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Non-invasive Associative Plasticity Induction in a Cortico-cortical Pathway of the Human Brain

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Contents

Short Abstract

Associative plasticity, which involves modification of synaptic strength by co-activation of two synaptic inputs, has been demonstrated in many species. Here I explore whether it is possible to induce associative plasticity within a corticocortical pathway in the human brain using a novel protocol that activates two brain areas repeatedly with double-site transcranial magnetic stimulation (TMS). The pathway between ventral premotor cortex (PMv) and primary motor cortex (M1) which computes hand movements for precision grasp was manipulated.

First, I selectively potentiated physiological connectivity between the stimulated brain areas. The effects as assessed with paired-pulse TMS were in accordance with principles of spike timing-dependent plasticity (STDP), pathway-specific and showed a different pattern of expression during rest and during performance of a naturalistic prehension task. Furthermore, I demonstrated that effects evolved rapidly, lasted for up to three hours and were reversible.

In a follow-up study, the protocol's effects on network interactions were investigated using functional magnetic resonance imaging (fMRI), specifically focussing on functional connectivity of network nodes within the wider parieto-frontal circuit controlling reaching-and-grasping. The study demonstrated that functional connectivity was *causally* modified between stimulated nodes and that those changes in coupling also affected parallel, functionally-related pathways. Comparison of neurophysiological (paired-pulse TMS) and functional (fMRI) connectivity between individuals revealed a linear relationship of these connectivity indices; the first can assess the physiological nature of the interaction, whereas the latter can elucidate global network effects, making the techniques complementary.

Neurophysiological interactions of ipsilesional and contralesional PMv-M1 were tested in chronic subcortical stroke patients during grasping. Patients showed a diminished facilitatory influence of ipsilesional PMv on M1 compared to healthy controls which might contribute to their motor disability. Application of paired-associative TMS "normalised" the reduced effective influence of ipsilesional PMv on M1 and this effect correlated with the patient's potential to improve their dexterity.

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Long Abstract

The brain is the source of behaviour and at the same time the brain's neural circuits are shaped by the behaviour that it produces. Those functional and structural modifications are referred to as experience-dependent plasticity. It is well-established that on the cellular level plastic changes are based upon synaptic plasticity. Modulation of synaptic efficacy can be achieved by inducing a persistent relationship between presynaptic and postsynaptic activity. Spike timing-dependent plasticity (STDP) has been demonstrated at various spatial scales of neural organization. This thesis aims to investigate the possibility of inducing increased corticocortical pathway efficacy in the human brain by means of paired associative transcranial magnetic stimulation (TMS). The novel experimental protocol employed here is based upon a protocol referred to as paired associative stimulation (PAS) which has been shown to modify motor output in line with principles of STDP. For PAS induction, however, peripheral electrical stimulation, as opposed to the cortical stimulation used in the current protocol, is paired with stimulation of primary motor cortex (M1) with TMS. Specifically in response to diseases such as stroke the brain exhibits an increased degree of reorganisation to establish adaptive networks that restore motor behaviour. The projects in this thesis utilise paired-pulse TMS and functional magnetic resonance imaging (fMRI) to explore the possibility of inducing increased pathway connectivity by means of corticocortical paired associative TMS in both the healthy and injured brain following stroke and to address questions of post-stroke network reorganisation in relation to grasping behaviour.

Chapter 1 is an introduction to the anatomy of motor cortical networks. It

provides a short overview of the evolution of the prefrontal cortex in primates, followed by an explanation of the motor system and its descending motor paths. It specifically covers the role of the ventral premotor cortex (PMv) in grasping behaviour in primates and how motor behaviour is represented in parieto-prefrontal circuits. The following subsection presents a review of neuroplasticity, and contrasts activity-dependent and associative plasticity. Examples of successful plasticity induction are presented for the human motor system. Subsequently, metaplasticity is described as a higher-order form of plasticity that ensures availability of synaptic plasticity and helps stabilise networks. The last subsection gives an overview of the dynamics of motor recovery, related motor network reorganisation and therapeutic neuromodulation approaches following stroke. The need for development of alternative rehabilitation strategies is discussed in the context of the heterogeneity of stroke recovery.

Chapter 2 presents results from a series of experiments focussing on paired associative plasticity induction in the ipsilateral PMv-M1 pathway. The study found that repeated pairing of TMS over PMv and M1 - at an interval that is thought to induce synchronous presynaptic and postsynaptic activity in the PMv-M1 synapses - can induce increased pathway efficacy. The effects were shown to be pathway-specific, and plasticity was expressed in accordance with the participant's cognitive state. Furthermore, reversal of the order of paired stimulation decreased pathway efficacy. Overall, the study suggested that increased corticocortical pathway coupling can be induced by means of repeatedly pairing activity of the areas and provides the first direct demonstration of cortical STDP-induction in humans.

In Chapter 3 the effects of the corticocortical paired associative stimulation protocol were further characterised. We demonstrate rapid evolvement of plasticity that persisted for at least an hour and began reversing after three hours. These findings imply a potential significance of the paradigm to help promote

and sculpt plasticity after stroke aiding motor recovery.

In Chapter 4 plasticity expression within the wider fronto-parietal motor network was investigated by means of functional imaging-based connectivity analyses. Functional connectivity between network nodes is indexed by correlations of activity of those brain areas. The study presents evidence that functional connectivity reflects short-term changes in synaptic efficacy induced in the PMv-M1 pathway. Simultaneously, pathway coupling was reduced in a parallel, non-stimulated pathway related to reaching-and-grasping. Paired-pulse TMS measures of connectivity and imaging-based functional connectivity may present two indices assessing the same connectivity, however, whereas this has always been implied, it has not yet been formally tested. We addressed the question here by correlating TMS-derived and imaging-derived connectivity measures for the PMv-M1 connection, and our results suggest that the measures correlate when the pathway is functionally active during the production of grasping behaviour. Both facilitatory and inhibitory causal influences of PMv onto M1 - during grasping and at rest, respectively - were reflected in positive functional coupling as measured with fMRI.

Chapter 5 advanced our understanding of the functional role of PMv in chronic patients with impaired hand function after subcortical stroke and tested whether our paired associative TMS protocol can enhance connectivity between PMv and M1 and improve dexterity. The facilitatory influence of ipsilesional PMv on ipsilesional M1 during execution of a grasping task was significantly reduced in patients compared to healthy controls which suggests that it may be contributing to the clinical deficits. Paired associative TMS "corrected" this reduced facilitatory influence in patients. Paired-pulse TMS connectivity measures were assessed during the performance of a grasping task. Intriguingly, the repeated execution of prehension movements improved upper limb function in all patients following both *verum* and *sham* pathway stimulation as indexed by

clinical test scores. However, the most significant overall improvements in hand function as assessed by the clinical test scores were achieved by those patients who showed the most strongly reduced effect of PMv on M1 before stimulation. Related to this, we demonstrated that the TMS-induced increase in ipsilesional PMv-M1 pathway connectivity was greatest in patients with the greatest overall improvements during the study. This suggests that there is a relationship between the a reduced effective influence of PMv on M1 and motor performance in stroke patients, and it may be possible that increases in PMv-M1 pathway connectivity can aid motor recovery that relies upon the stimulated pathway.

Finally, Chapter 6 presents a summary of the results and suggestions for future research. These include development of novel, pathway-specific non-invasive brain stimulation paradigms to promote functional recovery in stroke patients. The requirement of further investigation of adaptive and maladaptive network reorganisation is discussed in the context of a potential development of implantable neuroprosthetics.

1

Introduction

1.1 Motor cortical networks

Over the last decade it has become increasingly recognised that human behaviour and cognition are not represented in segregated, single brain regions, but that they are the result of connections and interactions between different brain areas. The generation of goal-directed grasping movements and manipulation of objects might initially appear to be a simple process, but it in fact represents a complex behaviour relying on the interaction of different neuronal network nodes. These neuronal networks not only are the cause of behaviour, but they are also, in turn, shaped by behaviour itself; learning a new motor skill is accompanied by plastic changes in brain structure and connectivity. Recent research has also raised the possibility of modulating connectivity in humans via non-invasive brain stimulation. This perspective is exciting in the context of potential therapeutic interventions following brain damage, particularly since - according to some authorities - the brain may be more susceptible to reorganisation after insult than a healthy brain.

The major aim of this thesis is to provide insight into how connectivity within the motor system can selectively be modulated by pairing of presynaptic and postsynaptic neural activity using transcranial magnetic stimulation (TMS). It also touches on the question of whether it is possible to correct abnormal inter-

actions among cortical regions selectively to improve motor impairment following stroke. This introduction will outline anatomical and physiological evidence for how the motor system produces goal-directed grasping movements via the interplay of specialised, spatially-distinct nodes within a cortical and subcortical network.

1.1.1 Evolution of primate prefrontal cortex

Phylogenetic history reveals that the basic organisation of circuits in the neocortex has dramatically changed in mammals (Kaas, 2008), and these changes include an increase in the number of cortical regions as well as drastic changes in hand physiology. Comparative evidence indicates that the cortical region that this work focuses on - the ventral premotor cortex (PMv) - evolved in early primates and is of great importance for manual dexterity. The area projects to the corticospinal cord and via this route specifically to motoneurons controlling forelimb, head and mouth movement (Passingham and Wise, 2012). The combination of greater tactile acuity and the possibility to oppose the pulpar surfaces of thumb and index finger (Napier, 1961, Jones, 1994) in tandem with the presence of PMv appears to have allowed primates to interact with the world by reaching towards, grasping and manipulating objects with their hand and to engage in hand-to-mouth-feeding (Passingham, 2008). There is some evidence that binocular vision evolved to subserve grasping movements in primates (Bloch and Boyer, 2002). Binocular vision ultimately allows for transformation of visual object features into appropriate hand configurations (visuomotor transformation) and if required, correction of prehension movements (Jeannerod, 1997). Sensory information - visual and tactile - is integrated within an expanded posterior parietal cortex which is anatomically and functionally connected to motor and premotor regions of the frontal cortex (Kaas, 2008). One of the aspects these cortical regions are concerned with is the control of grasp-

ing, and in the human brain they have nearly doubled in size relative to other primates (Passingham, 2008).

1.1.2 The motor system

The primate frontal lobe is traditionally subdivided into the rostral aspect (granular cortex) and the caudal portion (agranular cortex, based on the lack of granular cells in layer IV) (Rizzolatti et al., 2001). The caudal part of the frontal cortex is essentially involved in promoting motor acts and referred to as the frontal motor system. The frontal motor system is composed of the primary motor cortex (M1) in the precentral gyrus and seven additional non-primary motor areas found in the lateral and medial aspects of the neocortex immediately anterior to M1 (Dum and Strick, 2005, Hoshi and Tanji, 2007).

Motor-related activity in the frontal lobe has been located to PMv and dorsal premotor cortex (PMd) that are found in the lateral aspects of the frontal cortex. Another motor area lies in the dorsomedial aspect of the frontal cortex: the supplementary motor area (SMA or F3). The cingulate cortex is home to three distinct cingulate motor areas (CMA) with a rostral (CMAr, area 24c), ventral (CMAv, area 23c) and dorsal (CMAd, area 6c) subdivision (Picard and Strick, 1996). The lateral premotor areas are strongly interconnected with the CMA (Barbas and Pandya, 1987, Beckmann et al., 2009). All the above secondary motor areas are organised somatotopically and have direct access to the corticospinal tract.

The pre-SMA (F6) resides at the border between the premotor and the prefrontal cortex and constitutes the seventh motor area. Like the other premotor areas, the pre-SMA is active when movements are made, but in contrast to the other secondary motor areas lacks direct connections to the spinal cord or to M1 (Luppino et al., 1993). Pre-SMA might influence movements via its connexions to the pedunculopontine tegmental nucleus in the brainstem

(Matsumura et al., 2000) and M1 via it connexions with other premotor areas, such as F5 in the PMv and the CMAr (Gerbella et al., 2011, Luppino et al., 1993). Cortical neurons in M1 send their projections to motor neurons in the spinal cord which in turn directly control peripheral muscles.

The posterior aspect of the motor system encompasses the primary somatosensory cortex (S1), immediately posterior to the central sulcus, and the more posterior partitions of the parietal cortex, divided into the superior parietal and inferior parietal lobule. Some researchers have argued that the posterior parietal cortex integrates visual and tactile information to create spatial representations of the environment and three-dimensional shapes of objects suited for guided grasping, thereby making it part of the "vision for action" system (Milner and Goodale, 1995, Murata et al., 2000, Goodale et al., 2004, Fogassi and Luppino, 2005, Kravitz et al., 2011).

1.1.3 Primary motor cortex and corticospinal tract

M1 is the major cortical output site for the formation of structured movements. Sherrington and his colleagues mapped out motor representations in non-human primates in the early 20^{th} century by electrically stimulating the precentral gyrus and eliciting movements in the corresponding muscles (Grunbaum and Sherrington, 1908, Leyton and Sherrington, 1917). This somatotopy of cortical representations was also demonstrated in human M1 by the neurosurgeon Wilder Penfield who stimulated the motor cortex prior to surgical removal of epileptic tissue and tumours (Penfield and Boldrey, 1937). Representations in M1 follow two segregation principles; first, the medial to lateral axis captures the representations of leg and trunk, to arm and hand, to face and throat, and second, the motor cortex is divided into a phylogenetically older rostral (anterior, BA4a) and younger caudal (posterior, BA4p) zone (Geyer et al., 1996, Rathelot and Strick, 2009). A study using retrograde fibre

tracing in macaques characterised the rostral zone of M1 (BA4a) by a lack of direct corticospinal projections, and the caudal zone (BA4p) as a region encompassing direct, monosynaptic corticospinal motoneurons projecting to finger and arm muscles (Rathelot and Strick, 2006, Rathelot and Strick, 2009). The "motor homunculus" reveals a disproportionate representation of the hand area in M1 indicating the centrality of prehension as means of interacting with the world (Lemon, 1993). The simplifying concept of the homunculus only partially accounts for a fractured and overlapping digit representation in M1 as shown by electrical stimulation in monkeys and functional activations in humans (Schieber and Hibbard, 1993). The overlap of neural representations supports muscular activation patterns that subserve grasping of objects of different properties (Brochier et al., 2004).

The primary motor cortex exerts control over peripheral muscles via the spinal cord. Motor commands are directed from large pyramidal cells in layer V (Betz cells) through the corticospinal tract, with cortical pyramidal cells synapsing onto spinal alpha motoneurons or spinal interneurons in either the anterior horn or intermediate zone of the spinal cord, respectively (Rathelot and Strick, 2006). The corticospinal tract is composed of a lateral and anterior (or ventral) corticospinal tract (Fig. 1.1). Most pyramidal fibres (approximately 80 %) decussate on the level of the medulla oblongata into the lateral corticospinal tract, whereas approximately 20 % of fibres remain uncrossed and form the anterior (or ventral) corticospinal tract (Nathan and Smith, 1973, Dum and Strick, 1996). Motoneurons controlling hand and wrist muscles are located in the cervical enlargement of the spinal cord. Comparative phylogenetic analysis found that a higher number of direct corticospinal motoneuron projections along the corticospinal tract correlate with higher dexterity in primates (Heffner and Masterton, 1983). During their descent, motocortical axons pass through the internal capsule before transversing the pons and medulla.

Axonal injury in the descending motor pathways at the level of the internal capsule has been shown to correlate with motor deficits in stroke patients (Pendlebury et al., 1999).

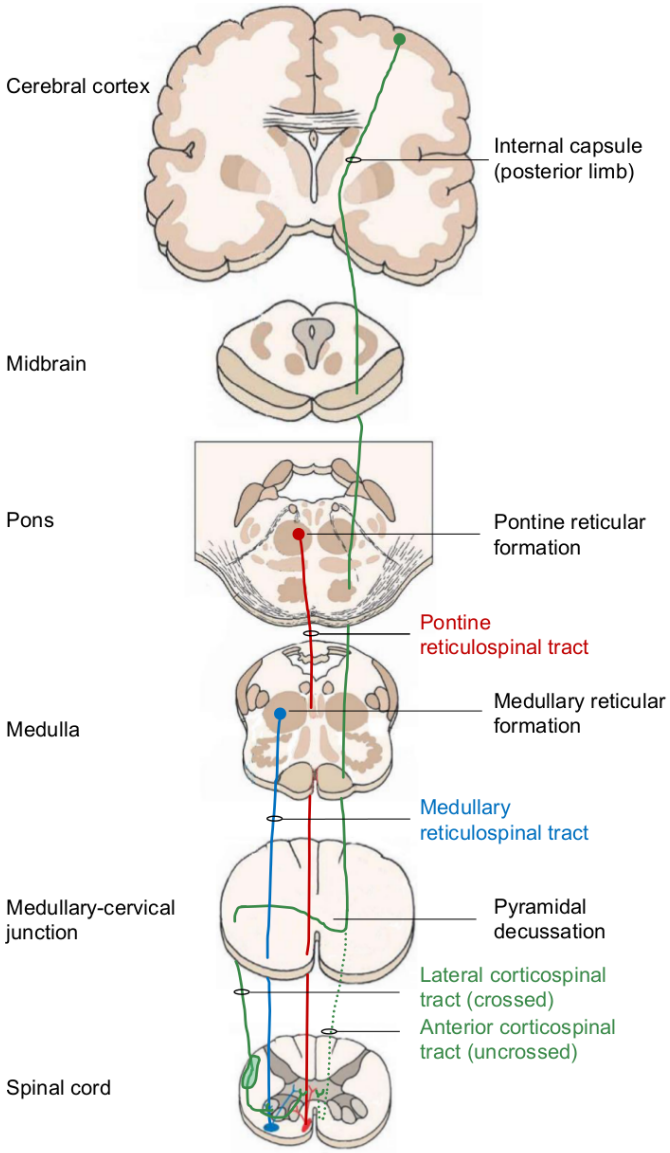

Figure 1.1: Parallel corticospinal projections. Axons from corticospinal neurons descend from the cortex via the posterior limb of the internal capsule. Their projections run through the midbrain, pons and medulla before crossing at the pyramidal decussation where they form the lateral corticospinal tract. Some fibres continue without crossing and form the anterior (or ventral) corticospinal tract. The reticulospinal tract descends ipsilateral from the pontine and medullary reticular formation.

1.1.4 Premotor cortex

The lateral premotor cortex (Brodmann area 6) is located immediately anterior to M1 and can be divided into PMv and PMd on the basis of microanatomy (i.e. cytoarchitecture and receptor distribution) and macroanatomy (i.e. connectivity) (Barbas and Pandya, 1987, Matelli et al., 1991, Dum and Strick, 1991, Geyer et al., 1998, Tomassini et al., 2007).

Connectivity of premotor areas

Up until the turn of the millennium, much of the knowledge about cortical anatomical organisation resulted from tracer injection studies in macaque monkeys. The anatomical nomenclature of the lateral frontal cortex has strongly been influenced by the terms defined through cytochrome-oxidase activity staining in monkeys (Matelli et al., 1985). In the macaque, ventral areas F4 (caudal) and F5 (rostral) compose PMv, and dorsal areas F2 (caudal) and F7 (rostral) correspond to PMd (Petrides and Pandya, 1994, Rizzolatti et al., 1998). The nomenclature will be referred to below since it is relevant for comparisons of anatomical areas and connectivity in monkeys and humans.

With the emergence of diffusion-weighted magnetic resonance imaging (MRI), classical anatomical tractography is now being compared to diffusion-weighted MRI *in vivo* results. Diffusion-weighted MRI not only allows for within-species comparisons, but also for comparisons of monkey and human anatomical connectivity. Connectivity also helps defining a brain region where a unique set of extrinsic inputs and outputs determines the functions a region can carry out, and this combination of afferents and efferents is referred to as a "connectional fingerprint" (Hudspeth et al., 1976, Passingham et al., 2002). Comparisons of anatomical connectivity of human and monkey PMv with other cortical regions have been carried out non-invasively in recent years employing diffusion-weighted MRI. Even more recently, diffusion-weighted tractography-

based *anatomical connectivity* of the frontal cortex was compared with *functional connectivity*, i.e. correlations in activity measured with functional MRI (fMRI) at rest, in macaques and humans (Sallet et al., 2013, Neubert et al., 2014).

Tracer studies in monkeys revealed that PMv and PMd have been shown to be nodes in segregated parietal and prefrontal circuits (Sanides, 1964, Matelli et al., 1986, Matelli et al., 1989, Wise et al., 1997, Tanné-Gariépy et al., 2002, Dum and Strick, 2005, Stepniewska et al., 2006). The connectivity fingerprint of PMv revealed similarities with macaque PMv connections (Matelli et al., 1986, Neubert et al., 2014); that is strong projections to the ventrolateral prefrontal cortex and strong interconnections with the anterior inferior parietal lobule (Rushworth et al., 2006, Tomassini et al., 2007), a pattern distinct from PMd connectivity.

Cortical areas immediately anterior to PMv, i.e. inferior frontal junction (IFJ), area 44d and area 44v are strongly coupled with PMv (Neubert et al., 2014). In addition to their coupling with PMv, those three regions are also interconnected with more anterior dorsolateral prefrontal areas as well as with visual association areas in occipitotemporal cortex. On the contrary, PMd is more sparsely connected with the dorsal aspect of prefrontal cortex (Tomassini et al., 2007).

PMv also receives projections from the parietal lobe. In addition to its major connections with anterior intraparietal area (AIP), area PF and area PFG in the anterior portion of the inferior parietal lobule, PMv receives input from the secondary somatosensory area and to a lesser extent from S1 (Tanné-Gariépy et al., 2002). Parieto-frontal connectivity between PMv and AIP is reciprocal as demonstrated for macaque brain (Matelli et al., 1986, Luppino et al., 1999).

Furthermore, PMv is densely interconnected with M1 and provides the strongest input into the hand area of M1 (Dum and Strick, 2005). PMv also sends direct corticospinal projections towards the cervical enlargement of

the spinal cord. Retrograde tracer injection into the cervical segments of macaque monkey spinal cord found corticospinal motoneurons in premotor areas (Dum and Strick, 1991). This labelling revealed that 48.5 % of the corticospinal projections come from M1, with PMv contributing 4.0 % to the corticospinal tract (referred to as "arcuate premotor area" (Dum and Strick, 1991)).

Functional specialisation of premotor areas

The ventral premotor cortex is subdivided into two areas based on histochemical staining: the more caudal aspect is referred to as area F4 in the macaque, the more rostral aspect as area F5 (Matelli et al., 1985). This subdivision is also matched by differential functional roles of F4 and F5. The caudal aspect of PMv (i.e. F4) shows somatotopic organisation with distinct representations of face, neck, and proximal arm movements (Gentilucci et al., 1988, Graziano et al., 1994, Fogassi et al., 1996), whereas F5 contains a movement representation for hand and mouth (Murata et al., 1997, Rizzolatti et al., 1998). Area F5, however, not only contains effector-specific motor neurons, but in more anterior aspects of F5 effector-independent visuomotor neurons can be found. Those visuomotor neurons of F5 become active upon the visual presentation of an object. Rostral aspects of F5 in macaques host "canonical neurons"that encode object-specific action plans (Rizzolatti et al., 1990, Raos et al., 2006, Umilta et al., 2007). "Mirror neurons" present another subset of visuomotor neurons that are active both when monkeys perform a particular action or when they observe this particular action (Belmalih et al., 2009).

