscript{60})). (For an schematic view of the timing-constraint pathways assumed for ipsiPAS\textsubscript{35} and ipsiPAS\textsubscript{60}, see Fig 3.4).

Moreover, as previously found for standard PAS\textsubscript{10}, ipsiPAS\textsubscript{25} could be considered
Figure 3.4: Schematic pathways (and consecutive timings) assumed for the ipsiPAS$_{35}$ and ipsiPAS$_{60}$ interventions. Please note that for ipsiPAS$_{60}$ the assumed pathway is the same as in ipsiPAS$_{45}$ with the only difference of the TMS - MNS interval.

as an asynchronous ipsiPAS intervention that lies in a similar time window of “effective asynchronicity” as that of standard PAS$_{10}$ (see Fig 3.6 for an schematic view of the TDP window), with the potential to induce either LTP-like or LTD-like after-effects. Finally, in order to count with a control condition outside of the presumably effective asynchronous time window for ipsiPAS (ipsiPAS$_{60}$, +15 ms asynchronous), we examined ipsiPAS$_{75}$, where the MNS and TMS arrival to the left M1 are around 30 ms apart. For all five interventions (PAS$_{45}$, ipsiPAS$_{25}$, ipsiPAS$_{35}$, ipsiPAS$_{60}$ and ipsiPAS$_{75}$) the procedures were comparable to PAS$_{25}$ and ipsiPAS$_{45}$ except for the respective ISI chosen.

3.3.5 Intracortical inhibitory and facilitatory neurophysiological mechanisms:
double-pulse TMS protocols

Since GABAergic mechanisms have been reported to be a key underlying factor in the induction of associative plasticity by standard contralateral PAS [79, 39], we decided to examine both GABA$_A$-mediated as well as GABA$_B$-mediated inhibitory mechanisms with double-pulse TMS protocols. Cortical inhibitory systems, through which cortical output is attenuated by GABA-receptor-mediated interneuron neurotransmission, can be indirectly assessed with TMS protocols such as short-interval intracortical inhibition (SICI; partially GABA$_A$-mediated [80, 81]) and long-interval intracortical inhibition (LICI; partially GABA$_B$-mediated [82, 83, 84]). Finally, we tested intracortical facilitation (ICF), which is considered to be possibly, although not exclusively, related to glutamatergic ex-
citatory circuits [85, 29]. We measured intracortical facilitation and inhibition at baseline before an ipsiPAS\textsubscript{45} intervention.

### 3.3.5.1 Short-interval intracortical inhibition (SICI) intracortical facilitation (ICF)

SICI and ICF were carried out according to the double-pulse paradigm first described by Kujirai et al. [24]. We used a subthreshold conditioning magnetic stimulus (CS) set at 80\% of RMT intensity, which was applied over APB motor hotspot 2 (SICI) and 10 ms (ICF) prior to the TS. Subjects received in a random order either the TS alone, or CS-TS for a total of 10 trials per ISI and 10 trials for TS alone. ISI 2 ms was chosen for SICI because intracortical inhibition has been reported to be maximal at ISIs from 2 to 5 ms. The ISI of 10 ms was chosen for ICF because intracortical facilitation is supposed to be maximal at ISIs of 10 to 15 ms [24]. SICI and ICF measurements were performed at baseline before ipsiPAS\textsubscript{45} intervention.

### 3.3.5.2 Long-interval intracortical inhibition (LICI)

LICI was measured applying a suprathreshold CS set at 110\% of RMT intensity over APB motor hotspot 150 ms prior to a TS [22], since intracortical inhibition has been reported to occur at ISIs ranging from 50 to 200 ms. Subjects received in a random order either the TS alone or CS-TS for a total of 10 trials per ISI and 10 trials for TS alone. LICI measurements were performed at baseline before ipsiPAS\textsubscript{45} intervention.

### 3.3.6 Online effects of MNS on ipsilateral M1 (ipsilateral SAI)

To directly investigate the online effects that the afferent stimulation has over the ipsilateral M1 MEP response, we designed a short-latency afferent inhibition (SAI) protocol [23] with an ipsilateral setup (MNS: left hand; TMS: left hemisphere (APB hotspot); ipsi-SAI). The protocol was performed with similar parameters as the ones used for ipsi-PAS interventions. An electrical stimulus was applied over the left median nerve (cathode proximal; ES of 200 ms duration and maximum voltage of 400 V) with a stimulation intensity adjusted in each subject to elicit a slight thumb abduction of approximately 1 mV peak-to-peak, which was approximately three times the perceptual threshold. Even though our target ISI in ipsi-PAS is 45 ms, a set of 3 different ISIs was included in order to account for potential interindividual differences in N20, S1-S1 IHI and S1-M1 interaction latencies, which are important for online measures such as SAI but do not play a relevant role in plasticity-inducing protocols [17]. Since synchronicity of inputs (MNS
and TMS) within the ipsilateral M1 can occur at ISIs as low as around 40 ms and as high as around 50 ms (latency for the MNS to excite contralateral S1 (around 20 ms), latency for the S1-S1 interhemispheric inhibition (between 20 and 25 ms) and latency for S1-M1 cortico-cortical interaction (between 2 and 5 ms)), all 42, 45 and 47 ms were considered near-synchronous ISIs. The TMS intensity for the TS was set to induce a MEP of the right APB muscle of around 0.5 – 1.0 mV amplitude when given alone. A total number of 50 paired CS-TS pulses (10 CS-TS pairs per ISI) and 10 TS alone were randomly applied at a frequency of 0.25 Hz.

### 3.3.7 Statistical analysis

Data analysis was performed using PASW for Windows version 18. Normal distribution of the data was assessed by Shapiro-Wilk tests (p > 0.05 indicating normal distribution of the data). When data was normally distributed, parametric tests (repeated measures ANOVAs and paired-samples t-tests) were used. When normal distribution of the data was not met, non-parametric tests (Spearman’s correlation) were used. An overall RMANOVA factor TIME x INTERVENTION (α = 0.05) was performed in order to explore general differences between interventions both for the target muscle (APB; 3 time points (pre, post1 and post2), 7 interventions) and for the control muscle (FDI; 3 time points (pre, post1 and post2), 7 interventions) [86]. RMANOVAs with factor TIME (α = 0.05; pre, post1 and post2) were used to study the effect of each PAS intervention (PAS\(_{25}\), ipsiPAS\(_{45}\), ipsiPAS\(_{35}\), PAS\(_{45}\), ipsiPAS\(_{25}\), ipsiPAS\(_{60}\) and ipsiPAS\(_{75}\)) separately. Subsequently, pairwise comparisons between time points (pre vs. post1 and pre vs. post2) using paired-samples t-tests were performed and Bonferroni corrected for multiple comparisons (since we performed two comparisons, a p-value of p < 0.025 was considered statistically significant) [87].

A RMANOVA with factor TIME (pre, post1 and post2) and PROTOCOL (PAS\(_{25}\), ipsiPAS\(_{45}\)) was performed to identify potential differences between each target protocols at all time points tested. Changes in RMT, maximum stimulator output (MSO) to elicit 1 mV peak-to-peak MEPs and MNS intensities within and between each intervention were analysed with paired-samples t-tests and univariate ANOVAs with factor PROTOCOL. Additionally, we performed correlations between baseline levels of GABA\(_A\) and GABA\(_B\)-mediated inhibition as well as glutamate-mediated facilitation (% of inhibition or facilitation) and ipsiPAS\(_{45}\) MEP changes on the same day (normalized to baseline; post1 and post2). The ipsi-SAI was analysed with normalized-to-test data (% of MEP change of each CS-TS condition with respect to the TS) using one-sample t-tests (test value set to 100; a Bonferroni corrected p < 0.0167 value was considered significant). Finally, we compared levels of attention (A), fatigue (F) and discomfort (D) before and after each PAS session separately using paired-samples t-tests (pre vs post; Bonferroni corrected p < 0.0167),
and between interventions using a univariate ANOVA. If necessary, a Greenhouse-Geisser sphericity correction was performed for the respective statistical analysis. Data of significantly effective interventions are presented as percentage of increase / decrease of the MEP amplitude after intervention with respect to baseline (mean ± s.d.m.).

3.4 Results

All subjects tolerated the interventions without reporting any unexpected discomfort and there were no adverse events during the study procedures. No significant differences for RMT, intensity to evoke \textit{InV}_{\text{MEP}} (MSO) and MNS intensities were found at any time point (pre/post, paired samples t-tests) or intervention (PAS$_{25}$, PAS$_{45}$, ipsiPAS$_{25}$, ipsiPAS$_{45}$, ipsiPAS$_{60}$, ipsiPAS$_{75}$, univariate ANOVA with factor PROTOCOL) comparison (p > 0.05 for all comparisons). A trend for the RMT at post1 to be significantly different from RMT-baseline was found in PAS$_{25}$ that, however, failed to reach significance after multiple comparisons correction (p = 0.032; pre/post comparison). Furthermore, no significant differences were found in the VAS measuring attention, fatigue and discomfort between interventions (RMANOVAs with factor ATTENTION, FATIGUE or DISCOMFORT pre vs. post; p > 0.05), although significant intra-protocol differences were found for fatigue in ipsiPAS$_{60}$ (p = 0.013) and ipsiPAS$_{35}$ (p = 0.008). However, the change in pre/post fatigue in these two interventions did not correlate with after-intervention MEP changes (Pearson’s correlation; p > 0.05 in both comparisons). For detailed group data see Tables 3.1 and 3.2.

An overall RMANOVA with factors TIME (APB MEP size; pre, post1-2) x INTERVENTION (PAS$_{25}$, PAS$_{45}$, ipsiPAS$_{25}$, ipsiPAS$_{45}$, ipsiPAS$_{35}$, ipsiPAS$_{60}$, ipsiPAS$_{75}$) revealed a significant TIME x INTERVENTION interaction (F(12,84) = 2.249; p = 0.016), indicating excitability changes in M1 that were dependent on the respective PAS intervention in the stimulated APB muscle. No such interaction was found for the FDI control muscle (F(12,60) = 0.187; p = 0.160).