Macaque area F5 can be subdivided further into F5c (convexity of F5), F5a (anterior F5), and F5p (posterior F5); more posterior F5c is believed to represent actions performed in a context-dependent way, while F5a may code actions at a more abstract level (Nelissen et al., 2005). Functional connectivity patterns of macaques and monkeys revealed parallels between F5c and human subregion

6v and F5a and human subregion 6r (Neubert et al., 2014). Area F5 is particularly important for the performance of prehension movements; upon observation of an object, the hand is shaped in an anticipatory manner so that the digits are in an appropriate position to lift an object as soon as the object is contacted (Jeannerod et al., 1995). Such anticipatory shaping of the hand according to an object's characteristics was demonstrated to depend on the anterior sector of F5 (F5a) by reversibly inactivating the area in trained monkeys; following inactivation, a deficit in hand pre-shaping was observed (Fogassi et al., 2001). Erroneous hand-object interactions were sometimes corrected upon contact with the object. Intriguingly, when M1 was disrupted, no grasping at all was possible. This subregion of PMv codes motor acts in a goal-centred manner; this is, where the final goal of an action is represented independent of which effectors are used (fingers versus tool) or which type of manipulation is required to obtain the goal (standard pliers versus "reverse pliers" that require opening of the hand to pick up an item) (Umilta et al., 2008).

Via its dense interconnections with the inferior frontal cortex PMv may also gain access to higher-level action control (Neubert et al., 2014) such as task-switching, stopping of initiated movements or motor caution (Mars et al., 2007, Chikazoe et al., 2009, Neubert et al., 2010, Buch et al., 2010, Wessel et al., 2013). The posterior aspect of the inferior frontal gyrus (pIFG) and IFJ in conjunction with pre-SMA present three nodes of a brain network of action inhibition (Aron, 2011).

PMd on the contrary has been associated with arbitrary stimulus-response mappings; this is the selection of an action based on a previously learned rule (Petrides, 1985, Passingham et al., 1998, Toni et al., 2001). In addition, PMd has been shown to be involved in action selection, preparation, and execution during visually-guided reaching (Wise et al., 1997).

Distinct roles of premotor areas in reaching and grasping

For hand-objection interactions under visual control, a distinction between the reaching component and grasping component can be made. Consistent with the division into two phases, it was suggested that the cerebrum contains two parallel visuomotor circuits, one concerned with reaching and one with grasping (Arbib, 1981, Jeannerod, 1997). Two largely segregated neuronal circuits have been mapped out that were traditionally believed to represent the two distinct aspects of prehension: The "dorsolateral circuit" composed of anterior area F5a and AIP, areas PF and PFG of the inferior parietal lobule has been linked to the "grasping" component, whereas the "dorsomedial circuit" composed of PMd (posterior aspect), medial intraparietal area (MIP) and anterior occipital-parietal sulcus (area V6A) has been associated with the "reaching" component (Jeannerod et al., 1995, Wise et al., 1997, Tanné-Gariépy et al., 2002, Galletti et al., 2003, Brochier and Umilta, 2007).

The dorsolateral stream processes visuomotor transformation required for an accurate pre-shaping of the hand to interact with objects of different shapes and sizes. Adequate interaction with a presented object requires the selection of finger muscle synergies: Temporary inactivation or TMS-induced disruption of AIP led to impaired pre-shaping of the fingers in both monkeys (Gallese et al., 1994) and humans (Davare et al., 2010) indicating the crucial role of AIP in the development of object-specific muscle-patterns.

Visuospatial information is also processed in the dorsomedial circuit of PMd, MIP and V6A allowing for controlled arm-reaching via projections to proximal arm muscles (Johnson et al., 1996, Fattori et al., 2001), with PMd encoding spatial information about hand and eye position during reaching (Pesaran et al., 2006). Area V6A encodes both visual and somatosensory information - such as form and motion as well as skeletomotor information regarding position of the upper limbs, respectively - and carries out early computations for

object-hand interactions (Galletti et al., 1997). The computations implemented by V6A in conjunction with MIP, gave this complex the label "parietal reach region" (PRR) in macaques (Batista and Andersen, 2001). A number of subdivisions of the human parietal cortex have been suggested to be functionally equivalent to the PRR; one region in the anterior precuneus (aPCu) showed activity related to sensorimotor aspects of reaching, a second region of the superior parieto-occipital sulcus (sPOS) was active in response to visual input during the same reach (Filimon et al., 2009). Area sPOS is suggested to be the homologue of macaque V6A (Fattori et al., 2001, Filimon et al., 2009).

However, a less stringent segregation of these cerebral circuits with respect to "grasp versus reach" components has been debated and functional evidence supports new computational models for visuomotor control with partial overlap of these circuits (Galletti et al., 2003). Adaptation of movements or on-line error correction appear to be differentially, and independent of the grasp-reach-dichotomy, influenced by the dorsolateral and dorsomedial network. When it comes to integrating perceptual information, the dorsolateral path responds to changes in perceptual conditions (Verhagen et al., 2008). In contrast, the dorsomedial network appears to be involved in fast on-line correction of movements (Desmurget et al., 1999, Galletti et al., 2003, Fattori et al., 2009). In summary, the dorsolateral stream is more involved in computations incorporating more complex perceptual information into a visuomotor transformation (Verhagen et al., 2008), whereas the dorsomedial stream appears to rely on advance visuospatial or metric information related to an object (e.g. object location).

1.2 Neuroplasticity

1.2.1 Principles of plasticity

The brain is the source of behaviour, and it is in turn modified by the behaviour it produces as well as by environmental and physiological changes and by experience (Zatorre et al., 2012). This dynamic loop means that the brain is adaptable. The mechanisms that the brain draws on for adaptation are functional and structural changes in organisation, for example, with modulations of synaptic transmission, neurogenesis, integrative properties of individual neurons, axon organisation and myelination, and extra-neuronal effects (for example, changes in astrocyte density and angiogenesis) (Thickbroom, 2007, Zatorre et al., 2012). These numerous mechanisms are referred to as plasticity. With present models of brain functioning emphasising the idea of interacting functional networks, it is assumed that on the whole-brain level neuronal plasticity is expressed in changes across distributed networks, alternative patterns of brain activation and changes in neuronal excitability, which in turn are associated with modifications of behaviour.

The seminal work of Hubel and Wiesel on cat visual cortex described how experience-dependent synaptic plasticity shapes neuronal networks in young kittens (Hubel and Wiesel, 1963). In their experiments, they deprived kittens from using one eye. Following this intervention, the primary visual cortex (V1) of the kittens showed a distinct visual development where ocular dominance columns were not established. Instead, columns in V1 that normally receive input from the deprived eye now processed information from the active eye. Areas that normally receive input from both eyes were not developed at all. Interestingly, ocular dominance could only be established early in childhood: opening both eyes in the adult cat did not lead to the development of ocular dominance at a later stage. The concept of a “critical period“ during early de-

velopment dominated the view that the adult brain was not capable of extensive changes in connectivity (Blakemore, 1995). However, more recently it was argued that plasticity is an intrinsic feature of the brain and retained throughout life (Pascual-Leone et al., 2005). The simplest movements when performed repeatedly, even for less than half an hour, can induce changes in motor cortical representations in adults (Classen et al., 1998). Contrary to the presumption that "you cannot teach an old dog new tricks" it is possible for elderly adults to learn new skills. Juggling-naive elderly learned how to perform classic three-ball cascade juggling over the course of three weeks, and this motor skill acquisition was accompanied by structural changes in the extrastriate visual cortex that is crucial for the perception of moving objects and visuo-motor transformation (Boyke et al., 2008, Scholz et al., 2009).

The notion that novel behaviours can be learned throughout life is not new. At the beginning of the 20^{th} century already, the Spanish neuroscientist Santiago Ramon y Cajal suggested that skill acquisition must have a neural substrate. He proposed a mechanism of plasticity with two time-scales on which dynamic changes in the human central nervous system can occur. The two steps of plasticity proposed by Cajal were, firstly the "reinforcement of pre-established organic pathways", and secondly "the formation of new pathways" (from Textura del Sistema Nervioso, (Pascual-Leone et al., 2005)). About a century later, Pascual-Leone and colleagues illustrated these two steps of plasticity by teaching healthy individuals a one-hand five-finger piano exercise for two hours for several weeks (Pascual-Leone et al., 1995). It was demonstrated that effective changes in cortical output maps to the muscles involved in the task already occur after a two-hour training session: cortical representations became continuously larger and excitability thresholds decreased as assessed with single-pulse TMS over M1. This form of rapid representational reorganisation is attributed to reinforcement of existing connections, and is believed to

form the first step of plasticity (Pascual-Leone et al., 2005). The second, slowly-evolving and long-lasting step of plasticity has been described by an increasing number of studies, both using TMS and fMRI. Expansion of the M1 representation can last over several weeks. This experience-dependent reorganisation was demonstrated in a study where cortical output maps as measured with single-pulse TMS increased following five weeks of a five-finger exercise on the piano (Pascual-Leone, 1996) . The enlargement of motor cortical maps over several weeks, following the daily practice of an serial reaction-time task (SRTT), was also evidenced by an fMRI study (Karni et al., 1995). Whereas the previous study provided evidence of functional reorganisation, longitudinal studies - investigating, for example, the effects of learning juggling on the brain - have also demonstrated structural changes in grey and white matter occur as a function of training (Draganski et al., 2004, Driemeyer et al., 2008, Scholz et al., 2009).

1.2.2 Principles of activity-dependent and associative plasticity

Functional and structural modifications to neural circuits ultimately require synaptic plasticity, which is the modification of synaptic strength. The Canadian psychologist Hebb postulated that *"when an axon of cell A is near enough to excite a cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased"* (Hebb, 1949). Two major types of plasticity, which are broadly consistent with Hebb's postulate for the cellular basis of plasticity, will be discussed in the following: activity-dependent plasticity and associative plasticity.

Activity-dependent plasticity refers to the induction of synaptic potentiation by means of repeated high-frequency electrical stimulation (tetanic) of a presynaptic site. The high frequency of the stimulation of the axon evokes action potentials postsynaptically, and thereby leads to concurrent presynaptic and

postsynaptic activity. This physiological model is referred to as long-term potentiation (LTP). Dynamic shifts in the strength of synaptic connections following tetanic stimulation have been extensively investigated in animal models. Bliss and Lomo (Bliss and Lomo, 1973) were the first to demonstrate activity-dependent plasticity in the rabbit hippocampus. This form of LTP was subsequently also demonstrated for neocortical circuits, including visual and motor cortex (Artola and Singer, 1987, Iriki et al., 1989, Caporale and Dan, 2008). Activity-dependent plasticity allows for bidirectional modifications of synaptic transmission, and the opposing effect of weakening of a synaptic connection, long-term depression (LTD), can be induced by application of low-frequency afferent stimulation (Artola and Singer, 1987, Dudek and Bear, 1992).

The concept of associative plasticity is possibly more in line with the original neurophysiological postulate of Hebb's that posits that temporal contiguity of presynaptic and postsynaptic activity is needed before changes in neuronal excitability and synaptic integration occur. Experimental support for associative plasticity was gained from studies where synaptic LTP was induced when the temporal order of weak presynaptic spiking precedes strong postsynaptic input (Kelso and Brown, 1986). Likewise, the inversion of stimulation-order induces depression (Levy and Steward, 1983). The dependence of synaptic modification on the order of pre- and postsynaptic spiking within a critical window is referred to as spike timing-dependent plasticity (STDP). STDP substantiates Hebb's theoretical framework of a causal relationship of presynaptic and postsynaptic activity for long-term changes in functional connectivity (Abbott and Nelson, 2000).

Subsequently, it was recognised that associative learning depends not only on temporal contiguity but also on contingency, whereby one neural event reliably predicts the other (Rescorla, 1968). Contiguous pairings of stimulation do not or do only weakly alter synaptic efficacy when the predictive relationship between two neural events is weak (Cook et al., 2014). Contingency of

pre-synaptic activity is required for both the induction of LTP and the induction of LTD; low-frequency presynaptic activity only evoked reduced synaptic transmission when the intervals between spiking activity were constant (Perrett et al., 2001).

1.2.3 Plasticity in human neocortex

Mechanisms of synaptic plasticity of human neocortex have been investigated by means of non-invasive brain stimulation, and experimental protocols have been designed to mimic activity-dependent plasticity and associative plasticity or STDP induction procedures used in animal studies (Thickbroom, 2007). Advanced understanding of adult brain plasticity increases the interest in the application of non-invasive brain stimulation as a therapeutic tool. TMS is well suited to modulate synaptic efficacy by activating either one pool of neurons at a given site (activity-dependent plasticity) or an input into a pool of neurons in synchrony with direct stimulation of another pool (associative plasticity or STDP).

Basics of TMS

In the following paragraph, a short technical introduction to TMS and some basics about neurophysiology in relation to brain stimulation are presented. Subsequent subsections will focus on experimental applications of TMS and particularly its role in the investigation of functional interactions. TMS excites neocortical cells via a brief, high-intensity magnetic field (field strength of up to 2 Tesla) through an isolated wire coil that is resting on the head (Hallett, 2007). The stimulation pulse passes through the skull and induces an electric current in the brain tissue below the coil. This activation is based on the principle of Faraday's electro-magnetic induction (Walsh and Cowey, 2000, Wassermann, 2008, Hellmann et al., 2008). Type (mono- or biphasic), amplitude, duration and di-

rection of the electric current will determine if the underlying neurons will be depolarised and generate action potentials (Siebner and Rothwell, 2003). Magnetic stimulation of M1 evokes a focal muscle twitch that can be recorded via electromyography (EMG) with surface-electrodes being attached to belly and tendon of the given muscle. The corresponding signal detected via EMG is referred to as a motor-evoked potential (MEP). An MEP can also be induced by stimulating M1 with transcranial electrical stimulation (TES).

By comparing responses evoked by TES and TMS, a difference in response latency is noted, which provides information about the different mechanisms of excitation (Hallett, 2007). Following TES only, the responses - descending volleys in the corticospinal tract - show an early D wave (direct wave) representing direct activation of descending axons. All following waves - indirect waves of different latencies: I_1 wave: 1.5 ms latency, I_2 wave: 2.5 ms latency, and I_3 wave: 4.5 ms latency - are induced by both TES and TMS. These waves are caused via trans-synaptic activation of corticospinal neurons; excitatory interneurons projecting to pyramidal cells are the first ones to be activated by an altered electric field, and their action potentials subsequently excite synaptically connected pyramidal cells.

The activation threshold for stimulated interneurons represents a measure of corticospinal excitability. The threshold is most commonly defined as resting motor threshold (RMT) which is the magnetic stimulation intensity required to elicit a small twitch, with 50 microV MEP amplitude with 50 % probability in a relaxed muscle (Rossini et al., 1994). RMT is usually expressed in percentage of maximum stimulator output. Application of Na^+- and Ca^{++}-channel modulating drugs (phenytoin, lamotrigine, and ketamine) influences RMT and thus, RMT is understood to reflect membrane excitability (Hallett, 2007, Ziemann, 2004). Since RMT is stable within individuals, but different across individuals, it can be used to ensure that participants are stimu-

lated with the same intensity with respect to their individual threshold in different TMS studies.

To assess changes in motor excitability, a specific stimulator output is defined for each individual and changes in MEP amplitude are related to changes in excitability. To compare effects across individuals within an experiment, one wants to standardise measures of excitability: one can either choose to titrate the stimulator output at the beginning of the experiment so that it evokes, for example, a 1 mV MEP or alternatively, administer a given proportion of RMT. The first methods allows for direct comparison of inter-individual measures and there is no need to normalise across participants to reduce inter-individual variability. This MEP amplitude is standardly used to assess upward or downward modulation of excitability. The disadvantage of this method is that different neuronal populations might be stimulated since different proportions of RMT were used in different individuals. In the following studies, the stimulator output was adjusted to evoke a 1 mV MEP in all participants.

TMS measures of motor excitability

Whereas spatial specificity of TMS might be on a centimetre scale, it is possible to gain further insight into which neuronal populations contribute to various cognitive processes and motor control or are modulated by neuroplasticity. Specific TMS protocols (see Table 1.1) have been developed that explore inhibitory and excitatory interactions within and across cerebral hemispheres, and these paradigms have been characterised with respect to their cellular and molecular agents (Ziemann, 2004, Reis et al., 2008).

Short-interval cortical inhibition (SICI) determines inhibitory neurotransmission mediated by $GABA_A$-receptors (Ziemann, 2004). The SICI stimulation protocol requires a conditioning stimulus (CS) to be applied to M1 at subthreshold intensity followed by a test stimulus (TS) over M1 1 - 5 ms later; this

stimulation results in a relative decrease of MEP amplitude (Kujirai et al., 1993). SICI is believed to be produced by the first sub-threshold CS, which activates low-threshold cortical inhibitory neurons that produce an IPSP at corticospinal neurons. This IPSP at the corticospinal neuron inhibits action potential generation which is technically elicited by the supra-threshold second pulse (Ziemann, 2004).

Paired-pulse effect	Conditioning pulse applied over	Inter-pulse interval	Conditioning pulse intensity	Neuronal populations
Short-interval intracortical inhibition (SICI)	Ipsilateral M1	1-5 ms	$\sim$ 70-80 % RMT	Inhibitory interneurons ($GABA_A$ receptor type)
Short-interval intracortical facilitation (SICF)	Ipsilateral M1	1-1.5 ms, 2.5-3.0 ms, 4.5 ms	$\geq$ RMT	Cortical interneurons, non-NMDA-receptor mediated transmission (late I-wave production)
Intracortical facilitation (ICF)	Ipsilateral M1	7-20 ms	$\sim$ 80 % RMT (slightly higher than SICI)	Net facilitation consisting of primarily glutamatergic interneurons, with some GABAergic effects
Long-interval intracortical inhibition (LICI)	Ipsilateral M1	50-200	$\sim$ 80 %	earlier effects result from spinal inhibitory mechanisms, later from cortical, $GABA_B$ receptor mediated
Interhemispheric inhibition (IHI)	Contralateral M1	7-10 ms	$>$ RMT; 110 % RMT	Transcallosal route, I_2 and I_3 waves are inhibited
Interhemispheric facilitation (IHF)	Contralateral M1	4-5 ms	$\sim$ RMT	Transcallosal route, I_3 waves are facilitated
Ipsilateral silent period (iSP)	Contralateral M1		$>$ RMT	Transcallosal route (posterior half of the trunk?), I_2 and I_3 waves are inhibited

Table 1.1: Paired-pulse TMS effects with their respective stimulation protocol and presumed underlying neurophysiological mechanisms (Kujirai et al., 1993, Hallett, 2007, Rossini and Rossi, 2007, Reis et al., 2008, Wassermann, 2008, Rothwell et al., 2009).

The mechanisms underlying intracortical facilitation (ICF), which tests excitability of excitatory neuronal circuits in motor cortex, are less well under-

stood. ICF is tested by the same protocol as SICI, but longer interpulse intervals (IPIs) of 7 - 20 ms (Kujirai et al., 1993). The leading hypothesis for the nature of ICF is that net facilitation is produced by stronger facilitation of corticospinal neurons via fast non-N-methyl-D-aspartate (NMDA) receptors and a slower component mediated by NMDA-receptors as well as a weaker, inhibitory $GABA_A$-mediated component (Ziemann, 2004).

Short-interval intracortical facilitation (SICF) enhances both early and later components of the I-wave, and thereby tests excitatory trans-synaptic activation of corticospinal neurons within M1 (Reis et al., 2008). SICF is measured at discrete IPIs that match I-wave latencies (Ziemann et al., 1998). Investigation of the impact of different drugs on SICF supports the idea that SICF represents the activity of excitatory, glutamatergic non-NMDA-receptor transmission onto corticospinal neurons (Di Lazzaro et al., 2003, Heide et al., 2006).

In the present thesis, paired-pulse TMS is used to investigate the effective influence of one brain region upon another, interconnected area; this is within and across hemispheres. The inter-areal glutamatergic projections from the first area, which receives the CS, modulate excitability in the second area, which is probed using the TS. Projection fibres within the motor system synapse onto excitatory and inhibitory interneurons within M1, where excitatory interneurons are likely to also be involved in I-wave generation (Cattaneo et al., 2005). IPIs of 6 -8 ms have been shown suitable for the investigation of direct cortico-cortical interactions of PMv and M1 in human paired-pulse TMS studies (Davare et al., 2008, Davare et al., 2009, Davare et al., 2010, Buch et al., 2010, Catmur et al., 2011) and are in line with accounts of approximate conduction times (Edgley et al., 1997, Olivier et al., 2002, Caminiti et al., 2013).

Experimental applications of TMS

TMS can be used to explore brain function on a temporal and spatial scale (Walsh and Cowey, 2000). Brain function can be elucidated on a millisecond temporal scale. This high temporal resolution has provided information about functional contributions of brain regions to the performance of cognitive tasks. TMS pulses given in a time-locked fashion during task trials interact with task performance. This interaction can either be positive, as evidenced by enhanced performance in response to single-pulse TMS (Grosbras and Paus, 2003), or negative, where a transient disruption to processing is introduced by several pulses of high-frequency TMS (Glover et al., 2005, Gould et al., 2012). The interaction of task and stimulation demonstrates that a region is causally involved in a cognitive task at this exact point in time (Walsh and Pascual-Leone, 2003). It has been shown that the critical time window for TMS disruption precedes peak-activations as measured with electroencephalogram (EEG) which is explained by the fact that only potentials of synchronously active neuronal populations can be picked up with EEG. TMS time-course estimates match estimates from single unit recordings more closely than do EEG measures; this suggests that TMS might provide a more accurate estimate of neuronal events than what can be obtained with EEG (Walsh and Rushworth, 1999). Alternatively, EEG might give an accurate estimate of neuronal events in the sense that it is a consistently late estimate. This may reflect the fact that generation of the EEG-dipole may be a later consequence of simultaneous activity of a large number of neurons. By contrast, TMS may give a relatively early timing estimate when examining its effects on behaviour. It may be necessary to stimulate a brain area at a time before it has executed any cognitive processing in order to detect behavioural effects. The spatial resolution of TMS is less accurate than it is for fMRI or positron-emission tomography (PET), but it allows for effective functional characterisation of a brain area in the order of millimetres to one centime-

tre (Hallett, 2007, Walsh and Cowey, 2000). TMS-induced effects on behaviour can be evoked first, by direct modulation of the stimulated area's activity and second, by the stimulated area's impact on activity in interconnected brain areas (Paus et al., 1997, Fox et al., 1997, Walsh and Cowey, 2000).

TMS has proven a particularly valuable tool in cognitive neuroscience where TMS-induced "virtual lesions" are used to investigate the role of a cortical area in a given cognitive task. It has been shown that activity in cortical regions is decreased following several minutes of 1 Hz repetitive TMS (rTMS) and that this activity-decrease outlasts the period of stimulation (Walsh and Rushworth, 1999). The spatial localisation of effective contributions of one area to a cognitive task can be obtained via subtractive inference whereby behavioural effects are compared following stimulation of different sites (Walsh and Cowey, 2000). The transitive nature of TMS-induced lesions has the advantage that investigation of brain function is not masked by plastic reorganisation that normally follows damage to brain areas (Walsh and Rushworth, 1999).

Investigation of functional interactions using paired-pulse TMS

Neurophysiological interactions between different brain regions can be examined with paired-pulse TMS (Civardi et al., 2001, Koch et al., 2006, Boorman et al., 2007, O'Shea et al., 2007a, Buch et al., 2010, Neubert et al., 2010). In the motor system, a CS is applied over a first brain area and followed milliseconds later by a TS over M1. If the first region is functionally interacting with M1, the CS will impact on MEP amplitude. This causal influence on M1 excitability can be facilitatory or inhibitory as indexed by MEP size increasing or decreasing, respectively. The exact parameters chosen for paired-pulse TMS (ppTMS) studies will crucially determine the effects and consequently the interpretation of the findings. These parameters are: (1) the intensity of the CS,

(2) the IPI between CS and TS, (3) the area to which the CS is applied, and (4) the cognitive state of the participant (e.g. functional interactions can be inhibitory at rest, but facilitatory during a task state (Davare et al., 2008, Buch et al., 2010).

Paired-pulse TMS and rTMS techniques - in isolation as well as in combination - have been used to explore how information is processed within the parieto-premotor circuit for goal-directed motor acts. Using ppTMS over PMv and M1, it was established that PMv exerts object-specific facilitation over M1 (Davare et al., 2008, Davare et al., 2009) which is consistent with the finding made in macaques (Prabhu et al., 2009, Davare et al., 2010). Visually-guided grasping also relies on interactions between AIP and PMv-M1; when information processing from AIP during hand-object interactions is disrupted by means of a virtual lesion of AIP, object-specific PMv-M1 interactions were significantly impaired without directly modifying corticospinal excitability (Davare et al., 2010). The lack of grasp-selective modulations can possibly be attributed to disruption of the rapid recurrent feedback loops between PMv and AIP (Davare et al., 2010). This explanation is supported by the reciprocal nature of AIP-PMv connections (Rizzolatti et al., 2001) and by the idea that "canonical neurons" of PMv might obtain their grasp-specific commands as well as information about requirement to update a motor command via area AIP (Rizzolatti et al., 1988). Buch et al. found that the influence of PMv on M1 motor output can switch during grasping from facilitatory to inhibitory when prehension has to be adapted online due to object change (Buch et al., 2010). During rest, PMv is also known to provide inhibitory influence over M1 (Davare et al., 2008). Moreover, another node of the action reprogramming network, the pre-SMA, modulates PMv's influence on motor cortical output from facilitatory to inhibitory when a prepared action must be inhibited and an alternative action selected (Mars et al., 2009, Neubert et al., 2010).

Plasticity induction using repetitive TMS (rTMS)

Beyond disruption of cortical activity using single pulses of TMS and probing of functional interactions using paired pulses of TMS, magnetic stimulation can be applied to promote cortical plasticity that outlasts the duration of stimulation. The concept of using repeated electrical stimulation of neural elements in order to artificially modulate synaptic efficacy has been transferred from animals to humans, and rTMS protocols are able to emulate frequency-dependent plastic changes on human motor cortex (Thickbroom, 2007).

High-frequency rTMS (5 - 10 Hz), especially at high stimulation intensities, leads to lasting, increased corticospinal excitability reminiscent of LTP (Thickbroom, 2007, Siebner and Rothwell, 2003, Pascual-Leone et al., 1994). As previously described for LTP in animal studies, LTP-like increases in excitability following high-frequency rTMS have been linked to NMDA receptors activity in humans (Huang et al., 2008). However, these after-effects are often weak and short-lived, with a duration rarely exceeding 30 minutes (Huang et al., 2005). Suppression of corticospinal excitability by means of low-frequency rTMS has also been described. Short periods (10 to 15 minutes) of rTMS at frequencies of 0.2 to 1 Hz can lead to a decrease in corticospinal excitability for at least 15 minutes (Chen et al., 1997, Hallett, 2007). The efficacy of induction of LTD-like effects depends on the stimulation intensity: motor output can be effectively suppressed by supra-threshold stimulation (Fitzgerald et al., 2006, Thickbroom, 2007), but less so by means of sub-threshold stimulation which is effective in modulating GABAergic, inhibitory synapses. The physiological mechanism of LTD-induction is understood to be primarily based on reduction of excitatory synaptic communication and not on modulation of intracortical GABAergic inhibition, which is believed to be largely unaffected by supra-threshold stimulation (Heide et al., 2006, Thickbroom, 2007).

Plasticity induction using theta burst stimulation (TBS)

TMS appears to be a tool well suited to induce cortical plasticity in the human brain; however, a noticeable shortcoming is the weak and short-lived nature of the after-effect. In order to address this issue Huang et al. developed a novel TMS paradigm using "theta burst stimulation" (TBS) patterns which is in analogy to high-frequency protocols used in animal experiments (Huang et al., 2005). TBS involves 3 pulses of stimulation given at 50 Hz that are repeated every 200 ms at sub-threshold intensity. Interestingly, different patterns of TBS produce different effects on synaptic connectivity. Application of intermittent TBS (iTBS), a 2 second train of TBS which is repeated every 10 seconds for a total of 190 seconds (600 pulses), produces facilitation of corticospinal excitability for 15 minutes. The high effectiveness of this short intervention on motor cortical output is understood to involve a reduction in efficacy of inhibitory synapses (Thickbroom, 2007). Intriguingly, when TBS is delivered continuously (cTBS; a 40 seconds train of uninterrupted TBS composed of 600 pulses), corticospinal output is suppressed. From spinal epidural recordings of the descending volleys evoked by single-pulse TMS before and after cTBS it is known that the reduction in motor excitability caused by cTBS occurs because cTBS depresses early I-waves (monosynaptic excitatory connection to pyramidal cells) (Di Lazzaro et al., 2005, Hamada et al., 2013).

1.2.4 Associative plasticity in human motor cortex

Models of associative, Hebbian plasticity emphasise the requirement for synchronous presynaptic and postsynaptic activity in connected neurons. Associative plasticity was described for a heterosynaptic connection involving peripheral median nerve, somatosensory and primary motor cortex. The experimental paradigm, referred to as paired associative stimulation (PAS), pairs low-frequency stimulation (0.05 Hz) of median nerve with TMS pulses over the M1

representation of abductor pollicis brevis (APB) at 25 ms and induces a facilitation of MEP amplitudes for at least 30 minutes following 90 paired stimuli (= 30 minutes) (Stefan et al., 2000). Evidence was provided that activity in the cortex is dependent on the relative timing of those two stimuli in order to facilitate MEPs; when M1 TMS was delivered 25 ms up to 35 ms after median nerve stimulation, afferent input was approximately coinciding with magnetic stimulation and effectively induced an increase in MEP amplitude. IPIs of 100 ms and above did not produce synchronous events and hence, did not lead to enhancement of motor cortical output. The temporal order of presynaptic and postsynaptic activity is crucial for the direction of excitability changes, and if the order of stimulation was reversed, so that afferent input arrives after motor cortex stimulation, excitability was depressed (Wolters et al., 2003).

Plasticity induction was found to follow strict topographical rules; cortical sub-regions within M1 did not express higher levels of excitability when they had not received repeated, synchronous peripheral afferent input via the somatosensory cortex and TMS over M1. Three lines of experimental evidence were provided that the observed increase in excitability was due to changes at a cortical rather than spinal cord level (Stefan et al., 2000, Wolters et al., 2003): (1) F-waves, that test excitability of a portion of spinal alpha-motor neurons of the median nerve, remained unchanged following intervention; (2) brainstem evoked MEPs, this is MEPs of non-cortical origin, showed no change; and (3) silent periods were prolonged indicating involvement of cortical interneuron circuits. The Hebbian model of plasticity is associated with requirements for cooperativity (simultaneous activation of a critical number of fibres), specificity (LTP only occurs in synapses that are activated) and associativity (weakly activated synapses can be potentiated if their activity coincides with an LTP-inducing stimulus onto the same cell) (Thickbroom, 2007). The cortical plasticity induced by PAS appears to follow these requirements. Whereas, at first sight,

PAS does not seem to have the temporal resolution to be caused by spike timing-dependent mechanisms with both 25 ms and 35 ms of IPIs inducing synaptic potentiation (Thickbroom, 2007), it is important to remember, as described above, that TMS induces a train of neural events that last over a time period, rather than being a completely circumscribed and isolated neural event.

Other corticocortical protocols derived from PAS stimulation protocols appear to be more compatible with spike timing-dependent mechanisms. Each single pulse of TMS over M1 elicits I-waves, a train of descending volleys at a periodicity of approximately 1.5 ms (Thickbroom, 2007). These volleys are believed to result from excitation of corticospinal neurons via excitatory interneurons within the same neural circuit. In close analogy to STDP protocols in animals, corticospinal excitability was increased for 10 minutes following application of TMS pulses to M1 at an IPI of 1.5 ms in accordance with I_1-wave latency (ppTMS was applied every 5 seconds for 30 minutes) (Thickbroom et al., 2006). This short-term increase in excitability is likely due to a spike timing-dependent enhancement of trans-synaptic efficacy between interneurons and corticospinal neurons within the stimulated region.

The possibility of inducing associative plasticity across hemispheres between homologous motor cortices was investigated by repeatedly stimulating the pathway using a ppTMS protocol which is designed to test the inhibitory influence of one M1 onto the contralateral M1 (Rizzo et al., 2009). The mechanism of interhemispheric inhibition (IHI) is tested with a ppTMS protocol in which the first TMS pulse is applied to one M1 followed by a second TMS pulse to the contralateral M1 at an IPI of 7 - 10 ms (Ferbert et al., 1992). The first TMS pulse of this IHI protocol is thought to excite transcallosal axons of *ipsilateral* M1 and their action potentials lead to excitation of inhibitory interneurons in *contralateral* M1; the second supra-threshold TMS pulse leads to concurrent activation at *contralateral* interneuron synapses. Synaptic strength in this pathway

was modulated by its repeated stimulation with an IPI of 8 ms at a frequency of 0.05 Hz for 30 minutes. However, contrary to the principle of associative plasticity - where synaptic strength is increased following correlated activity of a (weak) input to a postsynaptic cell with concurrent stronger activation of that cell - IHI was decreased for at least one hour (Rizzo et al., 2009). The attenuation of IHI was accompanied by an increase in corticospinal excitability of the conditioned M1 (corresponds to *contralateral* M1, tested by a single TMS pulse).

Given the results of plasticity induction effects reported with median nerve facilitation it is surprising that interhemispheric, inhibitory connectivity was not potentiated by repeatedly activating both motor cortices at intervals that are effective in producing an inhibitory influence of one M1 onto the contralateral M1. A possible explanation lies in the reciprocal nature of the interhemispheric connections. If, following repeated stimulation, excitability was increased in the hemisphere where the TS was applied (conditioned hemisphere), its transcallosal inhibitory influence onto the unconditioned hemisphere might increase. Via this route, excitability in the unconditioned hemisphere might be reduced and subsequently, IHI would be attenuated. Evidence for the hypothesis of increased IHI from the conditioned onto the unconditioned hemisphere could have been provided by testing the motor cortical output of the unconditioned M1 via single-pulse TMS and by probing of IHI in the direction opposite to the induction protocol. The lack of IHI modulation following application of a very short IPI of 1 ms might also be explained by activity travelling in both orthodromic and antidromic directions in transcallosal axons where an orthodromically moving action potential collides with back-propagating activity and thereby cancels out any effective activity.

Interaction of plasticity expression and network states

The unexpected after-effects of this interhemispheric ppTMS plasticity protocol underline the necessity of obtaining a better understanding of how TMS manipulates brain connectivity. Although it may be a common goal to selectively strengthen and weaken connectivity between specific brain areas in a controlled manner, it may be important to consider a number of issues in order to understand how the effects will arise. The after-effects of protocol are influenced by precise stimulation parameters but also by the network state. Network states, in turn, vary with: (1) previous network activity, (2) cognitive state, and (3) state of health or disease. An interaction of stimulation protocols and network activity is to be expected. (1) Previous network activity is crucial in that activity-dependent and persistent change in neuronal state shape the direction, duration and magnitude of future synaptic changes (Hulme et al., 2013) and will be covered in the following paragraph 1.2.5 about "Metaplasticity". (2) An example for the relevance of cognitive state is an experiment conducted by Fujiwara and Rothwell (Fujiwara and Rothwell, 2004) showing differential effects of a 5 Hz rTMS protocol over M1. During rTMS one of two different states of muscle contraction patterns were executed. If the targeted muscle is contracted during rTMS, SICI was decreased; whereas relaxation of the same muscle during rTMS resulted in an increase of SICI. Voluntary muscle contraction has been shown to influence plasticity induction in several other studies as well (Gentner et al., 2008, Huang et al., 2008). (3) The state of disease appears to be relevant for altered movement kinematics following a 10 Hz rTMS protocol applied over ipsilesional M1 in stroke patients. A beneficial effect of the stimulation on motor performance was only seen in patients with subcortical stroke, but not in patients who had had an additional cortical stroke (Ameli et al., 2009).

1.2.5 Metaplasticity

Having established that previous network activity determines the direction, duration and magnitude of activity-dependent and persistent excitability changes, I will now cover how plasticity itself is regulated and why its regulation is important. Metaplasticity presents a regulatory mechanism of plasticity and it is crucial for the maintenance of stable levels of neuronal activity in the face of increased or decreased network activity (Turrigiano and Nelson, 2000). In order for neuronal networks to encode and store information efficiently and produce meaningful behaviour, both LTP and LTD have to be readily available at any point. Metaplasticity regulates plasticity thresholds so that they remain within a dynamic range of modifiability (Hulme et al., 2013). The computational model of Bienenstock-Cooper-Munro (BCM) of synaptic plasticity illustrates how a synapse has the tendency to undergo potentiation or depression depending on decreased or increased excitability history, respectively (Bienenstock et al., 1982, Abraham and Bear, 1996, Bear, 2003). Prolonged increased activity raises the threshold for LTP induction, which in the BCM model is described by a sliding modification threshold.

In humans, metaplasticity has been investigated by applying a period of priming stimulation over a given cortical area; this priming stimulation has little or no effect on synaptic plasticity itself, but it influences the manner in which synaptic plasticity is induced by subsequent stimulation. One can test whether prior priming stimulation modulates the direction or size of the effects of a subsequent LTP- or LTD-inducing plasticity protocol. The priming and plasticity induction can be applied to the same cortical site. Iyer and colleagues showed that the cortical depression induced by a thirty-minute low-frequency (1 Hz) rTMS protocol was increased if it was preceded by priming stimulation employing a high-frequency (6 Hz) sub-threshold rTMS protocol (Iyer et al., 2003). Interestingly, excitability depression showed no evidence of decay after 60 minutes.

Transcranial direct current stimulation (tDCS) can also modulate cortical function by tonic stimulation with weak direct currents as well as cortical plasticity. Depending on the polarity of tDCS protocol used, corticospinal excitability can be enhanced (anodal) or decreased (cathodal) (Nitsche and Paulus, 2000). There is evidence that such tDCS after-effects are dependent on changes in synaptic efficacy in interneuron circuits that are both excitatory and NMDA-receptor-dependent (Liebetanz et al., 2002, Nitsche et al., 2003) and inhibitory and GABAergic (Nitsche et al., 2005). Again, the homeostatic nature of the mechanisms is underlined in tDCS studies; LTD-inducing low-frequency rTMS stimulation can be modulated by prior tDCS (Siebner et al., 2004). Ten-minute priming with facilitatory, anodal tDCS followed by application of a fifteen-minute 1 Hz rTMS protocol over M1 suppressed corticospinal excitability beyond the suppression observed without priming. By contrast, a cathodal tDCS priming which decreases motor network activity resulted in increased motor output following 1 Hz rTMS. Interestingly, the effect of subsequent plasticity induction was largest in those participants who had shown the greatest response to priming. In addition, tDCS-priming effects on subsequent high-frequency rTMS over M1 also exhibit a homeostatic pattern (Lang et al., 2004).

The impact of *prior* versus *concurrent* motor cortex activity on associative neuroplasticity induction was studied using *priming*-tDCS versus *concurrent*-tDCS with PAS, respectively. In contrast to effects described above, there was only evidence of homeostatic effects when tDCS and PAS over motor cortex were applied concurrently (Nitsche et al., 2007). Interestingly, effects observed with tDCS-priming followed by PAS could not be explained by homeostatic principles: this combination of the two excitability-enhancing protocols potentiated and increased motor cortical excitability even further.

The contrary effects of tDCS-priming on rTMS and PAS protocols give rise to the question as to how far mechanisms of plasticity are different when the

plasticity is induced over a single site (rTMS) as opposed to over interconnected areas (PAS). It is possible that rTMS induces patterns of plasticity that are similar to those induced by tDCS, and hence require homeostatic regulation, thereby preventing network destabilisation. PAS might induce plasticity in a timing-dependent, synapse-specific way and which ultimately benefits from an increase in global excitability. Understanding metaplastic mechanisms that dictate neuronal network stability is crucial to understanding behavioural plasticity as well as the effects of plasticity induction protocols.

1.3 Motor recovery

Plasticity is an intrinsic property not just of the healthy brain but also of the diseased brain. Functional recovery after a brain lesion, such as a stroke, has also been described as: "learning with a partially disrupted neural network" (Pascual-Leone et al., 2005). Stroke is the leading cause of adult motor disability. Despite rehabilitation therapy the degree of motor recovery is insufficient in two-thirds of patients; the dexterity impairments that follow their cerebrovascular accidents mean that they are unable to participate in activities that are normal aspects of daily living or to carry out a profession (Kolominsky-Rabas et al., 2001). Therefore, it remains desirable to understand the relationship between neuronal reorganisation and motor recovery and to develop targeted rehabilitative therapies to recover hand function. One main aim of this thesis is to explore the contribution of ipsilesional and contralesional PMv in recovery of goal-oriented reaching and grasping function and to understand whether modulation of PMv-M1 interactions using TMS can improve task-related hand motor performance.

1.3.1 Pathophysiology of ischemic stroke

Strokes are either ischaemic (80 %) or haemorrhagic, with haemorrhagic strokes presenting as either an intracerebral (15 %) or a subarachnoid (5%) haemorrhage (Thrift et al., 2001). Ischaemic stroke is caused by occlusion of a cerebral blood vessel and is clinically characterised by a rapid loss of brain function due to lack of oxygen in the brain tissue. The ischaemic core marks the region in which brain tissue is lost irreversibly. The damage in the ischaemic penumbra, which surrounds the ischaemic core, is reversible: the tissue is functionally impaired, but structurally intact (Ackerman et al., 1981). This tissue is a target for acute therapeutic interventions, and limiting neuronal loss within the penumbra is associated with neurological improvement and recovery (Donnan et al., 2008).

1.3.2 Processes mediating motor recovery

After the brain has suffered neuronal loss, different processes take place to mediate motor recovery. In acute stages, tissue of the penumbra is partially salvaged by recanalisation of occluded blood vessels and establishment of collateral flow as well as by reduction of inflammation. Behavioural compensation, this is changing the kinematics of motor strategies rather than recovering normal motor plans, is also a part of regaining motor function. For example, after motor strokes, patients tend to use trunk movements in order to compensate for reduced distal muscle control when performing a multi-joint pointing movement (Cirstea and Levin, 2000).

Beyond acute resolution of stroke pathology and adaptation of compensatory movement plans, plastic changes of the unaffected brain areas play a major role in motor recovery. One way of understanding the effect that a brain lesion has on neural networks is to adopt the perspective suggested by graph theory (Sporns, 2011) in which brain areas are regarded as “nodes“ on a graph and connexions between brain areas constitute graph “edges“. Brain lesions

perturb network function via the loss of network nodes (i.e. a brain area) or edges (i.e. connection or the disruption of communication between connected, non-lesioned structures) (He et al., 2007, Sporns, 2011). An ischaemic lesion can, for example, directly affect the descending motor pathway (corticospinal tract) that originates to a large degree from M1, but it might also affect the interconnections between other cortical areas beyond M1, which might be ipsilesional and/or contralesional (Murase et al., 2004, Hummel et al., 2005, He et al., 2007, Grefkes et al., 2008, Grefkes and Fink, 2011). The presence of redundancy within the motor network, however, constitutes a substrate for recovery. Different nodes of the network can process information directly and indirectly (e.g. transcallosal fibres from the non-lesioned hemisphere can influence homotopic regions in the lesioned hemisphere) and certain edges exist that may become important parts of the network after damage is sustained elsewhere (e.g. uncrossed fibres in the anterior corticospinal tract may become more important for arm control after damage). Neuronal plasticity basically allows for intact regions to "take over" the function of a damaged area or to "unmask" a pre-existing connection (Pascual-Leone et al., 2005, Bullmore and Sporns, 2009). Dramatic reorganisation in adjacent intact cortex and changes in connectivity with remote areas lead to physiological and structural reorganisation and have been found to drive behavioural recovery (Johansen-Berg, 2007).

Insights into cortical reorganisation after cortical damage have been gained from lesion studies in animals and from neuroimaging as well as neurophysiological measurements in stroke patients and have been related to motor recovery. However, neurobiological variability, such as time since stroke, lesion location, premorbidity or medication, makes it difficult to understand how the motor system reacts to damage. Nevertheless, it is certain that the main goal of functional reorganisation is the attempt to generate meaningful motor output. A key aim of understanding plasticity in the stroke-affected brain is, therefore,

related to the development of targeted brain stimulation therapies to drive motor recovery. Here, some important findings from animal studies will be covered. This will be followed by a review of human neuroimaging and TMS data that support the role of other motor areas (areas beyond the focus of damage) in mediating motor recovery. Lastly, interventional approaches that attempt to "normalise" motor-related activity in the stroke brain and behavioural outcome will be discussed.

1.3.3 Animal lesion studies

Combining lesions and surface stimulation in monkeys allows cortical mapping and remapping after damage. In 1950 Glees and Cole conducted an experiment in monkeys demonstrating local remapping after the removal of the M1 thumb representation (Glees and Cole, 1950). Two weeks after the lesion it was evidenced by electrical stimulation that the motor representation of the thumb had moved into regions of M1 adjacent to the lesion zone.

An interesting case for the impact of motor training on cortical reorganisation can be made when comparing this form of spontaneous recovery seen in the study of Glees and Cole with recovery that is accompanied with relearning. In two studies conducted by Nudo et al. (Nudo et al., 1996, Nudo and Milliken, 1996), monkeys received artificially induced ischaemic lesions to the hand representation in M1. Following four weeks of rehabilitative training, the representation of digits, wrist and forearm had expanded into cortical representations of the elbow and shoulder (Nudo et al., 1996). This is in contrast to the findings made for spontaneous reorganisation after infarct, where the reorganisation was associated with a loss of hand representations from the cortical area adjacent to the infarct (Nudo and Milliken, 1996). Active training of the affected limb improved hand function significantly and promoted the idea that use-dependent plasticity may play a beneficial role in functional motor recovery.

This finding, in combination with studies in stroke survivors that reported improved movement kinematics of the affected hand following neurorehabilitative training (Bütefisch et al., 1995, Taub et al., 1993), encouraged the idea that active training of the affected limb will benefit motor recovery. This notion challenged the idea of conventional physiotherapy that was based on the concept that the enhanced muscle tone of the impaired limb had to be reduced by refraining from active movements (Bobath, 1978).

Other more distant regions have also been implicated in network remodelling after ischaemic insult. Extensive rewiring involving secondary motor areas was found in squirrel monkeys with lesions to their M1 hand representation. Three months after tissue loss, the hand representation in PMv was expanded and new corticocortical connections from PMv to S1, areas adjacent to the lesion site, had been established as evidenced by retrograde labelling (Frost et al., 2003, Dancause et al., 2005).

Increased recruitment of secondary motor areas is not limited to the ipsilesional hemisphere, but also involves contralesional regions. Macaque monkeys recovered dexterity in association with increased ipsi- and contralesional brain activity a few months after unilateral transection of the direct corticospinal pathway at cervical level C4/C5 (this transection affects axons synapsing onto the muscle endplates of arm and hand) (Nishimura et al., 2007). The lesion to the corticospinal tract was below the medullary decussation, and lateralisation of cortical activity is referred to with respect to the affected limb. With recovery of precision grip, cortical activation patterns demonstrated increases in bihemispheric M1 activity four weeks after corticospinal transection. At later recovery stages - three months after the corticospinal transection - activity in contralateral M1 and bihemispheric PMv was found to be more extensive when contrasted with pre-operative fMRI data. Such a pattern suggests that contralateral M1 and both ipsi- and contralateral PMv are contributing to the recovered motor perfor-

mance that was observed in the animals. The genuine causal role of contralateral M1 and ipsilateral PMv in functional recovery at later stages was demonstrated by a marked slowing of movements following the reversible pharmacological inactivation of contralateral M1 and ipsilateral PMv. At earlier stages, inactivation of cortical areas revealed the functional relevance of both M1s as well as contralateral PMv. In addition to the finding that both ipsi- and contralateral cortical areas are involved in functional reorganisation and motor recovery, another interesting conclusion can be drawn from this study: Network reorganisation is a dynamic process with time-dependent changes.