3.4.1 Experiment 1

3.4.1.1 Facilitatory effect of standard PAS (PAS$_{25}$)

RMANOVA with factor TIME (pre, post1-2) revealed a significant effect within left-contralateral M1 (n = 11; RMANOVA factor TIME; F(2,18) = 4.339; p = 0.029) indicating a change in corticospinal excitability in at least one time point tested. Post-hoc comparisons revealed a significant increase in corticospinal excitability (indicated by an
Table 3.1: Mean resting motor threshold (RMT) intensities for each PAS protocol (PAS$_{25}$, PAS$_{45}$, ipsiPAS$_{35}$, ipsiPAS$_{25}$, ipsiPAS$_{45}$, ipsiPAS$_{60}$ and ipsiPAS$_{75}$) on each time point (pre indicating baseline, post1-2). Results are expressed as average maximum stimulator output (MSO) ± standard error.

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<tr>
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<th>pre</th>
<th>post1</th>
<th>post2</th>
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<tbody>
<tr>
<td>PAS$_{25}$</td>
<td>42.33 ± 1.60</td>
<td>40.77 ± 1.33</td>
<td>41.62 ± 1.65</td>
</tr>
<tr>
<td>PAS$_{45}$</td>
<td>40.70 ± 1.61</td>
<td>40.20 ± 1.80</td>
<td>40.50 ± 1.83</td>
</tr>
<tr>
<td>ipsiPAS$_{25}$</td>
<td>39.70 ± 1.59</td>
<td>39.90 ± 1.46</td>
<td>40.66 ± 1.95</td>
</tr>
<tr>
<td>ipsiPAS$_{35}$</td>
<td>41.70 ± 1.76</td>
<td>41.80 ± 1.94</td>
<td>41.90 ± 1.92</td>
</tr>
<tr>
<td>ipsiPAS$_{45}$</td>
<td>39.45 ± 1.18</td>
<td>39.90 ± 1.13</td>
<td>39.90 ± 1.20</td>
</tr>
<tr>
<td>ipsiPAS$_{60}$</td>
<td>40.50 ± 1.61</td>
<td>40.08 ± 1.58</td>
<td>40.50 ± 1.58</td>
</tr>
<tr>
<td>ipsiPAS$_{75}$</td>
<td>39.25 ± 1.14</td>
<td>38.75 ± 1.20</td>
<td>39.62 ± 0.92</td>
</tr>
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</table>

increase in the size of the MEP amplitude) relative to baseline for post1 (immediately after intervention; 53.64 ± 39.26 % MEP increase; p = 0.003) while significant differences were no longer observed at post2 (15-20 min after intervention; 28.26 ± 47.22 % MEP increase; p = 0.165) (see Fig 3.5 and Table 3.3). These results indicate a transient but significant increase in corticospinal excitability within left-contralateral M1 immediately after PAS$_{25}$ intervention that recovered to baseline conditions 15 min after the termination of PAS$_{25}$.

3.4.1.2 Interhemispheric inhibitory effect of near-synchronous ipsilateral PAS (ipsiPAS$_{45}$)

In contrast to PAS$_{25}$, there was a significant decrease in corticospinal excitability within left-ipsilateral M1 after application of ipsiPAS$_{45}$ (n = 11; RMANOVA factor TIME; F(2,18) = 4.508; p = 0.026). Post hoc comparisons revealed a significant decrease in corticospinal excitability within left-ipsilateral M1 both at post1 (25.91 ± 23.68 % decrease in MEP size; p = 0.017) and post2 (26.22 ± 26.91 % decrease in MEP size; p = 0.016), indicating that ipsiPAS$_{45}$ induced a reduction in the ipsilateral M1 corticospinal excitability that outlasted the intervention by at least 15 min (see Fig 3.5 and Table 3.3).

3.4.1.3 Differential effects between PAS$_{25}$ and ipsiPAS$_{45}$

RMANOVA revealed a significant TIME x INTERVENTION interaction (F(2,20) = 9.310; p = 0.001), indicating that PAS$_{25}$ and ipsiPAS$_{45}$ resulted in differential corticospinal excitability changes (LTP-like effect for PAS$_{25}$; LTD-like effect for ipsiPAS$_{45}$) within left M1. Post hoc comparisons between post1 and post2 measures revealed a difference between PAS$_{25}$ and ipsiPAS$_{45}$ both at post1 (p < 0.001) and post2 (p = 0.005).
Figure 3.5: Corticospinal excitability changes within left M1 for the PAS_{25} and ipsiPAS_{45} interventions. Changes are expressed as % change in 1mV MEP size relative to baseline (pre). Significant differences between interventions are indicated by an asterisk. (see Fig 3.5). In contrast, baseline 1mV\textsubscript{MEP} measurements were not significantly different between protocols (baseline p = 0.062, non-significant trend).

3.4.1.4 Topographical specificity of PAS\(_{25}\) and ipsiPAS\(_{45}\)

In 9 out of 11 subjects, we additionally recorded corticospinal excitability changes for the FDI muscle within left-contralateral M1 after PAS\(_{25}\) and left-ipsilateral M1 after ipsiPAS\(_{45}\) in order to study the topographical specificity of PAS-induced excitability changes. No significant differences in MEP amplitudes were observed after application of either PAS\(_{25}\) (RMANOVA factor TIME; F(2,16) = 0.835; p = 0.452) or ipsiPAS\(_{45}\) (RMANOVA factor TIME; F(2,16) = 0.572; p = 0.575, see Table 3.3). These results confirm and support the general view about a topographically restricted PAS effect for the conventional PAS\(_{25}\) protocol [76, 17] and extend this finding by showing a similar outcome for ipsiPAS\(_{45}\).
Table 3.2: Mean maximum stimulator output (MSO [%]) intensities used to elicit a 1mV peak-to-peak MEP in the APB muscle of the left M1 and intensities used for median nerve stimulation (MNS, [mA]) for each PAS protocol (PAS\textsubscript{25}, PAS\textsubscript{45}, ipsiPAS\textsubscript{25}, ipsiPAS\textsubscript{35}, ipsiPAS\textsubscript{45}, ipsiPAS\textsubscript{60} and ipsiPAS\textsubscript{75}) ± standard error.

<table>
<thead>
<tr>
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<th>1mV MEP MSO [%]</th>
<th>MNS [mA]</th>
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<tbody>
<tr>
<td>PAS\textsubscript{25}</td>
<td>52.63 ± 2.45</td>
<td>7.00 ± 1.19</td>
</tr>
<tr>
<td>PAS\textsubscript{45}</td>
<td>49.10 ± 1.64</td>
<td>6.16 ± 0.58</td>
</tr>
<tr>
<td>ipsiPAS\textsubscript{25}</td>
<td>49.60 ± 1.57</td>
<td>6.68 ± 0.48</td>
</tr>
<tr>
<td>ipsiPAS\textsubscript{35}</td>
<td>49.40 ± 2.21</td>
<td>6.19 ± 0.43</td>
</tr>
<tr>
<td>ipsiPAS\textsubscript{45}</td>
<td>48.63 ± 1.42</td>
<td>6.61 ± 0.49</td>
</tr>
<tr>
<td>ipsiPAS\textsubscript{60}</td>
<td>48.30 ± 2.15</td>
<td>6.13 ± 0.26</td>
</tr>
<tr>
<td>ipsiPAS\textsubscript{75}</td>
<td>46.75 ± 1.68</td>
<td>6.05 ± 0.54</td>
</tr>
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3.4.2 Experiment 2

3.4.2.1 Control PAS protocols did not change corticospinal excitability after intervention

RMANOVAs revealed no significant differences in corticospinal excitability for PAS\textsubscript{45}, ipsiPAS\textsubscript{25}, ipsiPAS\textsubscript{35} and ipsiPAS\textsubscript{75} within left M1 for any time points (pre, post1-2) tested (PAS\textsubscript{45} F(2,20) = 0.063, p = 0.940; ipsiPAS\textsubscript{25} F(2,20) = 0.257, p = 0.776; ipsiPAS\textsubscript{35} F(2,18) = 0.015, p = 0.985; ipsiPAS\textsubscript{75} F(2,14) = 1.956, p = 0.178; see Table 3.3). Closer inspection of the ipsiPAS\textsubscript{75} after-intervention MEP measures revealed no significant differences between either time point (post1-2) when compared to baseline (p = 0.919 and p = 0.069 (non-significant trend), respectively). These results indicate the existence of a constraint time-window for the induction of plasticity in both standard PAS (as previously found in [17] and [36]) and interhemispheric-mediated ipsiPAS (see Fig 3.6).

3.4.2.2 Interhemispheric facilitatory effects of asynchronous ipsilateral PAS intervention

Even though the RMANOVA did not result in a significant effect of time (pre – post1, post2) for the ipsiPAS\textsubscript{60} intervention (n = 10; F(2,18) = 2.320, p = 0.127), a closer inspection of the data revealed a significant increase in MEP amplitudes relative to baseline immediately after the intervention (32.26 ± 36.35 % increase in MEP size; post1: p = 0.016) that was no longer present 15 to 20 min after (23.76 ± 62.48 % increase in MEP size; post2: p = 0.188). These results indicate a tendency for an asynchronous ipsilateral PAS intervention (ipsiPAS\textsubscript{60}) to induce a reversed effect when compared to a standard asynchronous PAS\textsubscript{10} intervention [36] that however failed to reach significance possibly due to lack of power (see Fig 3.7) or to a potential reduced effectiveness of an ipsiPAS intervention to induce LTP-like effects. Such an increase in MEP size after ipsiPAS\textsubscript{60}
Figure 3.6: Summary of experimental findings. Mean corticospinal excitability changes within left M1 at post1 (expressed as percentage change relative to baseline (pre)) for each intervention. X-axis represents the assumed timing interval of asynchronicity [ms] between MNS and TMS pulse (either TMS before MNS, MNS before TMS or synchronous) arriving to M1. Please note that for ipsiPAS_{45} synchronicity of inputs resulted in a reversed effect as compared to PAS_{25}, indicating the existence of anti-Hebbian like plasticity in healthy volunteers. The data for PAS_{10} is taken out of a study by Wolters et al. 2003 just for display purpose. Significant effects for PAS (PAS_{25} and PAS_{10}) and ipsiPAS (ipsiPAS_{45} and ipsiPAS_{60}) interventions are displayed in bold.
### Table 3.3: Normalized MEP values [%] relative to baseline (pre) for the right APB and FDI muscle for all time points (post1: 0-5 minutes after intervention, post2: 15-20 minutes after intervention) and PAS protocols tested (PAS25, PAS45, ipsiPAS25, ipsiPAS35, ipsiPAS45, ipsiPAS60 and ipsiPAS75). Values are displayed as mean MEP values ± standard error. Asterisks represent significant differences relative to baseline (significance level p < 0.025, corrected for multiple comparisons). Significant results are displayed with italic bold font.