If contralesional motor areas are functionally relevant for motor recovery, the question is how do those motor areas contribute to hand function? One might consider four anatomical routes originating in the cortex by which the contralesional motor areas can gain access to motor output: first, direct corticospinal projections from contralesional M1; second, direct corticospinal projections from secondary motor areas (i.e. lateral premotor regions and medial premotor regions); third, transcallosal fibres connecting homotopic cortical regions, and lastly, transcallosal fibres connecting heterotopic cortical regions. (1) There is little evidence for the existence of direct ipsilateral corticospinal fibres from M1 to hand and finger muscles in primates (Soteropoulos et al., 2011). Therefore, other descending pathways conveying ipsilateral motor signals have to be considered. (2) Non-primary motor areas, such as the lateral premotor areas, SMA, dorsal cingulate motor area (CMAd) and ventral cingulate motor area (CMAv) also have direct anatomical access to spinal motoneurons (Dum and Strick, 1991, Dum and Strick, 1996). They project to the muscles of the fingers and wrist via spinal motoneurons and are in many aspects similar to M1 projections. Approximately 20 % of M1 efferents project to the ipsilateral side of the body, however those projections do not appear to innervate distal hand muscles (Kuypers, 1981, Dum and Strick, 1996, Soteropoulos et al., 2011). By contrast, a

greater proportion (23 %) of direct corticospinal fibres from SMA and 9 % of projections from CMAd/CMAv remain on the ipsilateral side and have direct access to hand control (Dum and Strick, 1996). These pathways from medial premotor areas potentially contribute to the generation and control of movement at the level of the spinal cord and may become of greater significance in recovery of motor function that follows damage to M1. Importantly, there is also a chance that secondary medial motor areas might not be affected by the same stroke that affects M1 since their blood supply comes from another source (anterior cerebral artery as opposed to middle cerebral artery). (3) Transcallosal fibres, be it from homotopic or heterotopic cortical regions, constitute two additional anatomical pathways that might mediate motor recovery. It might be possible to integrate the information processed in homotopic areas of the intact hemisphere into neural networks in the lesioned hemisphere and provide the necessary input for movement planning and execution (Grefkes and Ward, 2014). The hand representation of M1 has some transcallosal projections to contralateral M1, and the hand representation of SMA has relatively strong connections to the opposite SMA (Rouiller et al., 1994, Liu et al., 2002). (4) Lastly, heterotopic projections from the contralesional hemisphere might also undergo functional remodelling. Sprouting of novel corticocortical connections from PMv to S1, proximal to the hand representation of M1, has been found following lesions to the M1 hand area (Dancause et al., 2005). It may be that those novel projections from PMv target corticospinal motoneurons in S1, and via this route PMv may influence motor output in the absence of previously extensive connectivity with M1.

Ipsilateral spinal routes of non-cortical origin might also contribute to motor recovery after stroke. The reticulospinal tract, which starts in the pontine and medullary reticular formation and runs ipsilaterally in the medial longitudinal fasciculus of the spinal cord, provides a parallel pathway to proximal and distal hand muscles (Riddle et al., 2009). The reticulospinal tract has been

shown to be relevant for some of the functional recovery observed in macaque monkeys after selective lesioning of the pyramidal tract (Zaaimi et al., 2012). Changes in the size of excitatory postsynaptic potentials suggested relative increases in input from the reticulospinal tract into forearm flexors and intrinsic hand muscles, but not into forearm extensors, after the pyramidal tract lesion (Zaaimi et al., 2012). The reticulospinal pathway may play a pivotal role in functional recovery in severely affected stroke patients because it supports multijoint motor patterns such as flexor-synergisms that are commonly observed in patients (Grefkes and Ward, 2014). To conclude, animal models support the notion that motor recovery may involve a shift in the relative contribution of parallel motor networks, including corticospinal, subcorticospinal and corticocortical systems in order to establish some alternative input into spinal motoneurons.

1.3.4 Cortical reorganisation in stroke patients

A growing body of evidence confirms that functional reorganisation is present in stroke patients and that surviving neural networks are imperative for the recovery of motor function. Non-invasive techniques such as neuroimaging and brain stimulation are commonly used to investigate the neural mechanisms of recovery in patients.

Dynamics of reorganisation in stroke patients

Movements of the stroke-affected hand are associated with enhanced neural activity in the motor network in the lesioned as well as contralesional hemisphere (Weiller et al., 1992, Rossini et al., 2003, Ward et al., 2003a, Carey et al., 2006, Grefkes et al., 2008). Just as in animal studies, an evolution of cerebral reorganisation is present. Network reorganisation at initial stages after cerebral insult (sub-acute stroke) will briefly be discussed and will be followed by a review of network patterns observed in chronic stroke brains.

At initial stages, active movement of the hemiparetic hand evokes activation in ipsilesional and contralesional M1, SMA, premotor cortex, cingulate motor areas and cerebellum (Rossini et al., 2003, Ward et al., 2003a, Carey et al., 2006). Most recently, a meta-analysis of more than fifty fMRI studies demonstrated consistent over-activation in contralesional M1, SMA and bilateral PMv (Rehme et al., 2012). The involvement of secondary motor areas in motor recovery is plausible in the context of findings gained from animal studies: SMA, cingulate motor areas and premotor cortices form a direct corticospinal pathway that projects to both the ipsilateral and contralateral side of the body (Dum and Strick, 1991, Dum and Strick, 1996).

At chronic stages, the reorganised neuronal activation pattern is distinctly different from the functional patterns observed in the sub-acute stroke brain. Recovery-related increases in task-related brain activations have been described for M1 as well as for secondary motor areas, such as SMA, premotor and prefrontal cortex, and cingulate sulcus (Ward et al., 2003a). In addition to enhanced secondary motor area activity, another common finding has to be emphasised: a posterior shift in the somatotopic representation in M1. With recovery from hemiplegia following lesions of the posterior limb of the internal capsule, the hand representation extends more posteriorly into S1 (Weiller et al., 1992). This form of local remapping might represent the establishment of stronger connections between secondary motor areas and regions of M1 that still have access to spinal cord output (Ward, 2011).

Recovery depends upon severity of stroke

When reviewing research studies on stroke recovery, it is important to make a distinction between “poor recovery“ and “good recovery“. Different neural mechanisms appear to be involved in mediating different types of recovery. In general, the post-stroke motor system is constrained by the residual struc-

tural architecture. A redistribution of activity to secondary motor areas, with the aim to generate descending motor signals, is associated with more extensive damage of direct fibre projections from M1 to the spinal cord (Ward et al., 2006, Stinear et al., 2007). However, the integrity of the corticospinal system is crucial for the success of recovery overall and is a potentially useful tool for predicting outcomes after stroke (Stinear et al., 2012).

Patients with great impairments appear to depend on recruiting direct projections to spinal motoneurons from areas beyond M1, such as the premotor cortex. There is evidence that secondary motor areas in both the ipsilesional and contralesional hemispheres become functionally relevant for movement production. Experimentally, the causal influence of one area on motor function can be tested with disruptive TMS protocols. If activity in a given region is functionally relevant in a recovered stroke patient, disruption of cortical activity of that region should be associated with an impairment of performance. Activity-decreasing stimulation of the same site in healthy controls might not have any effect on motor output. Using TMS, ipsilesional and contralesional PMd have been shown to be functionally relevant for performance of a motor task in the subcortical stroke brain. When disruptive rTMS was applied over either ipsilesional (Fridman et al., 2004) or contralesional PMd (Johansen-Berg et al., 2002) it impaired task-performance to a greater degree than it did in healthy controls. The greater the task-related activity in contralesional PMd, the greater the slowing in reaction time induced by TMS to the same area, which suggests that the contralesional PMd activity was important for mediating the movements that patients were capable of. The relative slowing of movements following rTMS of contralesional PMd also correlated with the degree of motor impairment. Using fMRI, Johansen-Berg and colleagues furthermore showed increased neuronal activity in contralesional PMd (and contralesional M1) in the most poorly recovered patients during movement production. These two findings imply

that activation in contralesional PMd is functionally relevant in those stroke patients and that more severely impaired patients rely relatively more on recruitment of contralesional secondary motor areas (Johansen-Berg et al., 2002). Another study found that the effective influence of contralesional PMd on ipsilesional M1 was facilitatory in all patients during performance of a grip task (Bestmann et al., 2010). Yet, there was a positive relationship between the size of the influence of PMd on M1 and the severity of impairment. In addition, in more impaired patients the peak ipsilesional sensorimotor cortex activity was shifted posteriorly. This finding ties in with the idea of the "posterior shift in the somatotopic representation in M1" (Weiller et al., 1992) and can be interpreted as the consequence of an increased functionally relevant influence of PMd on descending motor pathways in the spinal cord (Ward, 2011). In addition to contributions from contralesional PMd, executive motor output in the poorly-recovered limb is also partially generated through activity in bilateral PMv (Ward et al., 2007).

In summary, involvement of secondary motor areas of the unaffected hemisphere is functionally relevant for the performance of motor acts in patients with worse clinical outcomes (Ward et al., 2003b, Calautti et al., 2007, Johansen-Berg et al., 2002). The shift in motor-related activity, most prominent in poorly recovered patients, is understood to result from greater damage to monosynaptic pathways and consequently, a greater need to recruit secondary motor area projections to generate output to motoneurons of the spinal cord (Ward, 2011). The evidence provided here might lead to the straightforward understanding that contribution of contralesional network nodes promotes motor performance in stroke patients. However, some evidence suggests that interhemispheric influences of different network nodes, namely contralesional M1 on ipsilesional M1, can have opposite - this is: impairing - effects.

In contrast with the hypothesis of a compensatory increase in secondary motor area activity in both hemispheres, there is evidence that contralesional

M1 hinders motor performance in some subcortical stroke patients. Murase and colleagues showed in patients that the inhibitory influence from contralesional M1 onto ipsilesional M1 upon movement onset was not turned into facilitation, as it is the case for healthy individuals, but remained inhibitory. Instead, contralesional M1 exerted an abnormally high, tonic degree of effective, inhibitory influence over ipsilesional M1 during movement of the affected limb (Murase et al., 2004). The degree of interhemispheric inhibition correlated with poor performance of the paretic hand (Murase et al., 2004, Grefkes et al., 2008). Upon suppression of contralesional M1 activity by means of rTMS, kinematics of finger and grasping movements in the affected hand were improved (Nowak et al., 2008). Suppression of over-activity within contralesional M1 was evidenced by reduced blood oxygenation level-dependent (BOLD) signal in the motor cortex. An fMRI study modelling effective connectivity of network nodes upon each other further suggested that the effective, inhibitory influence of contralesional M1 on ipsilesional M1 is reduced following application of rTMS over contralesional M1 (Grefkes et al., 2010). There was a significant correlation between behavioural improvement and reduced inhibitory influence from the contralesional M1 following contralesional M1 rTMS. One possible mechanism underlying this pathological over-activity in contralesional M1 is that circuits in ipsilesional M1 normally control activity in contralesional M1 immediately prior to movement, but that this inhibitory control from ipsilesional M1 onto contralesional M1 is absent following stroke (Ward, 2011).

The contradictory picture of the role of contralesional motor areas in mediating motor recovery might reflect a different "physiological signature" (Ward, 2011) of premotor and primary motor areas. Both types of influences are observed in the same type of patients: patients with subcortical strokes and greater motor impairment. Influence from premotor regions appears to become more facilitatory and functionally relevant for movement production

(Johansen-Berg et al., 2002, Bestmann et al., 2010), whereas contralesional M1 exerts a causal inhibitory effect on ipsilesional M1 and adversely affects motor execution (Murase et al., 2004, Nowak et al., 2008, Grefkes et al., 2010).

Whereas better recovery is associated with a "normalisation" of pathological activation patterns over time, it is not clear if normalisation of brain activity would be desirable for all patients. There is evidence from more than fifty neuroimaging experiments that recuperation of hand motor function is accompanied by a reduction of over-activity in motor network nodes (Rehme et al., 2012), independent of initial stroke severity and rate of recovery and despite high inter-study variance (resulting from different fMRI tasks and motor impairment levels). Given the evidence that over-activity of contralesional secondary motor areas is functionally relevant in patients with poor motor performance, it is likely that normalisation of brain activity at any stage post-stroke would only disturb motor output. In poorly recovered patients the motor system is constrained by the stroke in such a way that the brain relies on alternative access routes to spinal motoneurons, such as descending pathways from secondary motor areas.

1.3.5 Therapeutic approaches

Understanding the mechanisms of intra- and interhemispheric influences of different motor network nodes is essential to develop targeted neurotherapeutic tools that help to drive functional motor recovery. The notion that facilitatory and inhibitory interhemispheric balances are shifted post-stroke raises the idea of regulating cortical excitability using cortical brain stimulation paradigms.

Suppression of over-activity in contralesional M1 by means of low-frequency rTMS has been shown to improve the kinematics of finger and grasp movements of the affected hand (Mansur et al., 2005, Liepert et al., 2007, Talelli et al., 2007) and was associated with diminished over-activity in stimulated M1 and premotor areas and SMA (Nowak et al., 2008). Beneficial behavioural effects were

also demonstrated following a facilitatory 10 Hz rTMS protocol over ipsilesional M1 in patients with subcortical stroke (Ameli et al., 2009). The motor network showed dynamic remapping with a reduction of neural activity in the contralesional hemisphere when the affected hand was moved. Interestingly, in patients with subcortical *and* cortical damage, no improvement of motor performance was seen and activity in primary and secondary motor areas became widespread following rTMS stimulation (Ameli et al., 2009). This study indicates that the direction of the induced effects depends on the lesion location. It furthermore highlights how the same neuromodulation protocol does not induce the same effects in all types of patients, even if they have the same degree of motor impairment.

To conclude, one can expect that different treatment strategies have to be developed in order to account for variance in the stroke patient population such as location and extent of lesion, residual structural architecture and time post stroke. Transient behavioural improvements that are accompanied by dynamic network reorganisation following stimulation of the post-stroke brain have already been demonstrated and those developments are encouraging for the development of novel plasticity-inducing protocols designed to drive motor recovery.

All significant main effects and interaction effects are reported in this thesis.

2

Noninvasive paired associative plasticity induction in corticocortical pathway

2.1 ABSTRACT

Coincident pairing of pre- and post-synaptic activity selectively strengthens synaptic connections, a key mechanism underlying cortical plasticity. Here, we present a novel protocol using a prolonged period of repetitive paired pulses of transcranial magnetic stimulation (TMS) to selectively potentiate physiological connectivity between two human brain regions critical for precision grasp, the ventral premotor cortex (PMv) and the primary motor cortex (M1). The effect was anatomically specific: paired stimulation of the pre-supplementary motor area (pre-SMA) and M1 did not induce changes in PMv-M1 pathway connectivity. The effect was dependent on stimulation order: repeated stimulation of PMv prior to M1 led to strengthening of the PMv-M1 pathway,

*The work presented in this chapter was a collaborative project conducted jointly by myself (VMJ) and a previous fellow graduate student in our group, Ethan R Buch (ERB). ERB and VMJ are joint first-authors of the study published in the *Journal of Neuroscience* (Buch, Johnen et al., 2011). ERB designed Experiments 1 and 3 of the study, while VMJ and ERB both contributed to the design of all other experiments. ERB configured the experimental setup, and wrote the custom task stimuli and data analysis software used. VMJ was responsible for subject recruitment. Both VMJ and ERB jointly acquired the data, and contributed to the data analysis, figure creation, and preparation of the manuscript.

while repeated stimulation of M1 prior to PMv diminished the strength of the PMv-M1 pathway. The expression of the change in the pathway depended on the cognitive state of the participant at the time of testing: when the subject was tested at rest, paired PMv-M1 stimulation led to an increased inhibitory influence of PMv over M1, but when the subject was tested while engaged in a visuomotor task, PMv-M1 stimulation led to an increased facilitatory influence of PMv over M1.

2.2 INTRODUCTION

Synaptic plasticity refers to an activity-dependent modification of synaptic strength and might occur due to alterations in sensory experience or actions. In experimental manipulations, synchronous and asynchronous activity in interconnected neurons leads, respectively, to strengthening and weakening of their shared synapses (Hebb, 1949, Markram et al., 1997), and such changes may underlie behavioural plasticity. Evidence for spike timing-dependent plasticity (STDP), one of the classic Hebbian synaptic learning rules, has been found at various scales of neural organization (Caporale and Dan, 2008), from single synapses in slice preparations, to in vivo expression in mammals (Bliss and Lomo, 1973, Jackson et al., 2006). The aim of the current investigation was to test whether paired associative stimulation (Walsh and Pascual-Leone, 2003) TMS over two interconnected cortical areas might model similar types of changes in human subjects.

Typically in PAS experiments, electrical stimulation of median nerve is repeatedly paired with TMS over M1 which induces changes in M1 cortico-spinal excitability (Stefan et al., 2000). In accordance with the principle of STDP, dependent on the latencies of paired stimuli, coincident or non-coincident excitation of median nerve input and cortico-spinal output induce changes in motor cortical excitability; changes in excitability are also described as long-term potentiation (LTP) or long-term depression (LTD), respectively (Wolters et al., 2003).

PAS effects are measured in terms of excitability changes at the second stimulation site (M1). However, it is unclear whether such protocols induce changes in the strength of specific anatomical pathways as might be expected from animal models; it is possible that changes in excitability have solely occurred on the second stimulation site rather than within the connection. To examine whether changes in pathway strength have occurred, it is necessary to assess the impact of the first pulse on the effects induced by the second pulse, both before and after repeated pairings of stimulation. Only one study to date has examined whether repeated paired stimulation of brain areas results in STDP but the results were equivocal perhaps because it investigated interactions between homologous areas in the two hemispheres (the primary motor areas) which are known to have similar and reciprocal connexions (Rizzo et al., 2009). Other features of such plasticity, such as dependence on cognitive state, are unknown.

Neurophysiological interactions between premotor regions, such as PMv or pre-SMA, and M1 can now be characterized with paired-pulse TMS (ppTMS) (Davare et al., 2008, Mars et al., 2009). For example, at rest, an initial conditioning TMS pulse to PMv, followed by a subsequent test TMS pulse to M1, inhibits M1 cortico-spinal output, relative to that elicited by M1 TMS alone (Davare et al., 2008). This effect depends on cognitive state. When the PMv-M1 pathway is endogenously activated during reaching and grasping an excitatory effect is observed (Davare et al., 2009, Buch et al., 2010). Projections from premotor cortex to M1 are glutamatergic, but while some synapse directly onto cortico-spinal neurons, many synapse onto GABAergic interneurons within M1 (Tokuno and Nambu, 2000). The state-dependent effects of identical PMv TMS pulses probably reflect changes in input patterns to PMv that bias its output towards one of these two projection types (Duque and Ivry, 2009, Duque et al., 2010). The pathway from PMv and adjacent inferior frontal cortex to M1 is important when unwanted actions are inhibited (Swann et al., 2009,

Buch et al., 2010, Neubert et al., 2010). Given that the connectivity of PMv and M1 has successfully been investigated by means of ppTMS and that the effective influence of PMv on M1 is distinctly different depending upon cognitive state (i.e. excitatory during prehension and inhibitory during rest), this corticocortical pathway is a suitable model to investigate stimulation-induced modifications of effective connectivity and whether expression of plasticity is dependent on cognitive state.

We therefore examined effects of repeated pairings of PMv and M1 TMS on PMv-M1 physiological connectivity. In addition, we examined whether changes in PMv-M1 connectivity were pathway specific, by testing whether they were also induced by repeated pairings of pre-SMA and M1 TMS; whether such changes depended on cognitive state; whether such changes depended on stimulation-order during pairing.

2.3 METHODS

Volunteers

Thirty-three healthy, right-handed adults (age 26.0 ± 6.4 years (mean $\pm$ SD); 16 females) participated. All volunteers underwent high-resolution, T1-weighted structural MRI scans. Written informed consent was obtained prior to participation (National Research Ethics Service: Oxford REC C, No.05/Q1606/96). Some subjects participated in more than one experimental session for Experiments 1-5, but in these cases sessions were on average 123 ± 130 days (mean $\pm$ SD) apart. The number of naive and non-naive (participated in more than one experiment) volunteers were similar across experiments. Additional statistical analyses are presented to demonstrate that effects do not depend on subjects participating in more than one experiment.

Summary of experimental design

All experiments started with a baseline block, followed by a plasticity induction period, and a plasticity expression block (Fig. 2.1). Baseline and plasticity expression blocks consisted of 50 trials with volunteers either at rest (Experiments 1 and 2) or performing a reaching and grasping task (Experiments 3, 4, and 5). In all cases the effect of PMv stimulation on the effect of M1 stimulation was determined by comparing motor-evoked potentials (MEPs) recorded from right first dorsal interosseus (FDI) muscle, when either single-pulse TMS (spTMS; 25 trials) was applied to left M1 or ppTMS was delivered over both left PMv and M1 (25 trials; 8 ms inter-stimulus interval). Both spTMS and ppTMS trials were administered in pseudo-random order within the same block of trials. Precise inter-pulse timing is critical if both PMv and M1 pulses are to produce coincident influences on cortico-spinal activity; both resting-state and task-state interactions emerge at 6-8 ms intervals (Davare et al., 2008, Davare et al., 2009, Buch et al., 2010, Neubert et al., 2010). The plasticity induction period that intervened between baseline and plasticity expression periods consisted of 15 minutes of ppTMS applied at 0.1 Hz (90 total stimulus pairings) with the same 8 ms inter-pulse interval whilst the participants were resting.

Experiment 1 (11 volunteers; 6 females) tested for changes in PMv-M1 interactions at rest, following the PMv-M1 plasticity induction period. Experiment 2 (9 volunteers; 5 females) tested whether PMv-M1 potentiation also occurred after the same plasticity induction protocol was applied to a different pathway, between pre-SMA and M1. In Experiment 3 (12 volunteers; 5 females), we exploited the fact that PMv exerts an excitatory influence over M1 during grasping, and assessed whether plasticity induction would be expressed differently. Indeed, a different effect of plasticity induction was observed in Experiment 3, so Experiment 4 (10 volunteers; 6 females) was conducted as an analogous control experiment to Experiment 2 (induction protocol applied to pre-SMA-M1 path-

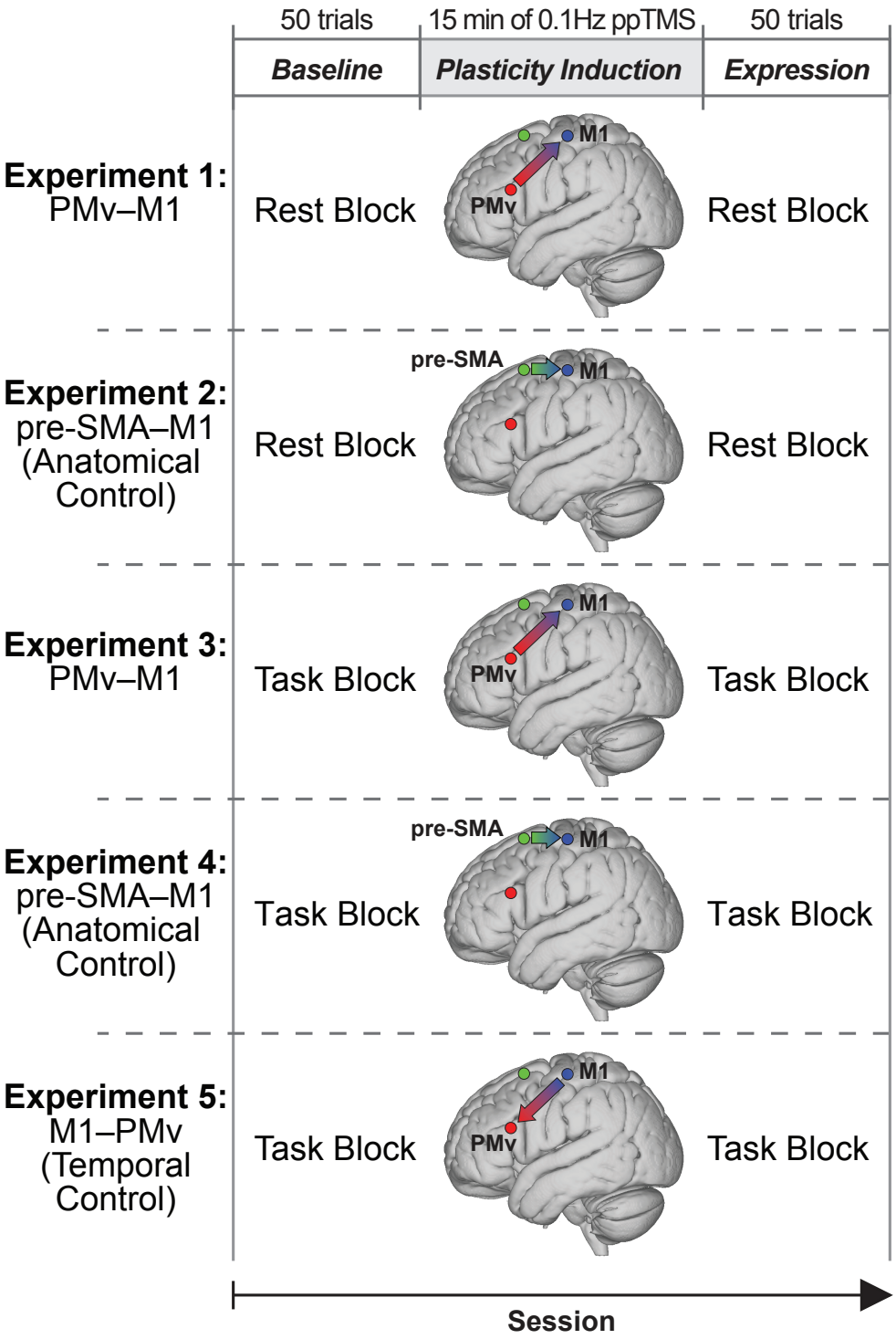

Figure 2.1: **Experimental design and set-up for all experiments.** Plasticity induction period, preceded (baseline) and followed (plasticity expression) by rest or task blocks.

way), and tested whether the effects observed during the expression task block in Experiment 3 were also specific to application of the induction protocol to the PMv-M1 pathway. In Experiment 5 (9 volunteers; 2 females), the order of stimulation during plasticity induction was reversed (each PMv pulse was preceded by an M1 pulse) to see if this diminished, rather than augmented, PMv-M1 connection strength during grasping.

Task blocks

Volunteers sat in a darkened room and made right-hand reaching and grasping movements (Fig. 2.2a) cued by illumination of one of two concentrically arranged cylinders (15 and 65 mm diameter) located 30 cm in front of the starting hand position (Buch et al., 2010). Each trial was initiated by pressing a touch-bar with the right hand. Inter-trial intervals (ITI) were therefore variable (6.90 ± 0.79 s (mean $\pm$ SD)) but did not differ significantly across phases of each experiment. Following a variable delay of 5-7 s (uniformly distributed), one cylinder was illuminated. Volunteers responded by grasping it with their thumb and index finger, before lifting it from its pedestal. Reaction and movement times were recorded. All trials were accompanied by either spTMS or ppTMS, with the pulse applied to M1 always occurring 100 ms after cylinder illumination, which was prior to movement onset (Fig. 2.2b).

Rest blocks

For rest blocks, volunteers attended to cylinder illumination while simply maintaining a static hand posture. To control for the overall temporal nature of the TMS, ITIs for rest blocks were defined as the sum of the ITI used in task blocks, plus a reaction and movement time sample drawn from probability density functions [based on (Buch et al., 2010)] defined for these variables. ITIs were therefore variable (6.23 ± 0.07 s (mean $\pm$ SD)) but did not differ signifi-

cantly across phases of each experiment.

Plasticity induction period

During plasticity induction, volunteers attended to cylinder illumination, while maintaining a relaxed hand position. Again, TMS pulses were applied to M1 100 ms after cylinder illumination.

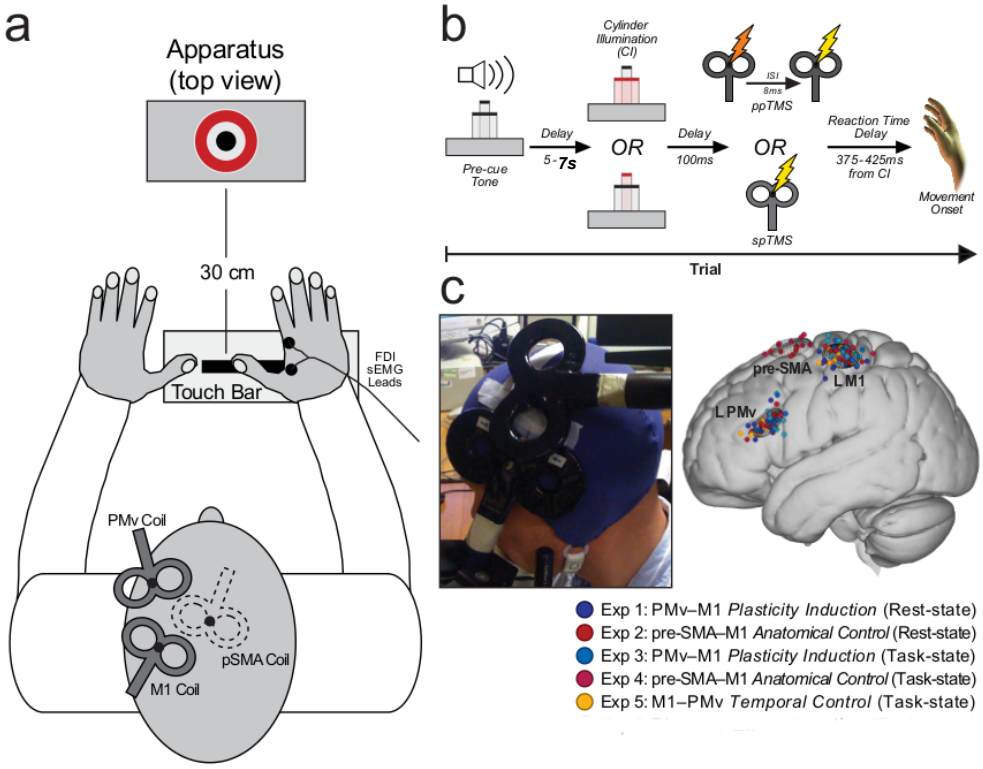

Figure 2.2: **Experimental setup.** (a) Stimuli and coil arrangement. (b) Event timeline for task trials. (c) Individual subject TMS locations (filled circles) and 95 % group confidence ellipses for TMS locations for all experiments in standardized MNI space. For Experiment 2 and 4, TMS was applied to PMv and M1 during the baseline and expression block and to pre-SMA and M1 during the 15 minute intervention.

TMS and electromyography recordings

TMS was applied using two Magstim200 (The Magstim Company Ltd) stimulators each connected to 50 mm figure-of-eight coils. The M1 "hot-spot" was

the scalp location where stimulation evoked the largest right FDI MEP amplitude. This scalp location was projected onto high-resolution T1-weighted MRIs of each volunteer's brain using frameless stereotactic neuronavigation (Brainsight, Rogue Research Inc.). The mean Montreal Neurological Institute (MNI) location for left M1 "hot-spot" [-38 -23 62] (Fig. 2.2c) was similar to that reported previously (Davare et al., 2009, Buch et al., 2010).

Since the TMS intensity used for PMv produced no measurable MEP, PMv coil location was determined anatomically. A marker was placed on each individual's MRI, and adjusted with respect to individual sulcal landmarks to a location immediately anterior to the inferior precentral sulcus. The mean MNI location [-54 21 23] (Fig. 2.2c) lies within the region previously defined as human PMv (Mayka et al., 2006), but is close to area 44, which resembles PMv in cytoarchitecture and connections (Croxson et al., 2005, Petrides et al., 2005). Plasticity induction in Experiment 2 and 4 involved pre-SMA TMS, with the coil located 4cm anterior to scalp vertex (Mars et al., 2009, Neubert et al., 2010) at mean MNI location [-2 7 68] (Fig. 2.2c).

Resting motor threshold (RMT) of left M1 was determined (38.5 ± 7.0 % stimulator output (mean $\pm$ SD)) as previously described (Rossini et al., 1994). As in previous ppTMS studies, PMv and pre-SMA TMS was proportional to RMT (110 %) (Mars et al., 2009, Neubert et al., 2010). M1 stimulation intensity during experiments was set to elicit single-pulse MEPs of $\sim$1 mV (45.5 ± 7.6 % stimulator output (mean $\pm$ SD)). TMS coils were positioned tangential to the skull. The M1 coil was angled at approximately 45° with respect to the midline of the head (handle pointing posteriorly); by evoking a current flowing postero-anteriorly this orientation is optimal to stimulate small hand muscles using a figure-of-eight coil (Brasil-Neto et al., 1992). The PMv coil was placed at approximately 0° relative to the midline (handle pointing anteriorly) (Fig. 2.2c). The pre-SMA coil was angled at approximately 30°, the handle pointing anteriorly towards the

right hemisphere. Coils were fixed in place by adjustable metal arms and monitored throughout the experiment. It is has been shown that PMv-M1 ppTMS effects cannot be attributed just to a direct effect of the PMv coil inducing activity in the M1 coil. We and others have tried applying two pulses of TMS to the M1 coil itself at the same inter-pulse latency while subjects are at rest, performing a similar behavioural task, and while they are performing other behavioural tasks (Davare et al., 2008, Buch et al., 2010, Catmur et al., 2011). In all cases an initial conditioning pulse that is applied directly to M1 does not exert the same task-dependent effects over M1 cortico-spinal excitability as does a PMv pulse. Similarly pre-SMA-M1 ppTMS effects cannot be attributed to the indirect activation of the M1 coil by the conditioning pulse because they are not mimicked by direct application of both conditioning and test pulses via the same TMS coil placed over M1 (Mars et al., 2009).

Right FDI electromyography (EMG) was recorded with bipolar surface Ag-AgCl electrode montages. Responses were band-pass filtered between 10-1000 Hz, with additional 50 Hz notch filtering, sampled at 5000 Hz, and recorded using a CED 1902 amplifier, a CED micro1401 Mk.II A/D converter, and PC running Spike2 (Cambridge Electronic Design, Cambridge, UK). Analysis focused on peak-to-peak amplitudes of MEPs. Trials in which test pulses failed to elicit reliable MEPs were discarded (i.e. an MEP $<0.2\,\mathrm{mV}$, $<5\,\%$ of trials in all subjects). Because for each individual stimulator output had been titrated to approximately evoke 1 mV MEPs, MEP amplitudes were not normalised any further. As no effect of cylinder size has been observed, MEPs from small and large cylinder trials were combined.

Statistical analysis

The median on MEP amplitudes was calculated for each participant, and because the MEP amplitude distribution was positively skewed, median MEP am-

plitudes were log-transformed (Nielsen, 1996). Analysis were conducted using repeated measures analyses of variance (ANOVAs). Greenhouse-Geisser corrections were used to correct for non-sphericity. Data is shown before log-transformation and represents the group mean of individual median MEP amplitudes.

2.4 RESULTS

2.4.1 Experiment 1: PMv-M1 plasticity induction and PMv-M1 interactions measured at rest

For both baseline and expression blocks, ppTMS inhibited MEP amplitudes relative to spTMS trials: ANOVA with within-subject factors BLOCK (baseline and expression) and PULSE (single or paired pulse) revealed a main effect of PULSE ($F_{1,10} = 5.83, P < 0.05$; Fig. 2.3). After plasticity induction, this inhibitory effect was potentiated (BLOCK by PULSE interaction: $F_{1,10} = 5.56, P < 0.05$), while overall cortico-spinal excitability remained unchanged (absence of main effect of BLOCK: $F_{1,10} = 0.65, P = 0.44$; Fig. 2.3). To further clarify that this interaction is driven by relative changes in ppTMS trial excitability, for each individual the mean ppTMS trial MEP amplitude was re-expressed as a ratio of spTMS amplitude. Direct contrast of ppTMS trial ratios across baseline and expression blocks confirmed the inhibitory effect was significantly lower after plasticity induction ($t_{10} = 2.34, P < 0.05$). Hence, repeated coincident activation of PMv and M1 did not induce a simple change in M1 cortico-spinal excitability, but instead selectively enhanced physiological connectivity between PMv and M1. To test if the potentiated ppTMS effect occurred due to changed coil placement in the expression block, we conducted a repeated measures ANOVA on the coefficient of variation on MEP amplitudes; the coefficient of variation is the ratio of the standard deviation to the mean. MEP variability did not change between the

baseline and expression block (no main effect of BLOCK $F_{1,10} = 0.13, P = 0.73$).

It is possible that plasticity is being induced during the baseline and expression blocks themselves. Such a possibility may be thought unlikely though because plasticity induction is expected to be maximal when there is a prolonged period of coinciding pre- and post-synaptic activity on the PMv-M1 synapse, and this is not the case in baseline and expression blocks where 50 % of M1 pulses are presented in the absence of any preceding PMv pulse. Nevertheless we checked for this possibility by re-running our analyses after dividing the baseline and expression periods into early and late periods. There was no significant main effect of block PERIOD (early versus late) or interaction of block PERIOD with the factors of pre- and post-plasticity induction BLOCK and PULSE for either baseline or expression blocks ($F_{1,10} \leq 2.522, P \geq 0.143$).

2.4.2 Experiment 2: pre-SMA-M1 plasticity induction and PMv-M1 interactions measured at rest

The absence of change in M1 cortico-spinal excitability in Experiment 1 suggested plasticity was specific to a particular pathway into M1. To further assess anatomical specificity, Experiment 2 tested whether PMv-M1 potentiation also occurred after the same plasticity induction protocol was applied to a different pathway, between pre-SMA and M1. Significant pre-SMA-M1 ppTMS interactions are also observed at 8 ms inter-stimulus intervals (IPIs) (Mars et al., 2009, Neubert et al., 2010), hence allowing application of the same repetitive ppTMS protocol. No changes in PMv-M1 interactions followed pre-SMA-M1 plasticity induction (absence of main effect of BLOCK: $F_{1,8} = 0.18, P = 0.68$, main effect of PULSE: $F_{1,8} = 3.10, P = 0.12$, and BLOCK by PULSE interaction: $F_{1,8} = 1.27, P = 0.29$; Fig. 2.4).

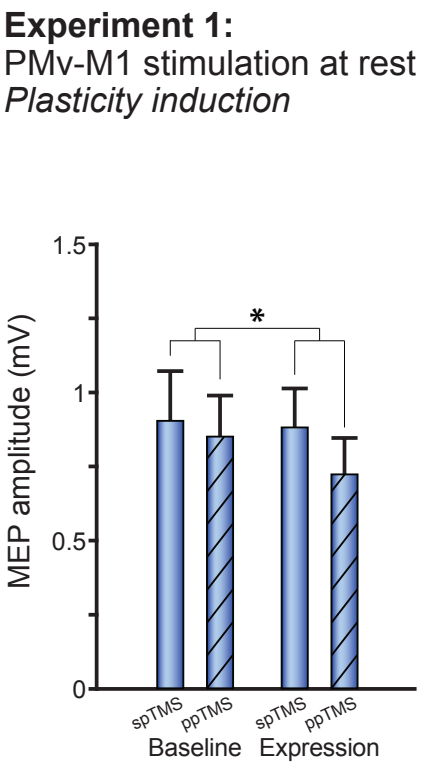

Figure 2.3: **Experiment 1.** Group mean of median MEP amplitudes measured with either spTMS over M1 or ppTMS over PMv and then, with an 8 ms IPI, M1 before (baseline) and after (expression) plasticity induction in the PMv-to-M1 pathway. Measurements were taken while the subjects were at rest and refrained from movement. Asterisks indicate significant effects (* $P < 0.05$). Error bars represent 1 s.e.m.

2.4.3 Comparison of Experiments 1 and 2

As a final check, we also compared the results of Experiments 1 and 2 directly in an ANOVA with the between-subject factor EXPERIMENT and within-subject factors BLOCK and PULSE. A significant three-way EXPERIMENT by BLOCK by PULSE interaction ($F_{1,18} = 5.93$, $P < 0.05$) confirmed potentiation of PMv-M1 physiological connectivity occurred selectively in Experiment 1. A main effect of PULSE ($F_{1,18} = 8.38$, $P = 0.010$) in the absence of a two-way interaction of PULSE by EXPERIMENT ($F_{1,18} = 0.54$, $P = 0.47$) suggested that PMv exerted an

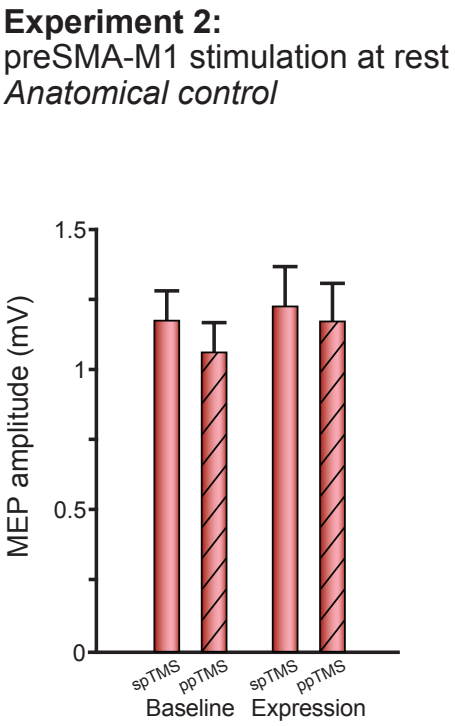

Figure 2.4: **Experiment 2.** Group mean of median MEP amplitudes measured with either spTMS over M1 or ppTMS over PMv and then, with an 8 ms IPI, M1 before (baseline) and after (expression) plasticity induction in the pre-SMA-to-M1 pathway. Measurements were taken while the subjects were at rest and refrained from movement. Error bars represent 1 s.e.m.

inhibitory influence over subsequent M1 pulses. Such an effect has been previously described (Davare et al., 2008, Davare et al., 2009). While statistically significant the effect reported here appears smaller in magnitude, but it should be noted that the coil sizes and procedures followed were not identical to those followed by Davare and colleagues.

Three subjects participated in both Experiments 1 and 2. Differences in results between the two experiments cannot be attributed to participation in more than one experiment for three reasons. First, the mean interval between participation was 75 ± 21 days (mean $\pm$ SD) and there is no evidence for TMS-induced meta-plastic effects over such a period. Second, the significant three way interaction remained after exclusion of all subjects participating in more than one

experiment ($F_{1,15} = 7.13, P < 0.05$), and equally after inclusion of all data plus a covariate indicating order of experimental participation (ANCOVA; $F_{1,17} = 5.60$, $P = 0.03$). In brief, plasticity induction was specific to a particular input pathway.

Background EMG activity could potentially confound MEP measurements if it differed systematically between experimental sessions at the time TMS pulses were applied. A three-way ANOVA (EXPERIMENT by BLOCK by PULSE) was conducted on medians of the root mean square (RMS) of the background EMG in 100 ms windows ending 10 ms before the first TMS pulse (first pulse artefact not included). There were no significant main or interaction effects related to these factors ($F_{1,18} \leq 1.90, P \geq 0.19$). The selective potentiation of PMv-M1 pathway strength at rest after plasticity induction cannot be explained by differences in background EMG activity.

2.4.4 Experiment 3: PMv-M1 plasticity induction and PMv-M1 interactions measured during movement

Up to this point, plasticity expression has been described as potentiation of PMv's resting-state inhibitory influence over M1. As stated earlier however, it has been documented in more than one laboratory with replicable results, that PMv-M1 interactions change when observed in the context of behaviour (Davare et al., 2008, Davare et al., 2009, Buch et al., 2010, Neubert et al., 2010). In Experiments 3, 4, and 5, we exploited the fact that PMv exerts an excitatory influence over M1 100 ms after the illumination of a target for grasping, and assessed whether plasticity induction would be expressed differently in a task context. If the PMv-M1 plasticity induction protocol induces pathway strengthening, then two dramatic changes to the expression of that effect should be observed in the task context. First, plasticity induction should potentiate the excitatory influence of PMv over M1. Second, this potentiation effect might also be apparent on spTMS trials, because MEPs induced by spTMS during grasping

also reflect PMv's endogenous functional influence over M1 (Grol et al., 2007).

In Experiment 3, PMv-M1 plasticity induction significantly increased cortico-spinal excitability on both spTMS and ppTMS trials (main effect of BLOCK: $F_{1,11} = 9.02$, $P < 0.05$; Fig. 2.5a). In this task context, the PMv-M1 pathway is intrinsically activated by grasping, and has been strengthened by the prior plasticity induction protocol. Hence, the impact of even spTMS to M1 is twice what it was prior to plasticity induction. Under such circumstances, it is not surprising that there was a ceiling effect, such that addition of another TMS pulse to PMv, on ppTMS trials, could not excite the PMv-M1 pathway in the expression block any more than it was already excited intrinsically by the act of grasping and by M1 TMS.

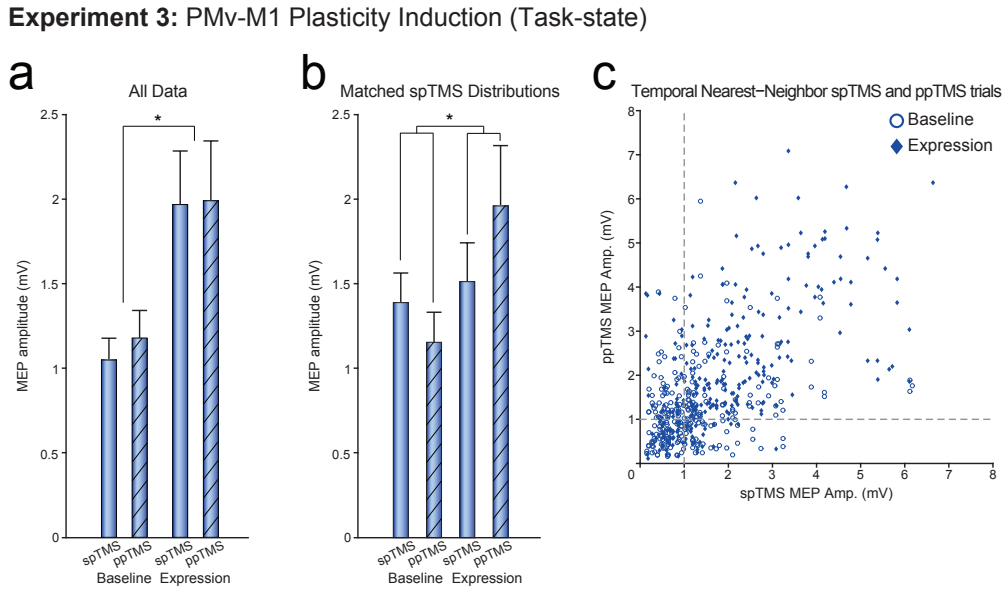

Figure 2.5: **Experiment 3.** Group mean of median MEP amplitudes measured with either spTMS over M1 or ppTMS over PMv and then, with an 8 ms IPI, M1 before (baseline) and after (expression) plasticity induction in the PMv-to-M1 pathway. Measurements were taken while the subject initiated reaching and grasping movements. Data from all trials (a) and after matching of spTMS MEP sizes in the baseline and expression periods (b) are shown. (c) spTMS trials are plotted against temporal nearest-neighbour ppTMS trials for baseline and expression block for all subjects. Asterisks indicate significant effects (* $P < 0.05$). Error bars represent 1 s.e.m.