<table>
<thead>
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<th>post1</th>
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<tr>
<td><strong>APB</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAS25</td>
<td>153.64 ± 11.83*</td>
<td>128.26 ± 14.23</td>
</tr>
<tr>
<td>PAS45</td>
<td>105.13 ± 10.26</td>
<td>99.45 ± 12.19</td>
</tr>
<tr>
<td>ipsiPAS25</td>
<td>93.10 ± 6.81</td>
<td>99.84 ± 12.09</td>
</tr>
<tr>
<td>ipsiPAS35</td>
<td>105.23 ± 14.81</td>
<td>103.96 ± 9.80</td>
</tr>
<tr>
<td>ipsiPAS45</td>
<td>74.09 ± 7.14*</td>
<td>73.77 ± 8.11*</td>
</tr>
<tr>
<td>ipsiPAS60</td>
<td>132.26 ± 11.49*</td>
<td>123.76 ± 19.75</td>
</tr>
<tr>
<td>ipsiPAS75</td>
<td>97.15 ± 12.04</td>
<td>81.87 ± 8.50</td>
</tr>
<tr>
<td><strong>FDI</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAS25</td>
<td>131.46 ± 14.64</td>
<td>122.77 ± 22.60</td>
</tr>
<tr>
<td>PAS45</td>
<td>112.49 ± 9.38</td>
<td>112.95 ± 9.90</td>
</tr>
<tr>
<td>ipsiPAS25</td>
<td>124.10 ± 10.68</td>
<td>125.68 ± 17.03</td>
</tr>
<tr>
<td>ipsiPAS35</td>
<td>94.82 ± 8.73</td>
<td>116.45 ± 10.80</td>
</tr>
<tr>
<td>ipsiPAS45</td>
<td>108.82 ± 12.48</td>
<td>105.03 ± 19.65</td>
</tr>
<tr>
<td>ipsiPAS60</td>
<td>115.00 ± 17.55</td>
<td>96.69 ± 27.65</td>
</tr>
<tr>
<td>ipsiPAS75</td>
<td>122.80 ± 9.92</td>
<td>106.37 ± 9.74</td>
</tr>
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</table>

intervention could not be shown in the FDI control muscle (n = 10; F(1.244, 11.193) = 1.498, p = 0.254, Greenhouse-Geisser corrected). Moreover, ipsiPAS60 was significantly different from ipsiPAS45 at time point post1 (paired samples t-test, p = 0.001), while post2 no longer showed significant differences between interventions (p = 0.072) (see Fig 3.7).

### 3.4.3 Experiment 3

#### 3.4.3.1 Correlation between baseline SICI, ICF, LICI and ipsiPAS45 after-effects

Here we wanted to test whether baseline inhibition (GABA<sub>A</sub> and GABA<sub>B</sub>-mediated) and facilitation (possibly glutamate-mediated) in left M1 (for a detailed description see experimental procedures) predicts the amount of ipsiPAS45-induced decrease in corticospinal excitability.

The ipsiPAS45 intervention resulted in significant decreases in MEP amplitude at time point post1 (37.85 ± 18.59 % decrease in MEP size; p = 0.002 (one-tailed), while significant differences could no longer be addressed at time point post2 (23.41 ± 39.28 % decrease in MEP size; p = 0.098 (non-significant trend).
Figure 3.7: Corticospinal excitability changes within left M1 for the ipsiPAS\textsubscript{45} as compared to the ipsiPAS\textsubscript{50} intervention.

At baseline there was a significant amount of GABA\textsubscript{A}-mediated (72.92 ± 11.16 % (mean ± s.e.m.); p = 0.004, one-tailed t-test) and GABA\textsubscript{B}-mediated (68.46 ± 13.18 %; p = 0.002, one-tailed t-test) inhibition as well as glutamate-mediated facilitation (83.80 ± 36.29 %; p = 0.036, one-tailed t-test).

Figure 3.8: Correlation between the amount of baseline GABA\textsubscript{B}-mediated inhibition (LICI) and decrease in 1 mV MEP size after ipsiPAS\textsubscript{45} (post 1).

Interestingly, a positive linear correlation analysis (rho = 0.943, p = 0.005) revealed that baseline GABA\textsubscript{B}-mediated but not GABA\textsubscript{A}-mediated inhibition (rho = 0.257, p = 0.623) influences ipsiPAS\textsubscript{45} after-effects at post1 (see Fig 3.8).

These results indicate that specific inhibitory circuits in M1 (as shown already for
standard PAS [39]) associated with GABA_B receptor activation might play an important role in associative plasticity in the living human brain. Furthermore, no such correlation could be observed for glutamate-mediated facilitation in M1 (rho = 0.029, p = 0.957). No such correlations could be observed for GABA_A- and GABA_B-mediated as well as for glutamate-mediated facilitation at post2 (p > 0.05 for all cases).

### 3.4.4 Experiment 4

#### 3.4.4.1 Online effects of ipsi-SAI

The normalized-to-test data (% of MEP change with respect to the TS) was normally distributed (Shapiro-Wilk p > 0.05 in all CS-TS conditions). Significant MEP changes were found at 47 (p = 0.002) ms ISI only, while no significant changes were observed at 42 and 45 ms ISIs (p = 0.405 and p = 0.300, respectively). More specifically, there was a significant inhibitory effect of the CS over the TS at the ISI of 47 ms (22.58 ± 21.04 % MEP change). It is noteworthy to point out that there was a rather high intersubject variability considering the ISIs that resulted in MEP inhibition at an individual level (number of participants that showed at least 5 % of MEP inhibition out of the total number of participants; 42 ms: 5 out of 13; 45 ms: 6 out of 13; 47 ms: 10 out of 13), with some participants showing no online effect or even facilitation at some of these intervals (see Fig 3.9).

![Figure 3.9](attachment:image.png)

**Figure 3.9:** Normalized-to-Test individual data of MEP percentage changes at each CS - TS interval tested (42, 45 and 47 ms ISI). Please note that only the ISI 47 (CS - TS) resulted in a significant overall change in the MEP amplitude with respect to the TS (Test).

Finally, the intensity to evoke a MEP response of 0.5 – 1.0 mV amplitude was 54
± 10.51 of m.s.o. (mean ± s.d.m.) and the intensity for MNS was 6.46 ± 2.58 mA (mean ± s.d.m.). No significant differences were found in attention, tiredness and discomfort before and after the ipsi-SAI protocol (p > 0.05 in all cases).

3.5 Discussion

In the present study, we provide novel evidence for reversed Hebbian-like plasticity (anti-Hebbian-like) in humans. More specifically, we show that inhibition of the sensorimotor network during associative stimulation reverses the outcome of an STDP-like protocol (LTD-like) in an anti-Hebbian-like fashion. Furthermore, we report that reversed associative plasticity critically depends on the timing interval between afferent and cortical stimulation.

IpsiPAS\textsubscript{45} over left-ipsilateral M1 resulted in a transient decrease in MEPs of the target (left APB) muscle immediately after termination of the intervention while PAS\textsubscript{25} increased corticospinal excitability. This differential effect recovered to baseline conditions approximately 15 minutes after termination of the standard PAS\textsubscript{25} intervention while persisted in the new ipsiPAS\textsubscript{45}. No such changes were observed for the FDI muscle of the left hand, confirming the topographic specificity of PAS-induced excitability changes in both interventions [17]. Based on these findings, we provide novel evidence that synchronicity of inputs in M1 during PAS does not exclusively lead to LTP-like effects. Instead, the synchronous arrival of inputs in M1 might induce either LTP- or LTD-like effects probably depending on the functional state of the sensorimotor network (up- or down-regulated) during the pairing of inputs (MNS and TMS). Moreover, we found a trend towards facilitation of the MEP amplitude after the application of an asynchronous ipsiPAS protocol (ipsiPAS\textsubscript{60}), which would resemble, in a reversed fashion, the effect of a standard asynchronous PAS protocol (PAS\textsubscript{10}).

3.5.1 Down-regulation of ipsilateral left SM1 during median nerve stimulation at the wrist

Apart from a contralateral activation of the SM1 after unilateral median nerve or tactile stimulation, a deactivation of the ipsilateral SM1 has been consistently reported in the neuroimaging literature. However, until now it still remains elusive whether the observed deactivation, inferred via a negative blood-oxygen-level-dependent (BOLD) response, underlies synaptic inhibition or whether this deactivation implies the existence of functional inhibition [88]. In our study, the hypothesis of M1 being down-regulated by an ipsilateral MNS is mainly based on neuroimaging (fMRI, PET and ASL) studies in humans under-
going similar peripheral stimulation settings, as well as tactile stimulation or voluntary thumb movements [62, 63, 61, 64, 65, 66, 67, 89, 68, 69]. Although most of these studies focused on S1, all of them report a concurrent deactivation of both S1 and M1 ipsilateral to the active hand that is even specific for each activated/deactivated finger [63]. In a recent study by Schäfer et al., additionally, they observed an enhancement of the sensory perceptual threshold of the conditioned, non-stimulated hand that accompanied the negative BOLD response, indicating that such a deactivation, at least in the case of S1 after MNS, is an indication of functional inhibition [68]. Moreover, in a transcranial electrical stimulation study performed by Brocke et al. [90] the authors found that M1-M1 interhemispheric inhibition was accompanied by a net reduction of the BOLD signal in the conditioned M1. In general, such imaging studies have shown that the negative BOLD response in the SM1 ipsilateral to the active hand correlates to neuronal, metabolic and / or vascular down-regulation, supporting the idea that net synaptic inhibition takes place in the deactivated SM1. Whether this holds for all cortical or subcortical areas in the brain is still a matter of current debate [91]. Furthermore, we could show that there was a significant inhibitory effect of the MNS over the ipsilateral M1 at near-synchronous arrival of inputs (47 ms ISI) to M1. This indicates that the MNS induces a neurophysiological observable decrease of the ipsilateral MEP response at an interval when both MNS and TMS inputs are presumed to arrive synchronously. The fact that we could not see any significant group inhibition at 42 and 45 ms ISI could be due to intersubject variability in the latencies taken into account for the MNS to reach the ipsilateral M1. Even though we did not find a significant group inhibition at 45 ms, it is known that exact synchronicity of inputs arriving to M1 is not required to induce a PAS effect [17].

3.5.2 Time specificity of ipsilateral paired associative stimulation

3.5.2.1 Near-synchronous interventions

Since we apply MNS above the motor threshold, we assume that there is an activation of the contralateral SM1 that is accompanied by a down-regulation (disfacilitation or inhibition) on the homologue SM1 (ipsilateral to the stimulated hand) as previously shown in animal and human studies [92, 93, 61, 67, 89, 69]. However, no study addressed so far the intra- and / or interhemispheric timing interval necessary for such an inhibitory communication between SM1 cortices.

The rationale for using a 45 ms ISI for ipsiPAS_{45} was motivated by a previous study from our group [70] where we could show that a peripheral conditioning stimulus reaching the right S1 after approximately 20 ms attenuates the early cortical N20 response in the homologous S1 20 to 25 ms later. This timing fits the reported transcallosal conduction time of around 20 ms (as measured with SEPs) between SM1 cortices reported
by Allison et al. [71]. Since S1 and M1 are interconnected via cortico-cortical pathways, it might take another 2 to 5 ms [78] for the signal to reach the ipsilateral M1 [17]. Based on these findings, we hypothesized that a critical time window ranging around 45 ms (near-synchronous arrival of both MNS and TMS pulse over M1 through corticospinal, subcortical and cortico-cortical pathways) might result in LTD-like plasticity in the left-ipsilateral M1 due to a significant down-regulation of the ipsilateral S1, which in turn might lead to a down-regulation of M1 in the same cortical hemisphere (see Fig 3.2 and Fig 3.3 for a schematic representation of timing of neurophysiological reported effects). This hypothesis was mainly driven by the STDP rules found in inhibitory circuits, where synchronicity of inputs does not always result in LTP changes of the synaptic weight [8] and where the outcome of pairing seems to depend on general network inhibition [94].

However, other potential candidate mechanisms might explain the reduction in corticospinal excitability within left-ipsilateral M1 after ipsiPAS45. For example, it might be reasonable to assume that the LTD-like plasticity in M1 could be induced directly through M1-M1 inhibitory interactions. Nonetheless, this seems unlikely since a control PAS protocol with a timing adjusted to fit M1-M1 interhemispheric inhibition (ipsiPAS35, see Fig 3.4) showed no significant after-effects, probably due to several reasons: interhemispheric inhibition (IHI) between S1 cortices has been shown at a critical timing interval ranging between 20 to 25 ms [70] while for M1 cortices, short-latency IHI is present at 7 to 10 ms ISI [25]. The pathway through which IHI between S1 cortices takes place probably involves Brodmann areas (BAs) 3b and 2 of both hemispheres, with the interhemispheric transfer of information most probably occurring between homologous BAs 2, for which sparse transcallosal fibres have been reported in the macaque [95]. This pathway seems likely since bilateral positive BOLD responses in BA 2 have been previously reported after unilateral hand stimulation [61]. The cortico-cortical interaction between BA 3b and BA 2 before (activated S1) and after (deactivated S1, probably via inhibitory influence of BA 2 over BA 3b) S1-S1 transcallosal communication could thus account for the longer timing interval in comparison to M1-M1 communication. Therefore, in order to induce LTD-like plasticity directly through M1 cortices in a near-synchronous fashion, an ISI ranging between 32 to 35 ms would be necessary to result in the observed after-effects seen for ipsiPAS45 (25 ms for peripheral stimulus to reach contralateral M1, 7 - 10 ms to reach ipsilateral M1, see Fig 3.4). The application of an ipsiPAS35 protocol, however, did not result in any significant change in corticospinal excitability after intervention most probably due to insufficient intensity of the MNS to induce transcallosal inhibition between M1 cortices because IHI between M1 cortices has only been shown with bihemispheric supra-threshold TMS paradigms [25].

As a further control, we applied an ipsiPAS35 intervention. The choice of this timing interval was motivated by the fact that both M1 cortices are presumably co-activated simultaneously during intervention (the MNS reaches the contralateral M1 after around
25 ms, roughly at the same time when the TMS pulse is applied over the ipsilateral M1). Previous studies have shown that a time window for the TMS pulses of 8 to 15 ms between homologous M1 cortices is necessary to elicit paired associative plastic changes \[58, 57\]. Nonetheless, such time window would be too short to account for the effect seen after ipsiPAS\(_{45}\). Moreover, ipsiPAS\(_{25}\) could be considered as well as an asynchronous intervention within the time-window seen for PAS\(_{10}\) (20 ms asynchronous with respect to ipsiPAS\(_{45}\), see Fig 3.6). The application of ipsiPAS\(_{25}\), however, did not result in any significant change of corticospinal excitability within ipsilateral M1. These results highlight that a specific timing between the MNS and TMS pulse (sufficient for interhemispheric communication to take place) seems to be a prerequisite to drive either LTD-like or LTP-like plastic changes within ipsilateral M1. LTD-like effects by pairing bihemispheric M1 magnetic stimulation, furthermore, have not been addressed so far \[58\]. Despite this aforementioned reasoning, we cannot rule out the possibility of a summation of ipsilateral S1-M1 and bihemispheric M1-M1 and S1-S1 interactions that could result in the LTD-like effect seen after ipsiPAS\(_{45}\), since the potential M1-M1 interactions occurring after 35 ms are contained within the 45 ms timing of ipsiPAS\(_{45}\) (see Fig 3.3).

### 3.5.2.2 Asynchronous interventions

In an asynchronous target intervention (ipsiPAS\(_{60}\); MNS before TMS) we observed, in contrast to the standard asynchronous PAS\(_{10}\) intervention, an increase in the MEP amplitude immediately after the intervention that however did not reach significance. The reason for this effect is, however, less clear than that of the LTD-like effect seen after ipsiPAS\(_{45}\), as it is not directly reversed with respect to the standard TDP time-window seen for conventional PAS interventions, but both reversed and shifted (see Fig 3.6). This differential effect could be related to the order of stimuli (TMS before MNS in PAS\(_{10}\), MNS before TMS in ipsiPAS\(_{60}\)).

Finally, we did not observe any significant corticospinal excitability changes within left-contralateral M1 after the PAS\(_{45}\) control intervention. This is in line with previous literature, showing a lack of significant corticospinal excitability changes after PAS with ISIs ranging between 35 and 50 ms \[36\], which highlight the effectiveness of asynchronous interventions only in the “TMS before MNS” side of the window (see Fig 3.6) of the TDP-window for standard PAS. This “TMS before MNS” asynchronicity seems not to be effective in the case of ipsiPAS interventions that lie in a similar frame of the standard PAS TDP-window (ipsiPAS\(_{25}\)). This, in turn, indicates a potentially different underlying mechanism taking place in the asynchronous ipsiPAS interventions (ipsiPAS\(_{60}\) vs. ipsiPAS\(_{25}\)) with respect to the asynchronous PAS (inhibition when TMS precedes MNS (PAS\(_{10}\)), no effect when MNS precedes TMS (PAS\(_{45}\))), probably due to the role of the down-regulation within the network in ipsiPAS interventions.
3.5.3 Timing-dependent plasticity mechanisms

Repetitively pairing of signals in a near synchronous way over the sensorimotor cortex in humans has been linked with LTP-like mechanisms similar to those taking place under Hebbian rules of plasticity in slice preparations \([37, 5]\). On the other hand, in the classic spike-timing dependent plasticity (STDP) experiments, timing of firing between two neurons results in differential effects. More specifically, when the presynaptic neuron excites the postsynaptic neuron and takes part in firing it repeatedly over a prolonged period of time, the synaptic weight between both neurons is strengthened (LTP). Moreover, when the order of spikes is inversed, the synaptic connectivity gets weakened (LTD). However, this timing-dependent directionality of effects has only been found when two excitatory neurons are paired, and shows reversed directionality when inhibitory neurons are paired with excitatory neurons (LTP when the firing of the postsynaptic neuron precedes the firing of the presynaptic neuron, and LTD when the presynaptic neuron fires before the postsynaptic neuron does, termed “anti-Hebbian”).

Non-invasive brain stimulation studies have focused on the synchronicity of inputs arriving to a certain cortical area of interest as an approximation to STDP excitatory-excitatory protocols \(\textit{in vitro}\), considering synchronous and asynchronous arrival of inputs as a critical factor for the induction of cortical plasticity. Therefore, STDP-like protocols constitute an attempt for Hebbian-like synaptic plasticity at the systems level in the living human brain. However, the timing-dependency of PAS-induced plasticity involves the interaction of broader neuronal populations and functionally relevant interconnected brain areas. This feature, in turn, reflects a plasticity mechanism that implies more general processing of information in opposition to the more focal cellular settings in STDP \(\textit{in vitro}\) and \(\textit{in vivo}\) in animal studies. In the present study, we could successfully induce reversed STDP-like plasticity through an interhemispheric pathway sufficient to generate LTD-like after effects.

Interestingly, in relation to the timing-dependency of PAS after-effects, the near-synchronous arrival of inputs over M1 has been linked with LTP-like mechanisms of plasticity, while LTD-like mechanisms are believed to occur when the arrival of inputs is temporally asynchronous \([36]\). Here we provide novel evidence that synchronicity of inputs might not exclusively lead to LTP-like effects as shown for Hebbian-like plasticity. Instead, the synchronous arrival of inputs might also lead to LTD-like effects depending on the internal state of the sensorimotor network (up- or down-regulation), the overall inhibition / disfacilitation taking place within the target cortical areas and the site of the node in the network. Similar findings about this inversed Hebbian plasticity rule have been reported in previous \(\textit{in vitro}\) (excitatory-inhibitory neuronal couplings), animal and computational studies only \([96, 8, 97, 98]\). In these studies, the overall level of inhibition seems to play an important role for the induction of either Hebbian or anti-Hebbian plas-
ticity [94], and it has been linked to adaptive filtering functions in the sensory domain (for reference see [99]).