If it is correct that the PMv-M1 plasticity induction protocol really does induce a change in pathway connectivity between the two regions, analogous to that seen in Experiment 1, but which is confounded by a ceiling effect related to task performance, then two predictions can be made. The first prediction is tested in the next experiment (Experiment 4, see below). The second prediction is that it should be possible to identify a subset of the data where the ceiling effect is minimized. Most subjects exhibit endogenous fluctuation in the sizes of their MEPs during testing sessions. If one assumes that these time-related fluctuations are common to both spTMS and ppTMS trials, then it should be possible to identify portions of the testing session when ceiling effects are least likely. To explicitly test for the presence of a ceiling effect, we devised an unsupervised method where a subset of the data, in which differences in spTMS MEP amplitudes for the Baseline and Expression blocks were minimized, was selected. It is commonly observed in TMS physiology experiments, that MEP amplitudes display stochastic fluctuations that appear unrelated to controlled experimental variables.

An iterative algorithm was used to alternately remove spTMS trial samples from each of the pre-induction and post-induction distributions, so that the scalar difference between the MEP amplitude medians was minimized. Next, the temporal nearest-neighbour ppTMS trials with respect to the selected spTMS trials were found. Thus, ppTMS trials included in the analysis were selected only on the basis of their proximity to spTMS trials of similar size in both blocks. The end result for each subject was a subset of 40 trials, with 10 each from the Baseline block spTMS and ppTMS conditions, and the Expression block spTMS and ppTMS conditions. First, to test if the selection algorithm worked as intended, baseline and induction period single-pulse MEPs were compared using a paired t-test. There was no significant difference between the distributions ($t_{11} = -1.32$, $P = 0.21$). A BLOCK by PULSE repeated measures ANOVA, how-

ever, again revealed the significant main effect for BLOCK ($F_{1,11} = 5.04$, $P < 0.05$) observed in the complete dataset, as well as a significant BLOCK by PULSE interaction ($F_{1,11} = 6.71$, $P < 0.05$; Fig. 2.5b). A scatter plot of spTMS MEP amplitude vs. the temporal nearest-neighbour MEP amplitude suggests that the data agree with our assumption, that endogenous fluctuations of MEP amplitudes are related to time, and affect both spTMS and ppTMS trials in a similar manner (Fig. 2.5c). The scatter plot also shows the part of the distribution from which the data are drawn when the sub-sampling procedure for creating Figure 2.5b is employed, i.e. spTMS-evoked MEP amplitudes of similar sizes in the baseline and expression block. In summary, the results support the contention that pathway specific plasticity induction effects occurred in Experiment 3.

2.4.5 Experiment 4: pre-SMA-M1 plasticity induction and PMv-M1 interactions measured during movement

If it is true that the facilitated MEPs in Experiment 3 reflected a pathway-specific change in connectivity in a pathway used to perform the reaching and grasping task, then the effects should be anatomically specific and should only occur following plasticity induction in the PMv-M1 pathway. To further assess anatomical specificity of the task-related PMv-M1 plasticity induction effects observed in Experiment 3, we tested whether an overall increase in cortico-spinal excitability would occur following plasticity induction in the pre-SMA-M1 pathway. The same induction protocol as in Experiment 2 was applied. While data from Experiment 4 were again consistent with the expected task-related facilitatory effect of PMv on M1 (main effect of PULSE: $F_{1,9} = 8.54$, $P < 0.05$), no changes in excitability or PMv-M1 interactions during performance of the task were observed in the expression block following pre-SMA-M1 plasticity induction (absence of main effect of BLOCK: $F_{1,9} = 0.46$, $P = 0.51$ and BLOCK by PULSE interaction: $F_{1,9} = 1.15$, $P = 0.31$; Fig. 2.6a).

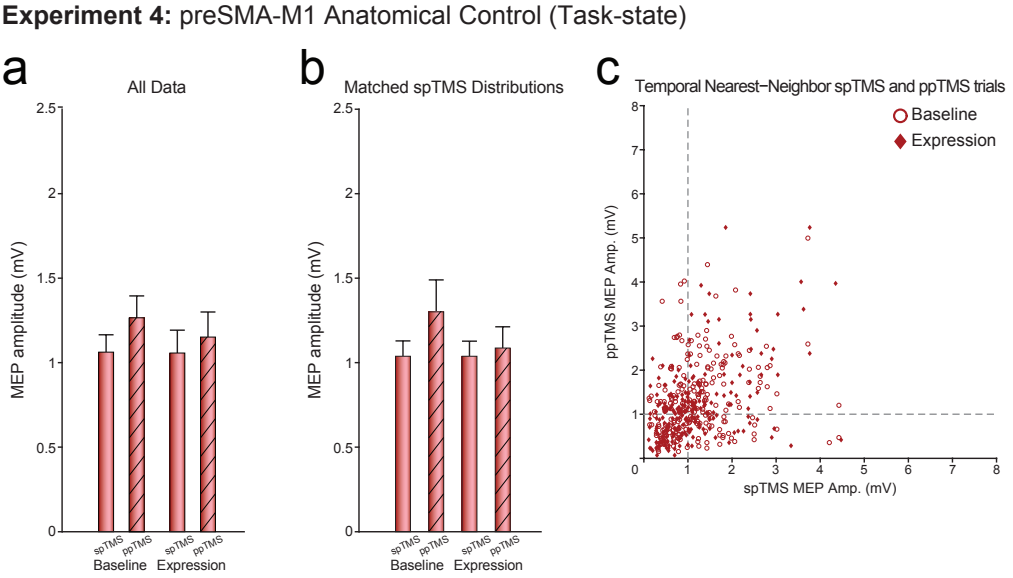

Figure 2.6: **Experiment 4.** Group mean of median MEP amplitudes measured with either spTMS over M1 or ppTMS over PMv and then, with an 8 ms IPI, M1 before (baseline) and after (expression) plasticity induction in the pre-SMA-to-M1 pathway. Measurements were taken while the subject initiated reaching and grasping movements. Data from all trials (a) and after matching of spTMS MEP sizes in the baseline and expression periods (b) are shown. (c) spTMS trials are plotted against temporal nearest-neighbour ppTMS trials for baseline and expression block for all subjects. Error bars represent 1 s.e.m.

Further supplementary analyses, of the sort used in Experiment 3, were carried out on the data from Experiment 4. The employment of similar analyses in both Experiments 3 and 4 tests if any biases in results are introduced via the process of data subset selection. When the post-induction spTMS trials were selected to be the lowest MEP trials or selected to be those that matched the pre-induction spTMS trials (there was no significant difference between spTMS effects pre- and post-induction: $t_9 = 0.10$, $P = 0.93$), a BLOCK by PULSE repeated measures ANOVAs revealed similar outcomes to the full dataset, as only a main effect for PULSE ($F_{1,9} = 5.08$, $P < 0.05$; Fig. 2.6b) was observed. Again, changes in MEP excitability appear to be time-related (Fig. 2.6c).

2.4.6 Experiment 5: M1-PMv plasticity induction and PMv-M1 interactions measured during movement

Hebbian plasticity depends on temporally precise activation of pre- and post-synaptic neurons. If potentiation of excitation in Experiment 3 is due to Hebbian-like strengthening of a specific corticocortical pathway, then changing the timing of PMv and M1 TMS to an interval that is less likely to lead to the same sequence of pre-synaptic and post-synaptic events may lead to a diminished, or potentially even a reversed, change in the influence of PMv over M1 during the induction period. In Experiment 5 we chose to reverse the order of stimulation of PMv and M1, but to keep the same relative latency of stimulation (M1 TMS followed 8 ms later by PMv TMS). Such a sequence of stimulation is unlikely to induce the same sequence of pre- and post-synaptic activity as in Experiment 3. In fact, it allows us to conduct an additional control test of whether PMv TMS exerted its effect during the plasticity induction period not via PMv, but instead via an effect on the nearby M1 coil. If, in Experiment 3, PMv had been exerting its influence over M1, during the 15 minute plasticity induction period, via a direct effect on the M1 coil, then it would not have mattered whether PMv or M1 were stimulated first during each paired pulse delivery in Experiment 5. In this scenario the M1 coil would have been activated twice in both Experiment 3 (indirectly via the PMv coil first and then directly) and in Experiment 5 (directly first and then indirectly via the PMv coil second) and the same pattern of plasticity induction would have been expected in both cases. This, however, was not what was observed. In Experiment 5, reversed order M1-PMv plasticity induction caused a significant decrease in cortico-spinal excitability (main effect of BLOCK: $F_{1,8} = 10.54$, $P < 0.05$, no main effect of PULSE: $F_{1,8} = 2.66$, $P = 0.14$ and no interaction effect of PULSE by BLOCK: $F_{1,8} = 1.44$, $P = 0.27$; Fig. 2.7a).

Further supplementary analyses, analogous to those used in Experiment 3

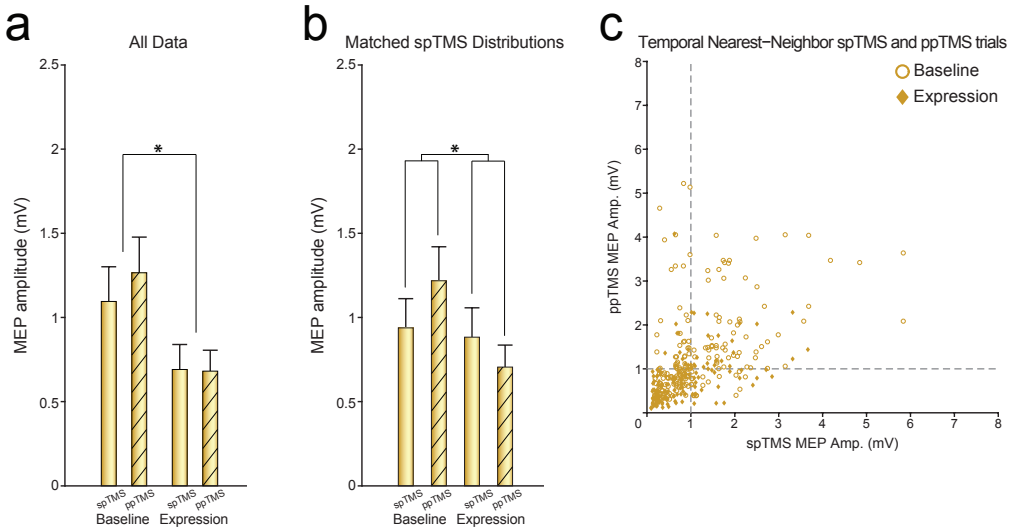

Figure 2.7: **Experiment 5.** Group mean of median MEP amplitudes measured with either spTMS over M1 or ppTMS over PMv and then, with an 8 ms IPI, M1 before (baseline) and after (expression) plasticity induction in the M1-to-PMv pathway (the opposite order of stimulation of PMv and M1 was used in Experiments 3 (Figure 2.5) and Experiment 5 (here). Measurements were taken while the subject initiated reaching and grasping movements. Data from all trials (a) and after matching of spTMS MEP sizes in the baseline and expression periods (b) are shown. (c) spTMS trials are plotted against temporal nearest-neighbour ppTMS trials for baseline and expression block for all subjects. Asterisks indicate significant effects (* $P < 0.05$). Error bars represent 1 s.e.m.

and 4, were also carried out on the data from Experiment 5. Again, using similar analyses in Experiments 3, 4 and 5, first, tests if any biases in results are introduced via this subset selection process and, second, makes it possible to remove a potentially confounding floor effect. A floor effect might obscure differential effects of the plasticity induction procedure on ppTMS and spTMS trials in the post-conditioning block. When the post-induction spTMS trials were selected to be the highest MEP trials or selected to be those that matched the pre-induction spTMS trials (after the manipulation there was no difference between pre- and post-induction spTMS effects: $t_8 = 1.60$, $P = 0.15$), a BLOCK by PULSE repeated measures ANOVA revealed the significant main effect for BLOCK ($F_{1,8} = 6.75$,

$P < 0.05$) as observed in the complete dataset, as well as a significant BLOCK by PULSE interaction ($F_{1,8} = 8.90$, $P < 0.05$; Fig. 2.7b). As in Experiments 3 and 4, the data supported the major assumption of this analysis, as changes in MEP excitability appear to be time-related (Fig. 2.7c). The scatter plot also makes clear the part of the distribution from which the data are drawn when the subsampling procedure for creating Figure 2.7b is employed. In summary, the relative change in spTMS and ppTMS effects after the plasticity induction period again suggests a pathway specific change in connectivity.

2.4.7 Comparison of Experiments 3, 4, and 5

Finally, direct comparison of Experiments 3, 4 and 5 with a three factor ANOVA confirmed that while the task-related ppTMS effect was consistent across experiments (significant main effect for PULSE; $F_{2,28} = 7.64, P < 0.01$), the plasticity induction protocols had quite different impacts on changes in excitability with respect to BLOCK, as a significant EXPERIMENT by BLOCK ($F_{2,28}$ =12.30, $P < 0.001$) interaction was observed. No EXPERIMENT by BLOCK by PULSE interaction was present ($F_{2,28}$ =0.11, $P = 0.90$).

The observed pattern of effects in Experiments 3 and 5 cannot be explained by the fact that four subjects participated in both experiments. First, the mean interval between participation was 159 ± 172 days (mean $\pm$ SD) and there is no evidence of TMS-induced meta-plastic effects over such a period. Second, the significant EXPERIMENT x BLOCK interaction remained after exclusion of subjects who participated in more than one experiment ($F_{1,15} = 29.01$, $P < 0.001$), and after inclusion of all subjects plus a covariate indicating order of experimental participation ($F_{1,18} = 39.92$, $P < 0.001$). In summary, repeated pairing of PMv and M1 TMS led to a strengthening of the PMv-M1 pathway during grasping, whereas inverting PMv-M1 temporal coupling during plasticity induction depressed PMv-M1 interactions during grasping. An analysis of background EMG

activity in Experiments 3, 4 and 5 found no effect of BLOCK, PULSE nor interaction of these factors with each other or EXPERIMENT ($F_{2,28} \leq 2.01$, $P \geq 0.15$). Thus, the changes in excitability in the expression blocks observed during performance of the reaching and grasping task cannot be explained by differences in background EMG activity.

Further additional tests were carried out to test whether the selection of subsets of data from each of experiments 3, 4, and 5 for the supplementary analyses investigating ceiling and floor effects had been carried out in similar ways. The average distance between spTMS and ppTMS trials selected for each pair of samples was 1.35 ± 0.60 trials (mean $\pm$ SD). A BLOCK by EXPERIMENT ANOVA revealed no significant main effects ($F \leq 1.00$, $P \geq 0.38$) or interaction effect (F=1.06, P=0.36) for nearest-neighbour distances. For the expression block, the data subset did not show a consistent tendency towards occurring predominately in the first or second half of the block, as mean location of the median subset trial was trial 24.92 ± 5.8 (mean $\pm$ SD) of the expression block (essentially the mid-point). This suggests that no consistent trend in changes to the post induction excitability occurred while the expression block task was being performed.

2.5 DISCUSSION

I have presented evidence for an altered causal influence of one cortical area upon another using paired-associative TMS over a specific corticocortical connection. Importantly, the stimulation protocol allowed selective strengthening or weakening of connectivity strength between the specific brain regions. The anatomical specificity of plasticity induction was demonstrated by showing that changes to PMv-M1 motor cortical excitability did not occur if another region, interconnected with M1, was subjected to paired associative stimulation in tandem with M1. The results were complemented by the finding that expression

of the effective influence of PMv on M1 was dependent upon the cognitive state at the time of testing (i.e. excitatory during grasping movements and inhibitory during rest).

Several TMS protocols have been shown to induce changes in excitability in M1. Some approaches entail repetitive stimulation of M1 itself (Chen et al., 1997). Others involve stimulation of premotor regions projecting to M1 (Munchau et al., 2002). In addition it is known that TMS-induced modulation of activity in one area, such as pre-SMA, can have a meta-plastic effect on M1 - it changes the ease with which plasticity is subsequently induced in M1 (Hamada et al., 2009). It is also known that changes in M1 cortico-spinal excitability are brought about by paired stimulation of an input into M1, such as the median nerve, and then of M1 itself (Stefan et al., 2000, Wolters et al., 2003). PAS is based upon Hebbian principles of synaptic plasticity and appears to modify connectivity in a controlled manner. Investigations that applied paired-pulse TMS over interconnected cortical sites, for example M1 and the supplementary motor area (SMA) (Arai et al., 2011) and M1 and posterior parietal cortex (Koch et al., 2013), demonstrated altered motor cortical excitability, but they did not present evidence that the effective influence of the first area over the second area was causally determining the observed change. What has been unclear, however, is whether non-invasive paired TMS protocols can induce plasticity in targeted neural pathways between two brain areas in the absence of induced changes in other pathways. The difference is not arcane. It is just such pathway-specific changes that occur in animal models of synaptic plasticity (Markram et al., 1997, Jackson et al., 2006). Second, it is just such pathway-specific changes that are likely to underlie the self-organization proposed to occur in mono-synaptically connected networks in response to regularly occurring input (Sussillo and Abbott, 2009). A potential example of such a network is the one composed of premotor areas centred on the M1 hand representation

(Dum and Strick, 2005). Experiments 1 and 3, in conjunction with control Experiments 2 and 4, demonstrated just such pathway-specific induction of plasticity between two cortical regions, PMv and M1.

The 8 ms interval between the PMv and M1 TMS pulses was chosen for investigation because this inter-pulse interval has been successfully used to probe functional connectivity between PMv and M1 with replicable results in more than one laboratory (Davare et al., 2008, Davare et al., 2009, Buch et al., 2010, Neubert et al., 2010). The effect of conditioning TMS pulses applied to PMv on M1 is anatomically specific and differs from the effects of TMS conditioning pulses applied over other adjacent regions, such as dorsal premotor cortex, pre-SMA and even from the effects of additional conditioning TMS pulses applied directly to M1 itself (Mars et al., 2009, Buch et al., 2010, Neubert et al., 2010). Such a degree of spatial specificity accords with that seen in other types of TMS experiments (Walsh and Cowey, 2000, Walsh and Pascual-Leone, 2003).

PMv-M1 interaction effects are seen throughout a range of IPIs. Although there is evidence that effects at longer IPIs of 12 or 18 ms are mediated by basal ganglia regions including the sub-thalamic nucleus, relatively short latency IPI effects of 8 ms used in the present experiment are transmitted via more direct corticocortical routes (Buch et al., 2010, Neubert et al., 2010). This is consistent with the latency of the fastest route through the basal ganglia, the hyperdirect route via subthalamic nucleus and globus pallidus, exceeding the 8 ms IPI (Nambu et al., 2000, Strafella et al., 2004).

Stimulation of the pre-SMA-M1 pathway was used as a control procedure in Experiments 2 and 4. It is an appropriate control for investigating PMv-M1 effects because interactions between pre-SMA and M1 occur at similar IPIs (Davare et al., 2008, Davare et al., 2009, Mars et al., 2009, Buch et al., 2010, Neubert et al., 2010). Pre-SMA-M1 interaction effects at short IPIs are, like short IPI PMv-M1 interaction effects, not mediated by the basal ganglia

(Mars et al., 2009, Neubert et al., 2010). In some cognitive states conditioning pulses applied over either pre-SMA or PMv facilitate M1 (Mars et al., 2009, Buch et al., 2010, Neubert et al., 2010) although there was little reason to expect the pre-SMA-M1 pathway to play a major role in the grasping task used in Experiments 3-5 or maintenance of a resting posture in Experiments 1-2.

It is well established that the impact of PMv stimulation on M1 stimulation depends on the cognitive state of the participant (Davare et al., 2008, Davare et al., 2009, Buch et al., 2010, Davare et al., 2010, Neubert et al., 2010). At rest, a PMv conditioning TMS pulse reveals the inhibitory influence of PMv on M1 as a decrease in MEP amplitude relative to an unpaired test pulse applied over M1. When performing a behavioural task, however, a conditioning pulse over PMv facilitates the effects of M1 stimulation when actions are being initiated. These effects, first reported by Davare and colleagues, were replicated in Experiments 1 - 2 and 3 - 5, respectively. Analogous state dependency has been demonstrated in other ppTMS experiments focusing on other brain areas (Koch et al., 2006, O'Shea et al., 2007a, Koch et al., 2008, Mars et al., 2009).

The differing impacts of the same PMv stimulation in different cognitive states are easily reconciled with the known anatomy and physiology of premotor connections to M1. Premotor projections to M1 are glutamatergic (Tokuno and Nambu, 2000). Although some projections synapse directly on M1 cortico-spinal neurons, the majority synapse upon GABAergic interneurons in more superficial layers of M1. If the task context results in patterns of input to PMv that biases its output towards projections to interneurons, for example when subjects switch from initiating to refraining from movements (Davare et al., 2008, Duque et al., 2010), then the effect of the PMv pulse will change from facilitating to inhibiting M1 cortico-spinal neurons.

The antagonistic effects of plasticity induction expressed in the rest-state (Experiments 1 - 2) versus task-state (Experiments 3 - 5) experiments can be ex-

plained in a simple and uniform manner as endogenous, state-related excitability changes in the excitatory premotor-M1 pathway. In both Experiments 1 and 3, paired stimulation of PMv followed 8 ms later by stimulation of M1 should potentiate the physiological impact of the excitatory premotor-M1 pathway. When subjects refrain from movement, as in Experiment 1, inhibitory interneurons within M1 are more active; hence, a potentiation of the excitatory premotor-M1 connection synapsing onto M1 inhibitory interneurons will result in a potentiation of suppression of cortico-spinal activity. When reaching and grasping movements are initiated, as in Experiment 3, inhibitory interneurons are less active (Duque and Ivry, 2009, Duque et al., 2010). A potentiation of the excitatory PMv-M1 pathway would, for this network state, result in a potentiation of facilitated cortico-spinal output.

Overall, the specificity of increased PMv-M1 pathway connectivity is less clear in the task-state than in the rest-state which we suggest is due to a ceiling effect. An additional control experiment, in which the stimulator output had been titrated to evoke 1 mV MEPs following spTMS in the Expression phase, might have produced clearer results pointing towards selective pathway enhancement, i.e. relatively greater ppTMS to spTMS measures post-induction compared to pre-induction.

In Experiment 5, a reversed order of stimulation led to weakening of the excitatory premotor pathway. Thus, although the task-state PMv-M1 pathway facilitation was still observed, the facilitatory effect was diminished following plasticity induction. Our results are in accordance with the notion that temporal order of pre- and postsynaptic activity determines the direction of synaptic modification, which is implied in Hebb's original postulate. Similar effects on the PMv-M1 pathway were not seen when plasticity was induced in another pathway involving pre-SMA, even though the end point of both pathways was M1 (Experiments 2 and 4). Rapid sequential activation of two brain areas, A

and B, during plasticity induction can alter the strength of influence A has over B. They are not seen if the same plasticity induction protocol is applied in another pathway into area B (for example from area C to B) with little role in the behavioural conditions examined. This finding is in line with idea that synaptic LTP is an input-specific process (Andersen et al., 1980, Cooke and Bliss, 2006).

One other experiment has looked at similar issues. Rizzo and colleagues applied paired pulses to M1 areas in both hemispheres, so that the first conditioning pulse inhibited the impact of the second on cortico-spinal activity. Repeated paired stimulation did not increase this inhibitory effect further. Instead, after repetitive stimulation, the conditioning pulse impact was diminished (Rizzo et al., 2009). It might be expected that a plasticity induction protocol should have had the opposite effect. Quite why effects emerged in this manner is uncertain. It may reflect induction of homeostatic changes that altered the potential for plasticity. Alternatively, the reciprocal nature of connections between both M1s may make M1-M1 PAS effects particularly complex; a stimulation order optimized for increasing the strength of a pathway from left to right M1 is also likely to be optimal for decreasing strength of the pathway from right to left M1. Either way, the current results are consistent with the suggestion of Rizzo and colleagues that a change in pathway strength can be induced by TMS.