3.5.4 GABA$_B$-mediated inhibition plays a role in inhibitory effects of ipsiPAS$_{45}$.

Interestingly, the baseline levels of GABA$_B$-mediated inhibition predicted the amount of inhibition induced by ipsiPAS$_{45}$. Those participants with more GABA$_B$-mediated inhibition within M1 before the intervention showed a larger ipsiPAS$_{45}$-induced decrease in M1 corticospinal excitability (see Fig 3.8), indicating that GABA$_B$ receptor activation might be an important underlying mechanism in the development of reversed associative plasticity in the human brain as previously reported for both PAS$_{25}$ (LTP-like effects) and PAS$_{10}$ (LTD-like effects). On the other hand, GABA$_A$-mediated inhibition and glutamatergic facilitation at baseline did not correlate with ipsiPAS$_{45}$ after-effects. GABA$_B$ receptors, due to their slow inhibitory effects, have been associated with the regulation of synaptic plasticity and the modulation of calcium and potassium channels [100], making them a preferential candidate in the regulation of long-term plasticity changes.

3.5.5 Short-term effect of paired associative stimulation protocols

In the original report by Stefan and colleagues [17] 90 pairs of stimuli were delivered at 0.05 Hz over 30 minutes, whereas other PAS studies used even higher stimulus numbers and / or rates [42]. PAS-induced after-effects are typically observed in a range of 30 - 120 minutes depending on the individual settings of the respective PAS protocol [37]. Here we used a modified PAS protocol consisting of 120 pairs of stimuli applied over the APB hotspot within left-contralateral M1 delivered at 0.20 Hz over 10 minutes that has shown to induce significant after-intervention effects in a prior study from our group [73]. In contrast to previous studies, here we showed that the PAS-induced effects on MEPS of the contralateral APB were much shorter and recovered already approximately 15 minutes after termination of PAS$_{25}$. Even though the amount of LTP-like (PAS$_{25}$) changes within left-contralateral M1 was comparable to previous studies [76, 17, 42], one potential explanation for the shorter after-effects may be related to our modified PAS settings [101, 46].

A limitation of the present study is the difficulty to draw an exact characterization of the ipsilateral latencies governing S1-M1 communication. Even though the latency of the MNS to excite contralateral S1 as well as the timing for interhemispheric inhibition to occur between S1s have been sufficiently addressed in previous studies [102, 70], it still remains elusive whether the 2 to 5 ms for S1-M1 communication described by Goldring et al. [78] applies to both the SM1 contralateral and ipsilateral to an active hand. For
example, although highly speculative, the effects seen after ipsiPAS{sub}_45 and ispiPAS{sub}_60 interventions could be explained under Hebbian-like rules of plasticity if we consider the ipsiPAS{sub}_60 intervention to be the near-synchronous one. For this to be true, we would need to consider a S1-M1 interaction taking place with a 20 ms latency. This seems implausible, though, as it constitutes a timing that lays way beyond the reported S1-M1 interaction latency seen contralateral to an active hand and there is no indication at present that the latency of S1-M1 cortico-cortical interaction after interhemispheric transfer of information is substantially longer as compared to S1-M1 interactions without prior interhemispheric communication. Furthermore, the S1-M1 ipsilateral interaction could potentially be even faster than in the homologous contralateral areas since BA 2 of S1 presumably mediates S1-S1 interhemispheric inhibition and directly connects in both hemispheres to BA 4 of M1 [103], probably being responsible for the inhibition seen in M1 after stimulation of the ipsilateral hand while concurrently inhibiting BA 3b of S1.
Chapter 4

Second Study
The role of cortical thickness in the effectiveness of a paired associative stimulation protocol

From the article: “Cortical thickness in primary sensorimotor cortex influences the effectiveness of paired associative stimulation” by Conde, V., Vollmann, H., Sehm, B., Taubert, M., Villringer, A. & Ragert, P. (NeuroImage 2012)

4.1 Abstract

Non-invasive brain stimulation protocols in general and paired associative stimulation (PAS) in particular seem to alter corticospinal excitability and thereby to influence behaviour with a high degree of intersubject variability. The cause of this variability is multidimensional and to some extent still unknown. Here, we tested the hypothesis that individual variations in cortical thickness can explain some of the variability of PAS-induced excitability changes. Ten minutes of a facilitatory PAS protocol (PAS$_{LTP}$) rapidly increased corticospinal excitability in the majority of the subjects (14/19 subjects) while others showed no such effect (5/19 subjects). A whole brain correlation analysis based on high resolution T1-weighted images revealed a significant positive relationship of PAS$_{LTP}$-induced excitability changes with cortical thickness of the underlying left sensorimotor cortex (SM1) only. Cortical thickness alone, among other potential influencing factors, explained about half of the PAS$_{LTP}$ variance, indicating that subjects with a strong after-effect were those with higher cortical thickness values in this region. Based on these findings, we provide novel evidence that local brain structure influences the individual amount of functional plasticity induced by PAS$_{LTP}$. While the underlying neurophysiological and/or anatomical reasons for this effect still remain elusive at this point, we conclude that cortical thickness should be considered as an important and until now not recognized modulating factor in studies employing non-invasive brain stimulation techniques.

4.2 Introduction

Although the majority of healthy subjects show a facilitation of corticospinal excitability after PAS$_{LTP}$, a considerable number of subjects also show no or even a reversed effect (LTD-like) [33]. The cause of this variability is to some extent still unknown. However, potential factors such as age [47], attentional state [51], time of the day in which the intervention is performed [44], intrinsic neuronal activity [104, 105, 106, 107], endophenotype characteristics [108, 101] and genetic factors [50] might play a crucial role. It
still remains elusive whether or not other factors such as individual differences in brain morphology might also partially account for inter-subject variability in response to non-invasive brain stimulation. In the present study we aimed to investigate the relation between PAS\textsubscript{LTP}-induced excitability changes and brain morphology. We hypothesized that individual variations in cortical thickness of motor-related areas influence the after-effects of PAS\textsubscript{LTP}.

### 4.3 Materials and Methods

#### 4.3.1 Subjects

Nineteen right-handed healthy subjects (24.11 ± 3.18 years of age (mean ± stdev.), 12 female) participated in the study. Prior to participation, all subjects underwent a comprehensive neurological examination to screen for exclusion criteria regarding TMS measurements and MRI recordings. Handedness was assessed by the Edinburgh inventory scale (laterality score range: +77 to +100 over a range of -100 (fully left-handed) and +100 (fully right-handed)). 13 subjects had a laterality score of +100 while the remaining subjects (n=6) had a laterality score between +77 and +90. All subjects were PAS naïve but had prior experience with other TMS experiments. Each subject fulfilled the inclusion criteria in agreement with the safety guidelines approved by TMS consensus \cite{74} and gave written informed consent to participate in the experiment according to the declaration of Helsinki. The local ethics committee of Leipzig approved the study. None of the subjects were using centrally acting drugs by the time of the study. Levels of attention, fatigue and discomfort were assessed for each subject before (pre) and after (post) the experiment using a visual analogue scale (VAS).

#### 4.3.2 Transcranial Magnetic Stimulation

Please see 3.3.2 for an overview of the TMS settings used in this study.

#### 4.3.3 Paired Associative Stimulation protocol

The PAS\textsubscript{LTP} protocol consisted of 120 peripheral stimuli applied over the median nerve followed by single-pulse TMS over the APB hotspot in the left hemisphere at a frequency of 0.2 Hz for a total duration of 10 minutes. An electrical stimulator (Digitimer DS7, Digitimer Ltd., Welwyn Garden City, Hertfordshire, UK) was used for median nerve stimulation (MNS, cathode proximal). The electrical stimulus had a duration of 200
and a maximum voltage of 400 V. The stimulation intensity was adjusted in each subject to elicit an APB contraction of approximately 1 mV peak-to-peak (resulting in a slight thumb abduction) which was closely three times the perceptual threshold. A single interstimulus interval of 25 ms between MNS and TMS was tested in all subjects (PAS\textsubscript{LTP}), which corresponded to the settings first described by Stefan et al. \cite{17}. The effect of the PAS\textsubscript{LTP} intervention on corticospinal excitability within the left M1 was studied using 1 mV MEP recordings and RMT (n=18) measurements at three different time points and were compared to baseline (pre): approximately 0-5 minutes (post1), 15-20 minutes (post2) and 30-35 minutes (post3) after intervention. For 1 mV MEP recordings, a total number of 10 consecutive TMS pulses per time point (pre, post 1-3) were applied over the left APB hotspot. For an schematic view of experimental setup see Fig 1.2 (please consider that this PAS protocol contained 3 post-measurements instead of 2). Corresponding MEP responses were analyzed offline. During intervention, subjects were asked to focus attention on the stimulated hand while counting the number of electrical stimuli applied to the median nerve \cite{51}. In order to ensure attention maintenance, we asked the subjects in three random time points about the number of stimuli received along the intervention. All subjects reported the correct number of stimuli applied.

4.3.4 Image acquisition

Structural MRI data was acquired on a 3 Tesla Magnetom Tim Trio scanner (Siemens, Erlangen, Germany) using a 32-channel head coil before PAS\textsubscript{LTP} intervention. T1-weighted images were acquired using a standard in-house MPRAGE sequence (TR = 1.3 s; TE = 3.46 ms; flip angle = 10°, FOV = 256 mm x 240 mm; 176 slices; voxel size = 1 x 1 x 1.5 mm). The acquisition time for the anatomical MRI scan was approximately 13 minutes for each subject.

4.3.5 Voxel-Based Cortical Thickness processing

Voxel-based cortical thickness (VBCT) processing was performed as previously described \cite{109}. Pre-processing of T1-weighted images was performed using SPM8 (Welcome Trust Centre for Neuroimaging, University College London, London, UK) running under a Matlab environment (Mathworks, version 7.7). Briefly, cortical thickness values are calculated at every volumetric point within the cortex in gray matter (GM) departing from previously segmented T1-weighted images (GM, WM and CSF) in native space. The thickness value at each voxel was calculated as the sum of the distances from that voxel, through non-straight line metrics, to the inner and outer borders of the GM (GM/WM and GM/CSF respectively) taking into account sulcal voxels where necessary \cite{109}. Cortical thickness and GM boundary maps were generated for each subject. Voxel-based cortical thick-
ness (VBCT) maps were in the space of the original input images with 1 x 1 x 1.5 mm resolution. The DARTEL algorithm [110] was used to warp the VBCT maps into a reference space which is an average of all subjects included, creating a template in which each VBCT map is warped by using a subject-specific deformation field generated in a previous step. The warped VBCT maps were scaled and smoothed with a 6-mm Gaussian kernel and then divided by the corresponding warped, scaled and smoothed masks. The resulting warped, smoothed VBCT maps were transformed to standardized Montreal Neurological Institute (MNI) space by changing the spatial transformation information only, thus cortical thickness values did not change (for a more detailed description of the VBCT analysis see [109]). Finally, an explicit gray matter mask covering the whole brain was generated from the warped and smoothed GM boundaries of all subjects.

4.3.6 Data analysis

Data analysis was performed using PASW for Windows version 18. A repeated measures analysis of variance (RMANOVA) with factor TIME (pre, post1, post2 and post3) was used to study differences in APB MEP amplitudes as well as in RMT for all time points tested using non-normalized raw data. Subsequently, pairwise comparisons between time points (pre vs. post1-3) using paired-samples t-tests were performed and Bonferroni corrected for multiple comparisons. Since we performed three comparisons, a p-value of p < 0.0167 was considered statistically significant. Post hoc comparisons between PASLTP responders and non-responders were carried out using non-parametric Mann-Whitney U Tests due to the small size of the non-responders group (n=5). A multiple regression whole brain analysis was performed using the warped, smoothed and normalized MNI VBCT maps generated for all subjects and the percentage change in corticospinal excitability immediately after termination of PASLTP (post1) relative to baseline which is a direct indicator for the effectiveness of the intervention. Age and gender were included as covariates. The explicit mask generated in the VBCT processing was used so that only voxels corresponding to GM in the cortex were analysed (excluding the cerebellum). Effects were reported for clusters of voxels exceeding a cluster size threshold of p < 0.05 family-wise error (FWE) corrected for multiple comparisons in the context of Gaussian random field theory [111] and a voxel-level threshold of p < 0.001 (uncorrected).

4.4 Results

All subjects tolerated the interventions without reporting any unexpected discomfort and there was no adverse event during the study procedures. No significant differences for attention, fatigue and discomfort, RMT, intensity to evoke 1mV MEP (expressed as the
maximum stimulator output) and MNS intensities were found within and between subjects (pre vs. post, paired samples t-tests p > 0.05).

RMANOVA revealed a significant effect for factor TIME (pre, post1-3) indicating a change in corticospinal excitability of right APB in at least one time point tested (RMANOVA factor TIME; F(3,54) = 3.146; p = 0.032). Post hoc paired comparisons showed a significant increase in corticospinal excitability relative to baseline for post1 (p = 0.005) while no such changes were observed at post2 (p = 0.162) and post3 (p = 0.149) (see Fig 4.1A). These results indicate a transient change in corticospinal excitability within left M1 immediately after PASLTP intervention that recovered to baseline conditions approximately 15 minutes after termination of PASLTP. No such changes could be observed for RMT of right APB (RMANOVA factor TIME; F(3,51) = 0.648; p = 0.588). A linear correlation analysis between baseline RMT and PASLTP-induced after effects (post1) revealed no significant relationship (r = 0.101; p = 0.689), indicating that baseline RMT levels did not account for the individual variance of PASLTP-induced excitability changes after intervention.

Among the 19 subjects tested, 14 subjects showed the expected facilitatory PASLTP effect (responders) while the remaining 5 subjects either showed no effect or a tendency towards a decrease in corticospinal excitability (non-responders) (see Fig 4.1B). Closer inspection revealed that PASLTP responders and non-responders did not differ with respect to stimulation intensities required to evoke 1 mV MEPs (Mann-Whitney U Tests: p = 0.96, n = 19), MNS intensities (p = 0.92, n = 19) and baseline RMT (p = 0.80, n = 18) (for one subject, RMT was not recorded). Furthermore, we compared the radial distance between the outer edge of the scalp and the cortex (hand area in left M1) between PASLTP responders and non-responders in Brainsight 2. As a departing point we used the TMS hotspot that was marked on the outer surface of the respective brain. This measurement, however, revealed no significant difference (PASLTP non-responders: 12.80 ± 0.55 (mean ± s.e.m., n=4); PASLTP responders: 13.31 ± 0.45 (mean ± s.e.m., n=14), Mann-Whitney U Tests: p = 0.51). Please note that due to technical reasons one subject (non-responder) could not be included in this specific analysis.

The multiple regression whole brain analysis (n=19) between GM VBCT and PASLTP-effects on corticospinal excitability immediately after termination of the intervention (post1) revealed a positive correlation only in the left primary sensorimotor cortex (SM1) (see Fig. 2; including BA 3b: x = -28, y = -34, z = 51; p = 0.001; Z = 4.19 as well as BA 4: x = -24, y = -31, z = 60; p = 0.001; Z = 3.63; cluster extend for SM1 (KE) = 316, Fig. 4.2A), according to the probabilistic brain atlas [112]. Within this cluster covering SM1, the peak in S1 alone accounts for 45 % of variance (r = 0.67, r2 = 0.45; p = 0.002) in our subject population, while the M1 peak accounts for 33 % of variance (r = 0.57, r2 = 0.33; p = 0.010). No negative correlation was observed.
Figure 4.1: (A) Ten minutes of PAS\textsubscript{LTP} (dashed box with figure representing the side of peripheral (median nerve) and cortical (TMS) stimulation) resulted in a significant increase in corticospinal excitability for at least 5 min after termination of the intervention relative to baseline (pre). No such changes could be observed after 15 (post 2) or 30 min (post 3). For details see text. Squares represent mean change in APB MEP amplitudes normalized to baseline [%]. Error bars represent standard error of the mean (s.e.m.). (B) Individual variations in MEP amplitudes 5 min after termination of PAS\textsubscript{LTP} at time point post 1. The thick horizontal line indicates the group average value of MEP changes after PAS\textsubscript{LTP}. Please note that approximately 73% of our subjects showed the expected facilitatory PAS\textsubscript{LTP} after-effect while 5 subjects exhibited no such effect or a trend to inhibition.
Figure 4.2: (A) Correlation analysis (whole brain) between VBCT values and PAS\textsubscript{LTP} effect (MEP amplitude change (post 1) relative to baseline (pre) [%]). The significant positive correlation is displayed on a structural warped T1 GM image of one participant constructed with DARTEL as well as on a rendered brain (MNI coordinate: x = -28, y = -34, z = 51). T1 GM images are displayed in coronal, sagittal and axial sections shown at p < 0.001 with an extend threshold of p < 0.05, FWE correction on cluster level. The colour bar indicates the respective T-score. Please note that there was a significant correlation in left SM1 only. (B) Scatter plots showing the correlation between individual variations in PAS\textsubscript{LTP} effect (post 1 relative to baseline [%]) and VBCT values within S1 (BA 3b, peak voxel at [x = -28, y = -34, z = 51]; r = 0.67, p = 0.002) and M1 (BA 4, peak voxel at [x = -24, y = -31, z = 60]; r = 0.57, p = 0.010).

In a further analysis, we compared VBCT values in SM1 between PAS\textsubscript{LTP} responders and non-responders. We found that PAS\textsubscript{LTP} responders had significantly larger VBCT values in the S1 cluster (1.63 ± 0.16 mm vs. 1.39 ± 0.14 mm, Mann-Whitney U Tests: p = 0.01) but not in M1 (1.40 ± 0.11 mm vs. 1.31 ± 0.12 mm, p = 0.15, see also Fig. 4.2B) when compared to PAS\textsubscript{LTP} non-responders (for an overview of individual VBCT maps see Fig 4.3). On the other hand, the mean global VBCT values across the whole brain did not differ between both groups.

We also observed a significant positive correlation between GM VBCT and PAS\textsubscript{LTP}-effects on corticospinal excitability at post2. More specifically, significant clusters were identified in left S1 (BA 2), left M1 (BA 4p), left SMA (BA 6), left superior parietal lobe (SPL) as well as in right superior temporal (STG) and medial temporal (MTG) gyrus (see Fig 4.4 and for details see Table 4.1).

No negative correlation was observed. Furthermore, no positive or negative correlation was observed for PAS\textsubscript{LTP}-effects on corticospinal excitability at post3.
Figure 4.3: Individual, non-normalized whole brain VBCT maps for each subject tested (n = 19) including PAS\textsubscript{LTP} responders (n = 14, first two rows) and non-responders (n = 5, last row). The colour bar represents VBCT values in [mm] ranging between 0.5 and 5 mm. The overall cortical thickness average value was not different between responders and non-responders.

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<th>Hemisphere</th>
<th>MNI Coordinates (x, y, z)</th>
<th>T score</th>
<th>Z score</th>
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</tbody>
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Table 4.1: Positive whole brain correlation analyses between GM VBCT values and \(PAS\textsubscript{LTP}\) after-effects (MEP amplitudes at post 1 and post 2). L, left; R, right. P values represent results with non-stationary correction on a cluster level (p < 0.05, FWE-corrected).
4.5 Discussion

This is the first study highlighting a relationship between the outcome of a plasticity-inducing brain stimulation protocol (PAS_{LTP}) and human brain structure. More specifically, the outcome of PAS_{LTP} was related to individual variations in cortical thickness of the left (underlying) sensorimotor cortex (SM1) only: subjects with a large facilitatory after-effect were those with larger cortical thickness values in SM1 and vice versa. This finding might have strong implications for the understanding of the effectiveness of non-invasive brain stimulation techniques with variable outcome. The fact that we exclusively found VBCT alterations in SM1 is in good accordance with the theoretical framework of PAS because both, the primary somatosensory cortex (S1; BA 3) as well as M1 (BA 4) are involved in the processing of inputs during intervention [17]. In line with previous findings, PAS_{LTP} over M1 increases MEP within the stimulated area and in parallel changes SEP components (e.g. increased N20-P25 amplitudes) in the ipsilateral S1 [40]. The fact that PAS_{LTP}-induced excitability changes occur not exclusively M1 but also extend into the ipsilateral S1 might be explained by dense cortico-cortical connections between both areas [113]. Based on these findings, it is reasonable to assume that our hypothesis about PAS_{LTP}-induced after-effects might extend to S1 as well, especially because S1 and M1 are important brain areas for the processing of inputs during PAS intervention.