It is becoming clear that plasticity can be induced in relatively specific ways, for example, by pairing TMS and endogenous brain activity (Thabit et al., 2010). The present study suggests PAS might be a useful tool for targeting specific corticocortical pathways, not just in healthy individuals but where recovery of movement depends on establishing new or re-establishing old activity patterns across motor networks (Grefkes et al., 2010). The pathways from PMv and adjacent inferior frontal cortex to M1 and from pre-SMA to M1 are known to exert complimentary roles in the inhibition of unwanted movements

(Aron et al., 2007, Chen et al., 2009, Swann et al., 2009, Neubert et al., 2010) and there have been attempts to modulate the strength of at least one of these areas with transcranial direct current stimulation (Hsu et al., 2011). The present results suggest a way in which directional activity within specific pathways within this network might be modulated.

3

Longevity of effects on motor cortical excitability as induced by paired associative cortico-cortical stimulation

3.1 ABSTRACT

Paired associative transcranial magnetic stimulation (TMS) selectively modulates the impact that activity in one cortical area will have on the activity of another cortical area in a pathway- and direction-specific manner. The current experiment focussed on understanding the longevity of the plasticity induction effect within the stimulated pathway. We demonstrated that plasticity evolved rapidly, lasted for at least one hour, and began to reverse three hours after intervention. These findings might be of importance for the development of potential, pathway-selective neurorehabilitation tools.

3.2 INTRODUCTION

Corticocortical paired associative stimulation (PAS) exploits the principles of associative neuroplasticity. In Chapter 2 it was shown that repeated paired pulses

of transcranial magnetic stimulation (TMS) can be used to selectively modulate corticocortical connectivity between the ventral premotor cortex (PMv) and primary motor cortex (M1). Plasticity in the human motor system is suggested to follow the principles of long-term potentiation (LTP), and in keeping with this PAS-induced cortical plasticity has been shown to evolve rapidly, to persist for a duration of 30 - 60 minutes and to be reversible (Stefan et al., 2000, Wolters et al., 2003, Rizzo et al., 2009). PAS is understood to induce synapse-specific plasticity.

In Chapter 2, I demonstrated a potentiation of the effect PMv exerts on M1 following paired associative stimulation of PMv-M1. The manner in which corticocortical pathway strengthening was expressed reflected the subjects' cognitive state: there was increased inhibition of M1 by PMv at rest and enhanced facilitation of M1 by PMv during the performance of a prehension task (Davare et al., 2008, Buch et al., 2010). The plasticity effects developed rapidly, but were investigated for a duration of only approximately 10 minutes. In order to show further parallels between the corticocortical pathway-specific plasticity induction with TMS and LTP, demonstration of persistence and reversibility of the effects is required. We therefore performed an experiment aimed at understanding the temporal development and longevity of TMS-induced excitability changes. Because the results of Chapter 2 indicated that plasticity effects were most robust when measured during the grasping task, we assessed the longevity of paired PMv-M1 stimulation-induced plasticity in the grasping task state. Measures were taken immediately after repetitive paired stimulation of PMv and M1 as well as 30 minutes and 60 minutes after intervention. Because it emerged that effects did not reverse after 60 minutes, we tested whether they reversed after three hours in a sub-group of five participants.

3.3 METHODS

Volunteers

In this experiment, 12 healthy, right-handed adults (23.3 ± 3.9 years (mean $\pm$ SD); 8 females) were included. All volunteers underwent high-resolution, T1-weighted structural MRI scans. All gave their written consent prior to participation (National Research Ethics Service: Oxford REC C, No.05/Q1606/96).

Summary of Experimental Design

In order to establish the duration of pathway-specific connectivity changes between PMv and M1, motor excitability was measured during the performance of the reaching and grasping task which had previously yielded robust results. The design of this experiment was identical to Experiment 3 in Chapter 2 (Result 2.4.4), with additional expression blocks measured 30 and 60 minutes following the conclusion of the plasticity induction period (Fig. 3.1 A) (as in the study by Rizzo et al., 2009). Each block was composed of 20 spTMS and 20 ppTMS trials. Since the effects appeared to still persist after 60 minutes, data from a fourth expression block commencing three hours after the end of the plasticity period were acquired in five subjects.

Measurement of cortical excitability

The mean MNI location for the left FDI "hot-spot" was [-34 -23 66] and for PMv [-55 18 24] (Fig. 3.1 B). The intensity of the M1 coil was set to 43.4 ± 5.6 % (mean $\pm$ SD) of the stimulator output in order to evoke an MEP of 1 mV amplitude; PMv was stimulated with 41.7 ± 7.0% of stimulator output. Stimulation intensities are similar to the previous experiments.

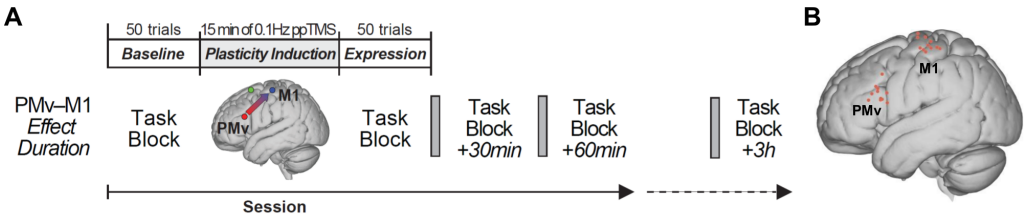

Figure 3.1: **Experimental design for Experiment 2.** At baseline, measures of corticospinal excitability and pathway-excitability were assessed during execution of the grasping task (with 25 spTMS and 25 ppTMS trials, respectively). Plasticity was induced by applying 15 minutes of paired TMS pulses over left PMv and left M1 at a frequency of of 0.1 Hz and with an IPI of 8 ms. Multiple plasticity expression task blocks were used to assess the duration of any plasticity effects (at 0, 30, and 60 minutes following completion of paired associative TMS), again with 25 spTMS and 25 ppTMS measurements. The effect duration was measured in an additional expression block three hours after completion of the plasticity period in five subjects. (B) Individual TMS stimulation locations in standardized MNI space (left hemisphere).

TMS plasticity induction

The protocol was identical to the one described in Methods 2.

Statistical analysis

Repeated-measures ANOVAs with independent variables PULSE and BLOCK and dependent variable MEP were calculated. The analysis was performed on each participant's median MEP amplitude; the figures represent the group mean of the median MEP amplitudes. For the correction of non-sphericity, Greenhouse-Geisser corrections were used. Two-tailed paired samples student's t-tests were performed to determine whether MEP amplitudes differed for each time block. Because of the clear directional nature of each hypothesis at 3 hours after plasticity induction, and because each test was predicated on the earlier results reported for this experiment (Results 2.4.4), we used one-tailed t-tests when examining the results from this sub-sample of subjects.

3.4 RESULTS

3.4.1 Temporal development of TMS-induced excitability changes as measured during grasping

Plasticity expression had been most robustly measured whilst participants were engaged in a reach and grasping task and for that reason the duration of PMv-M1 plasticity expression was assessed by collecting identical expression block data as in Experiment 3 in Chapter 2 (Result 2.4.4), during task performance on three occasions: immediately (0 minutes), 30 minutes and 60 minutes after plasticity induction.

As in the first analysis of the data from Experiment 3 in Chapter 2 (Result 2.4.4), we found an increase in cortico-spinal activity after plasticity induction in the current experiment (repeated-measures ANOVA with factors BLOCK (four levels: baseline and three expression blocks at 0, 30, and 60 minutes) and PULSE (spTMS and ppTMS): main effect of BLOCK ($F_{3,30} = 3.80, P = 0.028$)). It can be seen from Figure 3.2 A that the effect of plasticity induction remained at a fairly constant level across the 60 minute period. An additional planned comparison revealed that cortico-spinal activity remained higher even at the time of the 60 minute post-induction expression block in comparison to the baseline block ($t_{10} = -2.66, P = 0.024$ for spTMS and $t_{10} = -2.25, P = 0.048$ for ppTMS).

Because it soon became apparent that the impact of plasticity induction was protracted beyond 60 minutes, we administered an additional post-induction expression block 180 minutes after plasticity induction in a sub-group of five subjects whereby we assessed whether cortico-spinal excitability eventually began to reverse. First, we tested whether it was possible to detect an increase in cortico-spinal excitability between the baseline and the first expression block (0 minutes) in this small sub-sample. Second, we tested whether there was evidence of a decrease in plasticity induction in the 180 minute expression block

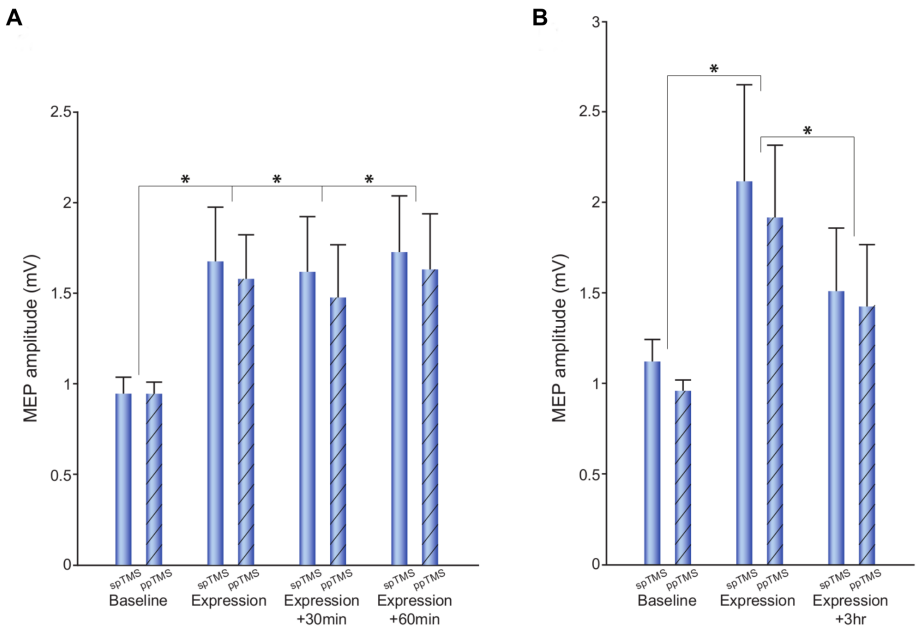

Figure 3.2: **Duration of induced plasticity effects as measured during grasping.** (A) Group mean of median MEP amplitudes measured with either spTMS over M1 or ppTMS over PMv and then, with an 8 ms IPI, M1 before (baseline block) and in three subsequent expression blocks collected 0, 30, and 60 minutes after plasticity induction in the PMv-to-M1 pathway. Measurements were taken while the subject initiated reaching and grasping movements. (B) Similar data are shown for a sub-group of five participants tested in expression blocks conducted 0 and 3 hours after plasticity induction. Asterisks indicate significant effects (* $P \leq 0.05$). Error bars represent 1 s.e.m.

in comparison to the 0 minute expression block. We carried out the test in this way because we reasoned that it was critical for a claim of reversal to be based on something other than a null effect. We focused on the 0 and 180 minute expression blocks with the argument that some degree of reversal of the effect of plasticity induction might occur a little earlier in some subjects than others (for example, at 60 minutes) and so the most sensitive test for reversal would entail a comparison of the 0 and 180 minute expression blocks.

Due to the clear directional nature of each hypothesis and because each test was predicated on the earlier results reported for Experiment 3 (Result 2.4.4) and the current Experiment 2 at time points 0, 30 and 60 minutes after plas-

ticity induction, we used one-tailed t-tests when examining the results from this sub-sample of subjects. In summary, we found that PMv-M1 plasticity induction did increase cortico-spinal excitability during a grasping task in this sub-group of subjects (comparison of baseline and 0 minute expression block; spTMS: $t_4 = 2.08, P = 0.053$, ppTMS: $t_4 = 2.40, P = 0.037$) and that this effect had significantly reversed 180 minutes later (comparison of 0 and 180 minute expression blocks; spTMS: $t_4 = 2.75$, $P = 0.025$; ppTMS: $t_4 = 2.48$, $P = 0.034$) (Fig. 3.2 B).

3.5 DISCUSSION

Our novel non-invasive plasticity induction protocol enhanced interregional connectivity in a pathway critical for object-hand interactions. Here, I investigated the longevity of excitability changes and replicated the findings of enhanced cortico-spinal excitability from Experiment 3 in Chapter 2. Plasticity was shown to last for at least one hour and to begin reversing at three hours after intervention when assessed during grasping. The sustained increased pathway connectivity for more than one hour makes the protocol an interesting tool for neurorehabilitation in stroke patients. By selectively enhancing connectivity in a pathway crucial for initiation and execution of grasping movements the protocol may prime the connection for motor learning and may favourably affect retention of newly learned motor behaviour. In recent years, the effect of combining neuromodulatory approaches with physical therapy have been investigated and shown to augment training effects in some stroke patients; the current protocol induces changes in neural coupling that are expressed for a period long enough to implement motor training.

Beyond understanding the duration of plasticity expression, it is crucial to understand the neurophysiological mechanisms of this plasticity-inducing protocol. In follow-up studies, one may want to explore whether pathway plas-

ticity induction leads to changes in specific interneuron populations of M1, such as inhibitory interneurons generating short-interval intracortical inhibition (SICI) or excitatory interneurons generating short-interval intracortical facilitation (SICF). Corticocortical pathways are known to project to later I wave-generating M1 populations, which can be investigated by with SICF stimulation protocols (Cattaneo et al., 2005). It may be possible that synapses between glutamatergic projections from PMv and late I wave-generating M1 interneurons are selectively potentiated by our paired-pulse plasticity induction protocol.

Combining protocols altering background excitability with the current protocol may be another means by which we can explore the neurophysiological mechanisms underlying this corticocortical pathway strengthening. A network's excitability state - as induced with transcranial direct current stimulation (tDCS) - has been shown to have a differential effect on subsequent plasticity induction.

There are several accounts of single-site stimulation paradigms (repetitive TMS (rTMS)) altering cortical excitability that interact *homeostatically* with tDCS-induced changes to background excitability (Siebner et al., 2004, Lang et al., 2004). One interpretation is that rTMS and tDCS induce similar effects on network excitability. Both rTMS and tDCS after-effects are dependent upon N-methyl-D-aspartate (NMDA) receptors (Liebetanz et al., 2002, Nitsche et al., 2003, Huang et al., 2007) and have been linked to shifts in intracortical inhibition and facilitation (Tergau et al., 1997, Nitsche et al., 2005). If rTMS and tDCS modulate synaptic strength within the same intracortical neuronal populations, it is likely that homeostatic processes have to occur in order to keep synaptic weights within a dynamic range (Nitsche et al., 2007). Homeostatic processes are important to protect neural networks from becoming destabilised (Hulme et al., 2013).

In contrast, tDCS-induced increases in excitability have been shown to

act *synergistically* upon induction of spike timing-dependent plasticity (STDP). When tDCS-network modification precedes classical PAS (i.e. peripheral nerve stimulation paired with M1 TMS) (Nitsche et al., 2007), excitability is enhanced and the effect prolonged. It may be that excitability increases as evoked by our paired associative TMS paradigm can be enhanced - in size and duration - by means of tDCS-induced tonic changes in membrane potential (Purpura and McMurtry, 1965) that modify neuronal firing probability (Bikson et al., 2004, Ruffini et al., 2013). Induction of LTP in glutamatergic synapses is dependent upon NMDA receptors which function as a coincidence detector of pre- and post-synaptic activity. Coinciding synaptic activity causes the removal of the receptor's Mg^{++} block and allows for influx of Ca^{++} ions into the postsynaptic cell (Mayer et al., 1984, Nowak et al., 1984). Preconditioning with tDCS may lead to a reduced membrane potential in the postsynaptic M1 neuron and ease Mg^{++} unblocking so that detection of presynaptic, glutamatergic PMv projections may be facilitated and lead to increased and prolonged neuroplasticity. Most artificially induced plasticity effects, induced with non-invasive brain stimulation protocols, are weak and rarely last longer than 30 minutes (Huang et al., 2005).

Importantly, there is a relationship between associative plasticity and learning. Associative plasticity, the modification of synaptic strength between neurons by synchronous activation of synaptic input, has been proposed to form the neurophysiological basis of learning (Rioult-Pedotti et al., 2000) and in humans, motor and visuomotor coordination learning can be improved by altering background network excitability whilst learning (Nitsche et al., 2003, Antal et al., 2004). If our associative plasticity protocol was to induce effects similar to learning, it may be that a combination of tDCS and corticocortical PAS evokes a synergistic effect on neuroplasticity. Overall, by testing whether a combination of excitability-increasing tDCS and corticocortical PAS results in either

synergistic or homeostatic effects on motor excitability, one may gain additional insight into the neural substrate of the pathway effect.

There is a growing interest in the neuroscience and clinical community to develop brain stimulation protocols to manipulate brain function with the ultimate aim of enhancing motor performance and cognitive performance (Krause and Cohen Kadosh, 2013) or to correct abnormal network interactions (Fox et al., 2012). Preliminary evidence exists that suggests that relatively-short lived plasticity effects are cumulative in nature and multiple sessions lead to prolonged increased facilitation (Bäumer et al., 2003, Valero-Cabre et al., 2008). Moreover, there is evidence that stroke patients who received daily rTMS-induced excitability modulation over the course of one week presented with better motor function one year later (Khedr et al., 2010). Different avenues are being explored for achieving changes in excitability that last for more than a short period. If excitability modulation opens a "sensitive" window in which, for example, neurorehabilitative motor training might be more efficient, it seems desirable to have a period of at least one hour in which manipulations could be carried out. Furthermore, it may be that prolonged increased synaptic efficacy leads to lasting anatomical changes via cellular mechanisms - such as axon sprouting, dendritic branching and myelin remodelling (Zatorre et al., 2012).

4

Causal manipulation of functional connectivity in a specific neural pathway during behaviour and at rest

4.1 ABSTRACT

Correlations in the activity of brain areas (functional connectivity) as measured with functional magnetic resonance imaging (fMRI) has been shown to reflect their underlying structural connectivity. Here we examine the possibility that functional connectivity also reflects short-term changes in synaptic efficacy. Paired transcranial magnetic stimulation (TMS) near ventral premotor cortex (PMv) and primary motor cortex (M1) strengthens physiological connectivity in a pattern consistent with Hebbian plasticity. Here we demonstrate that this potentiation of synaptic efficacy also leads to an increase in fMRI-measured functional coupling between PMv and M1. Moreover we show that strengthening connectivity between the two nodes has effects on a wider network of areas. We found increased coupling between PMv and the anterior intraparietal area (AIP), the parietal area providing key inputs into PMv. At the same time, functional

coupling in the other main cortical sensorimotor circuit (area V6A - dorsal premotor cortex - M1 pathway) was significantly reduced. Change in coupling strength varied as a function of whether or not functional connectivity was measured at rest or during a simple grasping task. An additional TMS experiment revealed that at rest the physiological influence of PMv on M1 was inhibitory whereas during grasping PMv facilitated M1. Both facilitatory and inhibitory causal influences of PMv onto M1 were reflected in positive functional coupling as measured with fMRI. A final control experiment revealed that application of an identical number of TMS pulses to identical brain regions at identical frequencies led to no change in fMRI-measured functional coupling when the inter-pulse interval was too long to expect Hebbian-like plasticity to occur.

4.2 INTRODUCTION

Correlations in spontaneous fluctuations in brain activity of functional magnetic resonance imaging (fMRI), often described as functional connectivity (Friston et al., 1995), have provided insights into the anatomical and physiological organisation of neural networks (Fox and Raichle, 2007, Smith et al., 2009, Smith et al., 2012, O'Reilly et al., 2013). Neural networks can be thought of as interconnected nodes that influence each other. While functional connectivity refers to the statistical correlations that exist between activity in different brain areas, effective connectivity is the term used to describe the causal influence that one neural network node has on another (Friston et al., 1995). One way to measure effective connectivity is paired-pulse transcranial magnetic stimulation (TMS), where a conditioning-pulse is applied to one cortical area and a subsequent test-pulse is applied to a second area (Pascual-Leone and Walsh, 2001). For example, when then test-pulse is applied to the primary motor cortex (M1) where it has an impact on corticospinal activity itself, then the modulating influence of the interconnected area on corticospinal activity can be assessed by means of a conditioning-pulse preceding the test-pulse (Mochizuki et al., 2004,

Koch et al., 2007, Boorman et al., 2007). Effective connectivity measurements made with paired-pulse TMS not only assess the strength of impact one node has upon another, but also provide information about the role of the pathway in a cognitive process (Neubert et al., 2010) and whether the net impact of one area on another in a given cognitive context is excitatory or inhibitory (Davare et al., 2008, Buch et al., 2010). Effective connectivity is thought to be based upon synchrony of activity levels of interconnected neuronal populations, which in turn can generate this synchronous activity through their interconnection via synapses (Passingham et al., 2002).

In a previous study, we have shown that we can manipulate effective connectivity by means of cortico-cortical paired-associative TMS (paTMS). The intervention was based on principles of spike-timing dependent plasticity (STDP). We were able to increase pathway connectivity in a selective manner in line with principles of synaptic plasticity (Buch, Johnen et al., 2011, Chapter 2).

The current study first seeks to establish if it is possible to *induce* changes in functional connectivity between specific areas within a neural circuit through the application of the paTMS protocol which has been shown to manipulate effective connectivity. Changes in functional interactions may be expected between directly stimulated network nodes as well as between more distant nodes that were not subject to paired stimulation. Contrary to TMS-evoked motor responses, which predominantly represent pyramidal cell output activity, fMRI measures of blood oxygen level-dependent (BOLD) responses indirectly reflect neural input into a cortical area (Logothetis et al., 2001, Goense and Logothetis, 2008). fMRI is a brain imaging technique that is based on the idea of neurovascular coupling whereby there is relationship between local neural activity and subsequent changes in cerebral blood flow. Importantly, the BOLD fMRI signal cannot distinguish between excitatory and inhibitory neural processes (Logothetis, 2008).

Second, the study attempts to establish a link between fMRI-derived functional connectivity measures and paired-pulse TMS-derived effective connectivity measures by comparing correlations of the two measures across subjects. Connectivity indices derived from fMRI and from paired-pulse TMS are related to and constrained by anatomical connectivity (Passingham et al., 2002, O'Reilly et al., 2013). They furthermore have in common that they have established that network node interactions are context-dependent. Different cognitive states - e.g. resting-state and task performance - are associated with shifts in connectivity weights, and thereby with different patterns of neural network organisation (Hampson et al., 2002).

In Experiment 1, we induced increased coupling in a specific cortico-cortical pathway by paTMS of left ventral premotor cortex (PMv) followed by left M1 at a 8 ms interpulse interval (IPI). The protocol was identical to the one used previously that has provided evidence that it specifically enhances the causal influence of PMv on M1 (Buch, Johnen et al., 2011, Chapter 2). The stimulated network nodes are two regions within an extended parieto-frontal circuit important for object-hand interactions during movement (Jeannerod et al., 1995, Fogassi et al., 2001, Davare et al., 2006). The pathway is distinctly coupled during performance of reaching-and-grasping movements and during resting. PMv exerts a predominantly excitatory influence over M1 when subjects are reaching and grasping. This influence becomes inhibitory at rest (Davare et al., 2008, Buch et al., 2010). Since characteristic coupling modes within the stimulated connection are related to different cognitive states, the circuit serves as a suitable model system to investigate whether paTMS affected the pathway differently during prehension performance and during resting-state.

STDP models assume connectivity changes occur when neurons fire in quick succession. In Experiment 2, we therefore examined whether changes in functional connectivity between PMv and M1 could still be induced with a 500 ms

IPI, an interval many times longer than the longest one at which PMv-M1 interactions have been shown to occur (Davare et al., 2008, Neubert et al., 2010). Despite the longer IPI, Experiment 2 employed an identical number of TMS pulses applied at an identical frequency (0.1 Hz) to identical brain areas.