Interestingly, we also identified a significant correlation between GM VBCT val-
ues and PAS\textsubscript{LTP}-effects on corticospinal excitability at post2. These results indicate that the individual PAS\textsubscript{LTP} recovery process might depend on the thickness of multiple GM areas including not only SM1 but also other motor-related as well as non-motor temporal cortical areas. While the interpretation of this finding is difficult at this point, future studies should provide deeper insight into the underlying mechanisms of the recovery phase of PAS\textsubscript{LTP} at a network level.

4.5.1 Variability in the outcome of non-invasive brain stimulation protocols

Among recently developed TMS protocols aimed to induce plastic changes in the human brain, PAS seems to be particularly relevant due to its foundation in basic neuroscience research. PAS in humans has been adapted in accordance to spike timing dependent plasticity (STDP)-inducing protocols in slice preparations and neuronal cultures. Additionally, PAS seems to share many common features with Hebbian mechanisms of plasticity since it is capable of inducing LTP/ LTD-like effects in a time-dependent manner and depends on NMDA receptor activity \[77\]. Thus, non-invasive associative stimulation seems to be the most efficient way to study Hebbian-like mechanisms of plasticity in the living human brain (for a more detailed review see \[5, 16, 33\] at present.

Overall, PAS has been considered clinically relevant to identify maladaptive processes in a huge variety of neurological disorders \[33\]. However, such protocol as well as other plasticity-inducing brain stimulation protocols display large inter-subject variability even in healthy subjects. Hence, the potential comparison of PAS-induced after-effects between patients and healthy subjects becomes challenging and might limit the clinical relevance of the protocol.

In the present study, 73 \% of our subjects showed the expected PAS\textsubscript{LTP} facilitation (responders) while the remaining subjects (n = 5) showed no such effect (non-responders). The amount of variance and onset of PAS-induced after-effects in our study is in good accordance with previous studies \[101\]. However, the observed PAS\textsubscript{LTP}-induced after-effects seem to be shorter than previously reported. We believe that one obvious reason for the faster recovery is, in accordance with a previous study \[105\], mainly related to the fact that PAS\textsubscript{LTP} in the present study was applied for 10 min only.

The variability in PAS interventions \[33\] and in other plasticity-inducing TMS protocols \[46, 43\] has been previously addressed by focusing on different potentially relevant external (suitable for manipulation) or internal (not suitable for manipulation) factors. Among these factors, age seems to play an important role \[47\]. For example, PAS\textsubscript{LTP}-induced after effects have been shown to be more pronounced in healthy young and middle-aged subjects as compared to elderly subjects, suggesting an age-dependent
reduction of cortical plasticity. In the present study, however, we used a relatively narrow age cohort ranging between 19 and 32 years of age to minimize this potential influencing factor. Furthermore, it has been shown that the outcome of PAS\textsubscript{LTP} depends on the respective time of the day when the intervention is applied \cite{44}. According to the results of Sale and colleagues, the outcome of PAS\textsubscript{LTP} seems to be more effective in the afternoon as compared to an intervention applied in the morning. Even though we did not explicitly control for this PAS\textsubscript{LTP}-dependent daytime variation in our study, it seems rather unlikely that this factor is the sole reason explaining that some subjects were PAS\textsubscript{LTP} non-responders. More specifically in our study, 4 out of 5 PAS\textsubscript{LTP} non-responders were tested in the afternoon, while 6 out of 14 responders were tested in the morning. Therefore, we are confident that daytime variation cannot explain the interindividual differences in PAS\textsubscript{LTP} outcome in our study.

Moreover, genetic factors seem to largely influence functional plasticity \cite{46}. One of the genetic factors (amongst others) that has been shown to influence the effect of non-invasive brain stimulation protocols are polymorphisms of the brain derived neurotrophic factor (BDNF). A common polymorphism in the BDNF gene (val\textsubscript{66}met) has been shown to affect the learning of simple motor tasks \cite{48} and is related to inter-subject variability in plasticity-inducing TMS protocols such as PAS \cite{49}. Furthermore, in a recent study by Missitzi et al. \cite{50}, the authors applied PAS\textsubscript{LTP} in a set of either genetically identical (monozygotic) or genetically different (dizygotic) twins, revealing significantly larger intra-pair variability for the dizygotic group and thus emphasizing the importance of genetic factors in TMS-induced plasticity. Additionally, a recent study provided compelling evidence that carriers of the BDNF val\textsubscript{66}met polymorphism show structural brain alterations predominantly in hippocampus and prefrontal brain areas, i.e. not in primary sensorimotor cortex \cite{114}. Therefore, it seems unlikely that individual differences in VBCT within SM1 are mainly dependent on variations of the BDNF gene. Nevertheless, it is possible (even though beyond the scope of our research question here) that individual variations in cortical thickness in the area where we found the correlation (SM1) are (co-) determined by genetic predisposition.

Another potentially relevant external factor affecting the outcome of PAS\textsubscript{LTP} is the attentional state of the subjects during intervention \cite{51}. In the present study we asked our subjects to focus their attention to the stimulated hand while counting the number of electrical stimuli applied to the median nerve in order to ensure attention maintenance during intervention. In fact, all subjects reported the correct amount of stimuli applied to the cortex when randomly asked during the PAS intervention. While we cannot exclude minor variations in attention, we believe it is rather unlikely that differences in attentional states co-correlate with cortical thickness to explain the observed variability of the PAS effects.
Finally, it is reasonable to assume that the variable outcome after PAS\textsuperscript{LTP} might be related to the fact that, especially in non-responders, the pairing between MNS and TMS was not in an optimal time window due to differences in neuronal travel time for the afferent signal to reach contralateral S1 \cite{115, 39, 104, 42}. Since adjusted PAS protocols also show a rather high amount of variability in PAS-induced after-effects \cite{101}, suboptimal time windows for \textsuperscript{PAS}\textsuperscript{LTP} in our study cannot be the only reason for subjects being responders or non-responders.

Thus, even though we were obviously not able to control for all potential factors influencing the outcome of \textsuperscript{PAS}\textsuperscript{LTP}, we tried to exclude as many influencing factors as possible in order to identify other yet unknown determinants of individual variations in human brain structure. We cannot entirely rule out that the correlation between \textsuperscript{PAS}\textsuperscript{LTP} and individual variations in cortical thickness in SM1 (partially) depends or interacts with other known factors influencing the outcome of non-invasive brain stimulation protocols. Therefore, future experiments have to be performed in order to more closely relate our findings to known influencing factors \cite{46} such as genetic factors, differences in (baseline) synaptic history and/or sensorimotor skills. Furthermore, it would also be important to investigate if a similar structure-function relationship can be established for inhibitory PAS protocols as well as for other plasticity-inducing protocols. An open yet unsolved issue is the interpretation of the “bigger is better” finding in the present study.

From a neurophysiological perspective, we believe that the interpretation of the present findings have to be done with caution because the structure-function relationship is complex and might critically depend on the respective stimulation type and/or parameters. Nevertheless, it seems reasonable to assume that a thicker SM1 cortex might be a “marker” of better processing resources thereby enhancing synaptic efficacy, which in turn would translate into more prominent \textsuperscript{PAS}\textsuperscript{LTP} effects as compared to subjects with a thinner SM1.

4.5.2 Brain structure and function interplay

The linkage between brain structure and function has been widely examined since the first discovery of learning-related neuroanatomical changes in the adult human brain (for review see \cite{116}). Subsequently, long-term as well as short-term learning has been shown to induce structural changes both in grey and white matter \cite{117, 116, 118, 119}.

Apart from learning-related brain alterations, structural baseline variations of certain brain regions have been shown to predict individual motor learning success \cite{120}. Since PAS and motor learning seem to share similar underlying mechanisms \cite{16}, it becomes reasonable to assume that individual variations in brain structure of healthy
subjects might also account for the effectiveness of plasticity-inducing TMS protocols. Recently, several studies aimed to identify a relationship between baseline measures of corticospinal tract excitability and structural properties of white matter [121, 122]. The novelty of our study is that we compared functional plasticity changes after PAS_{LTP} intervention and not individual baseline excitability as done previously [123].

We used VBCT instead of voxel-based morphometry (VBM) as an alternative measure of brain structure in human subjects. VBCT has shown to be highly sensitive and powerful in detecting changes in GM thickness in comparison to VBM, because VBCT is not dependent on surface area or cortical folding and the analysis seems not to be susceptible to differences in brain size among subjects [109]. Furthermore, the concept of cortical thickness initially appears less conflicting than GM density since it mainly addresses the extent of GM between adjacent boundaries without measuring how tightly the contained mass is arranged. However, the cortical thickness results have to be interpreted with caution especially in areas such as SM1, where neuronal density seems to correlate negatively with cortical thickness values (for a more detailed explanation see [109] and [124]).

In summary, this is the first study that disclosed a link between the outcome of plasticity-inducing protocols such as PAS_{LTP} and human brain structure. We conclude that cortical thickness should be considered as an important and until now not recognized modulating factor in studies employing non-invasive brain stimulation techniques.
Chapter 5

Clinical relevance of the studies
In general, studies applying non-invasive brain stimulation of the human cortex with the aim of inducing corticospinal excitability changes are of high importance for both basic and clinical research. For one, the development of new protocols focusing on basic mechanisms of plasticity can reveal important features of the human brain *in vivo* that cannot be addressed via invasive stimulation in animal models or *in vitro* studies, such as interhemispheric cross-talk in the highly specialized sensorimotor hand areas. Furthermore, the combination of plasticity-inducing protocols and the search of factors determining such intervention-induced effects allow us to understand the way in which the brain can be effectively and successfully altered, an important issue when dealing with potential clinical applications of these techniques.

### 5.1 Relevance of the new ipsilateral paired associative stimulation protocol

Until now, previous PAS studies mainly focused on the induction of plasticity over an activated area (for review see [37]). In the present study, however, we were able to induce plastic changes within a presumably down-regulated cortical area (M1) as a result of interhemispheric inhibition between homologous brain areas (S1-S1). With respect to ipsiPAS\textsubscript{45}, after-effects were reversed as compared to standard PAS protocols, indicating that STDP-like mechanisms at the systems level in humans might be more complex than previously thought and might depend upon broader facilitatory and inhibitory interactions within the target cortical areas. Furthermore, interhemispheric inhibition between M1 cortices has been linked to the prevention of mirror movements in order to avoid maladaptive interhemispheric cross-talk [125] and, although speculative, could be proposed as a potential functional basis for the LTD-like effect observed after ipsiPAS\textsubscript{45}. This movement cancellation driven by interhemispheric inhibition could, moreover, relate to the “sensory image cancellation” suggested as functional correlate of the anti-Hebbian plasticity mechanisms observed in animal studies.

IpsiPAS\textsubscript{45}, in turn, provides a novel tool for investigating sensorimotor integration, interhemispheric processing, plasticity-related disorders or corpus callosum abnormalities as well as in clinical populations where the reported SM1 activation / deactivation pattern is altered. Inhibition of the sensorimotor network during associative stimulation, finally, opens the door for the induction of plasticity within other functionally relevant networks where specific facilitation / inhibition interactions take place, as in the case of the bihemispheric SM1 network in the present study.
5.2 Implication and potential clinical relevance of the correlation between cortical thickness and paired associative stimulation after-effects

The present study provides novel evidence that the outcome of PAS\textsubscript{LTP}-induced excitability changes might at least be partially explained by individual differences in cortical thickness within contralateral SM1. This knowledge, in turn, might have important implications for the use of plasticity-inducing protocols in clinical studies and neurorehabilitation [126, 127, 128, 129]. One of the most challenging and difficult issues in clinical studies is to design individual therapies for the specific needs of each patient. An increasing knowledge of the determinants affecting the outcome of such protocols might help to individualize and optimize therapy measures based on intraindividual factors as mentioned above. For example, a patient who shows only little response or benefit to non-invasive brain stimulation might benefit from prior exercise over several days or weeks before intervention, which by itself has already been shown to modulate brain morphology in healthy subjects [117, 119]. Since the present study indicates that especially subjects with larger cortical thickness in SM1 are those that showed the largest increase in corticospinal excitability after PAS\textsubscript{LTP}, this might be one approach to facilitate and optimize recovery processes in neurological diseases when combined with non-invasive brain stimulation. In the past few years, it has been shown that various neurological disorders are associated with abnormal changes in cortical thickness [130, 131, 132, 133, 134]. Furthermore, PAS-induced plasticity has been shown to be altered in several neurological disorders that are associated with abnormal brain morphology as well [135, 136, 137, 138]. Moreover, both cortical thickness and PAS after-effects are age-dependent [139, 47], showing a progressive decrease from young to elder subjects in both structural and functional plasticity. As a result, PAS-induced effects might be related to cortical thickness both in healthy and pathological state, and could potentially serve when combined together as a more powerful tool in clinical studies as compared to neuroimaging techniques alone. Finally, future studies have to be performed for inhibitory PAS protocols in order to investigate whether or not a similar structure-function relationship can be established.
Summary

Spike timing-dependent plasticity (STDP) has been suggested as one of the key underlying mechanism for learning and memory. Due to its importance, timing-dependent plasticity studies have been approached in the living human brain by means of non-invasive brain stimulation (NIBS) protocols such as paired associative stimulation (PAS). PAS consists of a low frequency stimulation of the median nerve followed by transcranial magnetic stimulation over the contralateral primary motor cortex (M1). PAS in humans has been considered as a model of STDP-induction since (a) it takes into account local interaction between two inputs reaching a target cortical area and (b) the after-intervention effects are critically dependent on the timing of arrival of these two inputs, resembling a comparable time-window as that seen in STDP cellular studies. However, contrary to STDP studies at a cellular level, functional plasticity induction in the human brain implies the interaction among target cortical networks and investigates plasticity mechanisms at a systems level.
This thesis comprises of two independent studies that investigate the role of network interactions in the outcome of plasticity-inducing NIBS protocols such as PAS. Both studies aim at understanding the importance of considering broad cortical networks when predicting the outcome of timing-dependent associative plasticity induction in the human brain.

In the first study we developed a new protocol (ipsilateral PAS (ipsiPAS)) that required timing- and regional-specific information transfer across hemispheres for the induction of timing-dependent plasticity within M1 (see first study in chapter 3), while in the second study we tested the influence of individual brain structure, as measured with voxel-based cortical thickness, on a standard PAS protocol (see second study in chapter 4).

Overall, we observed that the near-synchronous associativity taking place within M1 is not the only determinant influencing the outcome of PAS protocols. Rather, the online interaction of these cortical networks integrating information during a PAS intervention determine the outcome of the pairing of inputs in M1.

More specifically, with these studies we have found that:

- The network state (up- or down-regulation) of the areas being activated during paired associative stimulation interventions play a role in the directionality of after-effects. In our first study, we have shown that an overall inhibition of the network where associativity takes place (mediated by interhemispheric inhibitory drive) reverses the outcome of a PAS intervention in an anti-Hebbian-like fashion.

- These results suggest that the near-synchronous arrival of inputs to the target area does not guarantee a potentiation-like after-effect in all interventions, but rather the outcome will depend upon the intrinsic network interactions during pairing of inputs.

- Timing and order of inputs in the human brain in vivo determines the effectiveness of plasticity-inducing interventions targeting broad interconnected cortical areas (see Fig 3.6 for an overview of the overall effects).

- The individual brain structure of the cortical areas integrating information during standard contralateral PAS (SM1) partially determines the effectiveness of the intervention, indicating that the integrity of the area where associativity occurs is not the only structural factor driving the after-PAS effects. Rather, both the somatosensory and motor areas (receiving input information during PAS intervention) are responsible for the seen increase in M1 corticospinal excitability.
The shown relevance of the “active network” during pairing of inputs, therefore, underpins the essential difference between STDP in neuron-to-neuron communication and the timing-dependent network plasticity as studied at a systems level in the human brain.

Even though we were able to successfully induce bidirectional corticospinal excitability changes after effective PAS interventions (standard as well as ipsiPAS, see first study in chapter 3), it still remains elusive whether the observed changes in MEP amplitudes (increase or decrease in size) are exclusively cortical or a mixture of effects at both cortical and spinal levels. Commonly, it has been thought that PAS effects have a cortical origin since excitability changes are induced in MEPs but not F-waves, and the amplitude of MEPs generated by electrical brainstem stimulation does not change after PAS [17]. In addition, Quartarone et al. directly measured TMS-induced corticospinal descending volleys in patients with cervical epidural implanted electrodes and found that only the amplitude of later descending volleys (which relate to the intracortical TMS activation of pyramidal neurons transsynaptically) were affected after PAS [76, 16, 140]. However, studies that measured both MEPs and H-reflex have reported parallel modifications of spinal and cortical excitability after PAS interventions [140, 141] based on the finding that both the MEP and the H-reflex are increased after PAS. Additional measures of spinal excitability after our new ipsiPAS protocols, thus, are necessary in order to elucidate the origin of the observed MEP amplitude changes. Moreover, the specific role of other structures such as subcortical nuclei remain unknown. Follow-up experiments employing whole-brain imaging techniques such as concomitant EEG, fMRI or PET and TMS could help clarifying which other cortical or subcortical areas apart from the sensorimotor cortex are involved in PAS after-effects. Finally, since our new ipsiPAS protocol includes interhemispheric interactions, measures of corpus callosum structure could be of potential interest in order to correlate the degree of interhemispheric inhibition-dependent decrease in MEP size after ipsiPAS45 with the density and integrity of transcallosal fibers, as done with sensorimotor cortical thickness in standard PAS (see second study in chapter 4).

Regarding the correlation between cortical thickness values and the percentage of MEP increase after a standard PAS intervention (see second study in chapter 4), several questions remain open when trying to interpret the significance of cortical thickness as a cytoarchitectonic measure. Voxel-based cortical thickness techniques are highly dependent upon T1-weighted image segmentation in cerebrospinal fluid, grey matter and white matter, where boundaries between segments are established based on image contrast. This segmentation process is especially delicate in the sensorimotor areas, where the transition from white to grey matter is not sharp but rather smooth with a high density of radial myelinated axonal fibers [142]. Thus, several grey matter voxels can be labeled as white matter due to these myelinated axons with a consequent underestimation of the grey mat-
ter cortical thickness in these areas. Cortical thickness values in M1 are therefore to be taken as a relative measure of cortical thickness, potentially meaning that a high content of these radial myelinated axons (resulting in an apparent thinner cortex) is the structural correlate of uneffective PAS response. Further measures of cortical thickness based on more sensitive brain segmentation processes are thus needed in order to determine the nature of the observed correlation.

Finally, a potential limitation of human whole-brain plasticity studies is their theoretical background, which almost completely lies on rules applied to synaptic plasticity. Although neuron-to-neuron communication represents indeed the grounds towards which network plasticity arises, it fails at learning the peculiarities of network plasticity as a distinct form of plasticity. For example, one might assume that network plasticity is merely the result of neuron-to-neuron interactions (pre- and post-synaptic pairs), or consider that the plastic changes seen after interventions at a network level (targeting neuronal populations rather than neuronal pairs) are governed by rules that emerge from the nature of the interactions taking place within larger populations of neurons. Thus, the result of neuronal pairs forming neuronal networks might be more (or different) than the sum of its parts. Future studies specifically addressing mechanisms of plasticity emerging at the network level are necessary in order to understand the whole-brain plasticity interactions that have been found at the systems level of the human brain.

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