In Experiment 3, we directly linked paired-pulse derived connectivity measures with fMRI-derived functional connectivity measures for the PMv-M1 pathway. We tested for correlations in the two measures across subjects, both during rest and during grasping movements since different cognitive states have been associated with reversal of the sign of net effective influence of one neural node over another.

4.3 METHODS

Volunteers

Twenty-three healthy volunteers participated in the study (age: 24 ± 4 years (mean $\pm$ SD), twelve males). Written informed consent was obtained before participation for TMS stimulation (Berkshire REC, REF 11/SC/0537, version 1.2) and fMRI acquisition (MKREC REF 07/Q1603/11, version 2.0). Fifteen subjects (eight males) participated in Experiment 1 (8 ms IPI). Fifteen subjects (nine males) participated in Experiment 2 (500 ms IPI). For Experiment 3, paired-pulse TMS data was obtained for ten participants from Experiment 1 (five males).

Summary of experimental design

We used fMRI to explore changes in functional coupling of network nodes and altered network dynamics following the application of a corticocortical plasticity induction protocol. Cortical plasticity was induced with the same protocol as previously (Buch, Johnen et al., 2011, Chapter 2). paTMS stimulation over left PMv and left M1 at a frequency of 0.1 Hz for 15 minutes (Fig. 4.1 A and D)

has been shown to selectively enhance the neurophysiological connectivity of the two stimulated, connected brain regions. Each stimulation pulse over PMv preceded the stimulation pulse over M1 by 8 ms. The protocol is designed to evoke concurrent presynaptic and postsynaptic activation and thereby induce synaptic plasticity in the stimulated pathway. The paTMS protocol was applied outside the MR scanner ("off-line") (Fig. 4.1 C). Using a within-subject design, participants underwent two separate fMRI scans in counterbalanced order, one scan before paTMS (baseline) and one scan after paTMS (post-TMS). For all participants the two scans were acquired on different days. This ensured that TMS-induced effects, which last for at least one hour (Buch, Johnen et al., 2011; Chapter 3), were no longer present when acquisition of the baseline scan followed acquisition of the post-TMS scan. The expression of plasticity has been shown to depend on the participant's cognitive state: if the participant is at rest the influence of PMv on M1 is predominantly inhibitory whereas during the performance of a naturalistic grasping task the effect of PMv on M1 is predominantly facilitatory (Buch, Johnen et al., 2011; Chapter 2). To understand if the paTMS plasticity induction protocol evokes distinct changes in functional coupling or in network connectivity depending on cognitive state - i.e. in the resting state and in the task state - fMRI data were collected during blocks when subjects were reaching and grasping and blocks when they remained at rest. Acquisition of resting-state and continuous grasping fMRI data (task-positive) was counterbalanced (Fig. 4.1 D).

My previous study (Buch, Johnen et al., 2011) demonstrated that the causal influence of the first node on the second node can be manipulated via the paTMS intervention in a controlled manner according to the principles of STDP. STDP refers to the way that synaptic modification is dependent on the order of presynaptic and postsynaptic spiking within a critical window of tens of milliseconds. Synaptic efficacy is augmented when presynaptic activity pre-

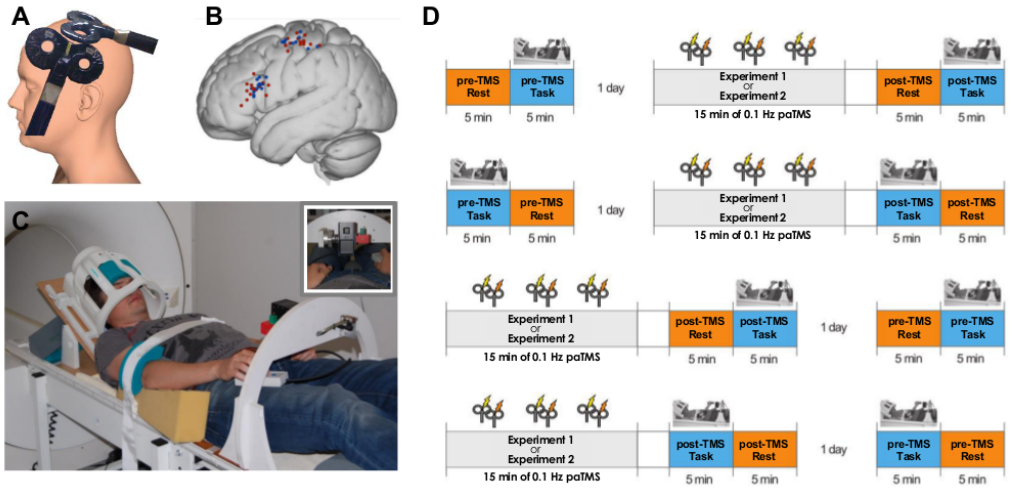

Figure 4.1: **Experimental design.** (A) Ninety paired-pulses of TMS were delivered over left PMv and M1 with an IPI of 8 ms (Experiment 1) or an IPI of 500 ms (Experiment 2). (B) Individual stimulation locations (red circles: Experiment 1; blue circles: Experiment 2) in standardized MNI space. (C) Participants performed visually-guided grasping movements towards one of two objects (small or large; see inset) while lying supine in the MRI scanner. The head coil was tilted forward by 30° to allow for direct line of sight of the objects to be grasped. A response button box was positioned on the upper leg. (D) Experimental design and setup for all experiments (for either Experiment 1 or Experiment 2). Order of resting-state and task-positive fMRI as well as baseline and post-TMS were counterbalanced.

cedes postsynaptic activity by such an interval. It is plausible to expect synchronous presynaptic and postsynaptic activation when TMS pulses are applied over PMv and M1 at an interval of 8 ms if we consider likely PMv-M1 pathway length (Schmahmann et al., 2007), and approximate transmission times established in previous studies (Edgley et al., 1997, Olivier et al., 2002, Caminiti et al., 2013), as well as parameters successfully used in previous paired-pulse TMS studies of human PMv-M1 interactions (Davare et al., 2008, Buch et al., 2010, Buch et al., 2011). A crucial aspect of STDP is its temporal asymmetry: synaptic strength is diminished when stimulation is reversed and occurs on the postsynaptic site prior to presynaptic activity. The PMv-M1 paTMS plasticity induction effect exhibits similar characteristics (Buch, Johnen

et al., 2011; Chapter 2). To test whether changes in functional connectivity were dependent on the IPI of 8 ms, I conducted a control experiment with an IPI of 500 ms whilst keeping all other parameters identical (i.e. intensity of the stimulation, total number of pulses, and total duration of stimulation).

Transcranial magnetic stimulation (TMS)

TMS was applied using two Magstim 200 stimulators each of which was connected to a 50 mm figure-eight coil. On a day prior to the day of the combined TMS-fMRI experiment, resting motor threshold (RMT) was determined for each participant for the left M1 "hot-spot", which is the scalp location where stimulation evoked the largest motor-evoked potential (MEP) amplitude in right first dorsal interosseous (FDI) (Rossini et al., 1994) (40 ± 7 % stimulator output, mean $\pm$ SD). Electromyography (EMG) activity of right FDI was recorded with bipolar surface Ag-AgCl electrode montages. Responses were bandpass filtered between 10 and 1000 Hz, with additional 50 Hz notch filtering, sampled at 5000 Hz, and recorded using a CED 1902 amplifier, a CEDmicro1401 Mk.II A/D converter, and PC running Spike2 (Cambridge Electronic Design).

Plasticity induction using paired-associative TMS

On the day of application of the paTMS protocol, the scalp location of the left FDI "hot-spot" was confirmed and the location was projected onto the high-resolution, T1-weighted MRI brain scan of each participant using frameless stereotactic neuronavigation (Brainsight; Rogue Research). The mean Montreal Neurological Institute (MNI) location for left M1 "hot-spot" [-40 -18 59] was similar to that reported previously (Davare et al., 2009, Buch et al., 2010) (Fig. 4.1 B). For plasticity induction, PMv coil placement was determined on the basis of sulcal anatomy, and was just ventral to the convergence of the inferior precentral sulcus and inferior frontal sulcus and therefore at the border of dysgranu-

lar premotor-prefrontal transition area inferior frontal junction (IFJ) and ventral premotor area 6v (Neubert et al., 2014). For brevity we refer to the area as PMv. The mean MNI location for PMv was [-56 19 19] (Fig. 4.1 B).

As in our previous paTMS study (Buch, Johnen et al., 2011), left PMv was stimulated with 110 % of RMT and left M1 with a stimulation intensity to elicit a 1 mV MEP following a single TMS pulse (stimulator output 47.0 ± 6.7 (mean $\pm$ SD). The paTMS lasted for 15 minutes and was applied at a frequency of 0.1 Hz, with an IPI of 8 ms in an attempt to induce corticocortical plasticity (Experiment 1) and an IPI of 500 ms in Experiment 2 (for details see 4.3 "Summary of Experimental Design"). For all stimulation experiments, TMS coils were fixed in place tangentially to the skull by means of adjustable metal arms and monitored throughout the experiment.

Experimental setup

For image acquisition during resting as well as during grasping, participants lay supine in the MR scanner with the eight-channel head coil being tilted forward by 30°, (Fig. 4.1 C). This setup enabled participants to perform a naturalistic visually-guided reaching-and-grasping task in front of their bodies. Participants were allowed to move their eyes in order to guide their movements. An optical response button box was placed on their right upper leg and served as a start-and-finish position. Reaction times and total movement times were recorded. With the aim of avoiding movement artefacts, the participant's upper arm lay on a wedge-shaped polyfoam cushion and was firmly, but comfortably strapped to the side of the participant's chest. This setup constrained rotation movements in the plane between the button box and the target objects. The head was supported with foam wedges. The participants had received extensive training in the reaching-and-grasping task at least one day prior to the first MRI acquisition outside the MR scanner. The target object, which consisted of a large red

cube and a small green cube (Fig. 4.1 C, inset), was held in place through an arc-shaped device positioned over the participant's hips. Participants had been instructed to grasp for one of the two cubes, to slide it out of its supporting rail on a rectangular box, and to return it into the same supporting rail. On a given trial, either the large red or the small green cube was to be grasped. A red or green light-emitting diode (LED) in the middle of the rectangular box instructed the participant which cube to grasp. MR-compatible switches on the device recorded the time at which the object was removed from the supporting rail and the time at which the object was returned into the supporting rail. Control of LEDs and recording of movement-related responses was performed with a computer running Presentation 15.0 (Neurobehavioral Systems, San Francisco, CA). TMS stimulation was conducted outside the MRI scanner room. Participants walked over to the MRI scanner and scanning was started after a short delay of approximately three to four minutes. We have previously shown that plasticity expression effects last for at least one hour (Buch, Johnen et al., 2011; Chapter 3) and therefore, TMS-induced effects should not be attenuated by a delay of several minutes.

Image acquisition

MRI data were acquired on a Siemens 3T Trio MRI scanner at the Oxford Centre for Clinical Magnetic Resonance Imaging (OCMR). For purposes of neuronavigation-guided TMS stimulation, all volunteers underwent high-resolution, T1-weighted structural MRI scans that included nose and ears. For each condition - resting-state and grasping-task - five minutes of whole-brain T2*-weighted gradient echo planar images (EPIs) sensitive to BOLD were acquired with the following parameters: repetition time = 3.000 ms, echo time = 30 ms, flip angle = 87°, isotropic voxels of 3.0 mm, no slice gap, 45 slices in axial direction. Participants were instructed to keep their eyes closed during resting-

state fMRI. During the reaching and grasping task, participants performed 66 reaching-and-grasping trials for either a small or a large cube positioned in front of them (Fig. 4.1 C). A new trial sequence was generated for every participant and for each session, with an intertrial interval (ITI) of 4295.5 ms to 4795.5 ms (4545.5 ± 145.5 ms; mean $\pm$ SD). This ITI was such that every participant could perform the movement within the time window.

Image pre-processing

Resting-state and grasping-task fMRI data were pre-processed in the same manner using tools from the FMRIB Software Library (FSL; fmrib.ox.ac.uk/fsl) (Smith et al., 2004). Pre-processing involved: motion correction, brain extraction, spatial smoothing with a Gaussian 5 mm full-width at half-maximum kernel, and high-pass temporal filtering at 100 s.

Selection of regions of interest

The majority of fMRI analyses focussed on regions of interest (ROIs) with a well-established association with object grasping (Fig. 4.2). Grasping of an object is controlled via an anatomically well-defined fronto-parietal network comprising the areas that we stimulated with TMS, PMv and M1, as well as the anterior intraparietal (AIP) area, the anterior aspect of the occipito-parietal sulcus (area V6A) and the caudal part of PMd; a relatively early visual area, V3A, provides input into the network (Tanné-Gariépy et al., 2002, Galletti et al., 2003, Brochier and Umilta, 2007). We assessed (1) how functional connectivity between stimulated nodes, PMv and M1, changed in response to the repeated application of paired TMS pulses; (2) how the functional connectivity of stimulated nodes with other grasping-related nodes changed; (3) what other changes in functional connectivity evolved between other fronto-parietal areas after PMv and M1 were stimulated together. Because all of these areas are likely to be in

distinct functional configurations during rest and during grasping, we hypothesised that functional coupling might be modulated differently in the two cognitive states by the repeated application of paired TMS pulses. We used 6 mm diameter masks to extract BOLD time series at each ROI. ROI masks were created in MNI space. The ROI mask for M1 was based upon the meta-analysis of functional brain imaging data of motor control (Mayka et al., 2006). The PMv ROI location was then identified by finding the region in which BOLD activity was significantly correlated with activity in M1 at the group level, both during rest and during the grasping task. Selecting a ROI that was correlated with M1 activity both at rest and during grasping allowed demonstrating the TMS-evoked manipulation of an existing correlation. The peak resulting MNI coordinate [-58 4 30] was located in what is defined as PMv by Mayka et al. (Mayka et al., 2006) which had a centre-of-mass at [-52 4 24]; more specifically, it comes to lie in the 6v/F5c subdivision of PMv identified by Neubert and colleagues (Neubert et al., 2014). The ROI masks for AIP, PMd, V6A, and V3A were the same size, but were centred around the group peak activation average coordinates of an fMRI study that employed a similar visually-guided grasping task (Grol et al., 2007). Masks were registered to individual EPI space in a two-step process: the mask was transformed into individual, high-resolution structural space via non-linear registration (FSL FNIRT) (Andersson et al., 2007) and then into individual functional space via affine registration (FSL FLIRT) (Jenkinson and Smith, 2001).

Network analyses

We conducted three analyses that focussed on correlations of spontaneous low-frequency oscillations in BOLD activity to investigate how in response to PMv-M1 paired TMS pulse plasticity induction. We extracted the BOLD time courses from the ROIs described above and examined their correlations before and af-

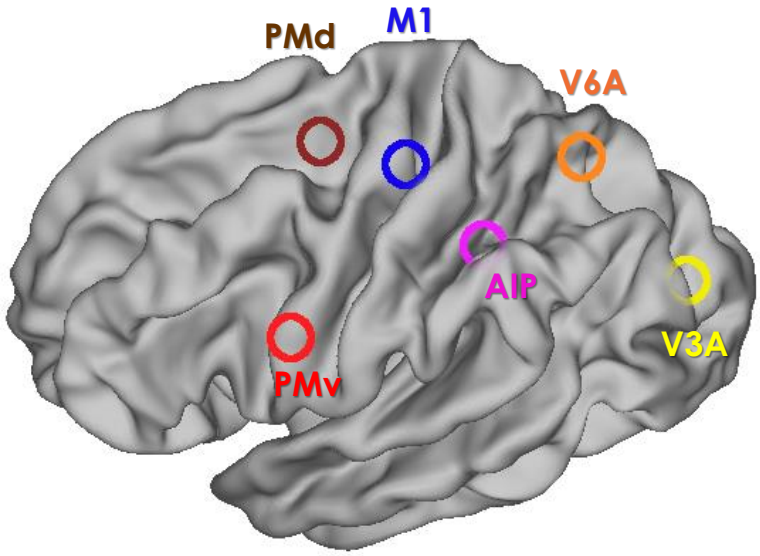

Figure 4.2: **Regions of interest in standard MNI space.** Plotted on the "Human Connectome Project Workbench". MNI coordinates for M1 [-36 -22 60], PMv [-58 4 30], AIP [-44 -42 46], PMd [-22 -4 58], and V6A [-22 -64 54], and V3A [-26 -86 18].

ter paired TMS pulse plasticity induction by means of two hypothesis-driven analyses: (1) How does PMv coupling with the co-stimulated M1 alter (seed-based correlation analysis (SBCA)) and (2) how do pairs of nodes within the grasping-network change their coupling (partial correlation analysis). In a third, exploratory analysis (3), we examined the connectivity changes in large-scale networks as defined by their shared spontaneous low-frequency fluctuations (dual-regression analysis).

Seed-based correlation analysis (SBCA)

SBCA maps the functional connectivity of one "seed" ROI across the entire brain in a voxel-wise manner on the basis of the correlation between the seed

ROI's BOLD time series and the BOLD time series at each voxel in the rest of the brain (O'Reilly et al., 2010). We employed SBCA to assess if paTMS-based modulation of the PMv-M1 pathway dynamically altered the functional interactions of either of these two nodes with each other and/or with other nodes within the reaching and grasping network. We assessed the functional connectivity of a 6 mm diameter seed mask in left PMv with the whole brain (target mask) before and immediately after paTMS and contrasted PMv-M1 connectivity at baseline versus connectivity during post-TMS (for details about statistical analyses see below). The analyses of resting-state and grasping task fMRI data were conducted independently. All analyses conducted for Experiment 1 and Experiment 2 were identical, which allowed us to directly contrast the effects in a higher-level analysis. For the first step of SBCA, statistical connectivity maps for every individual and for each of the four conditions (resting-state baseline / resting-state plasticity expression and task baseline / task plasticity expression) were created using the SBCA tool implemented in FSL (fsl_sbca). The time series for the left PMv seed mask was calculated. The SBCA model also accounted for the time series resulting from structured noise in the average BOLD signal in WM, GM and CSF and head movement (six regressors resulting from McFLIRT motion correction). WM, GM, and CSF masks were derived from individual T1-weighted structural images using the FSL segmentation tool FAST and registered to EPI space using FLIRT (Zhang et al., 2001). The resulting connectivity map described the correlation between the average BOLD time series of the PMv mask and the time series for each voxel within the whole brain. The individual correlation maps were transformed into MNI space, using FLIRT affine registration of EPI to structural space and subsequently FNIRT non-linear registration to MNI space. Standard space group correlation maps (z-score maps) were generated by entering SBCA-derived individual correlation maps into a group general linear model (GLM) and thresholded at $Z > 2.3$ with

a significance threshold of $P < 0.05$. These thresholded group z-score maps were projected onto the Midthickness.32k CaretBrain as provided by the Human Connectome Project Workbench using the "surf_proj" algorithm as implemented in FSL and then visualised using the Human Connectome Project Workbench (http://www.humanconnectome.org/connectome/getconnectome-workbench.html).

As the next step, we computed the average time series resulting from these statistical connectivity maps for M1 ROI. We then compared time series correlations of PMv with M1 at baseline and during post-TMS. For statistical comparisons we conducted a paired t-test, contrasting PMv-M1 interactions at baseline versus during post-TMS. Prior to statistical analysis, correlation coefficients were Fisher z-transformed. We also conducted a higher-level analysis, contrasting Experiment 1 with Experiment 2, using a mixed-model ANOVA with within-subject factors TIME (baseline / post-TMS) and between-subjects factor PROTOCOL (Experiment 1 / Experiment 2). We used a significance level of $P < 0.05$. Fifteen participants contributed to the resting-state group z-score map (for both Experiment 1 and Experiment 2); 14 participants contributed to the grasping task group z-score map, since the data of one participant had to be removed due to excessive head movement during data acquisition (for both Experiment 1 and Experiment 2).

Partial correlation analysis

To investigate changes in functional connectivity between pairs of grasping-network nodes we conducted a partial correlation analysis between the BOLD time series of directly connected nodes of the left hemisphere using custom made software written in Matlab (MathWorks). Partial correlation analysis-generated correlations represent only correlations specific to the pair of cortical regions in question by regressing out the time series of all other network nodes

under investigation. We focussed on pairs of regions thought to be monosynaptically connected (Tomassini et al., 2007, Mars et al., 2011): M1-PMv, PMv-AIP, AIP-V3A, M1-PMd, PMd-V6A, and V6A-V3A. Individual BOLD time series for each network node mask (6 mm diameter) were generated using a GLM-based design that incorporated regressors denoting potentially confounding factors such as variation in white matter (WM), grey matter (GM), and cerebrospinal fluid (CSF), and whole brain BOLD signal as implemented in FSL (fsl_glm). Individual partial correlations were normalised using Fisher's z-transform.

Analogous to statistical tests used in SBCA, we conducted paired t-tests contrasting pairwise interactions at baseline versus during post-TMS on Fisher z-transformed partial correlation coefficients (independently for Experiment 1 and Experiment 2). At a later stage, we also subjected Experiment 1 and Experiment 2 to a direct comparison by means of a mixed-model ANOVA with factors PROTOCOL (Experiment 1 / Experiment 2; between-subjects factor) and TIME (baseline / post-TMS; within-subjects factor). We used a significance level of $P < 0.05$. Resting-state and grasping task MRI data sets were analysed in an identical way, but were not compared directly due to a categorical difference in movement artefacts (movement artefacts were larger in the grasp task than in the resting-state MRI). The severity of movement artefacts required the removal of one grasping task data set for both the Experiment 1 and Experiment 2 condition.

Dual-regression analysis

To understand if co-activation patterns of large-scale networks of functional connectivity change dynamically in response to plasticity induction, we investigated networks defined by their shared spontaneous low-frequency fluctuations (< 0.1 Hz). Coherence within (1) resting-state networks (RSNs) and (2) networks during task performance were analysed before and after paTMS in-

tervention using a whole-brain corrected approach. Whereas SBCA and partial correlation analyses focussed on nodes of the fronto-parietal grasping-network, this approach has the potential to identify any networks (defined as areas sharing BOLD signal temporal correlations) in which connectivity is changing as a result of the TMS intervention.

This approach proceeds in three stages. To begin, concatenated multiple fMRI data sets are decomposed using independent component analysis (ICA) to identify large-scale spatial patterns of functional connectivity. We used the baseline fMRI data sets of all 23 participants who participated in this study to obtain group-averaged ICA-network maps. For those seven participants who contributed to both experimental conditions, only one baseline data set was contributing to the generation of "group-averaged baseline" network masks; therefore, twelve "baseline" data sets were drawn from Experiment 1 and eleven "baseline" data sets were drawn from Experiment 2. By identifying ICA components based on data from both experiments, we avoided biasing our analysis as a result of any possible differences in the two groups of subjects. At the second stage, two regressions are carried out in which the ICA-derived components are regressed back against the BOLD time series from the baseline and post-plasticity induction periods in the two experiments (8ms IPI and 500 ms IPI): firstly, to identify subject-specific temporal dynamics for each group-averaged ICA spatial component via a linear model fit (spatial regression) and, secondly, to compute subject-specific associated spatial maps by using the generated time series as a regressor against the associated fMRI data (temporal regression). At the final stage, the resulting individual spatial component maps are collected across subjects into single 4D files (1 per original ICA network map, with the fourth dimension being subject identification).

The resulting maps - baseline and post-TMS - with one for each of the two experimental conditions, i.e. Experiment 1 and Experiment 2

- were then tested for voxel-wise statistical significance against the ICA maps generated from all 23 participants baseline fMRI data sets ("group-averaged baseline") using nonparametric permutation testing (5,000 permutations) (Nichols and Holmes, 2002) and cluster-based thresholding and normalisation of the design matrix columns to unit standard deviation. Voxel-wise testing excluded the cerebellum.

The only difference in how resting-state fMRI and grasping task fMRI were treated lay in the dimensionality estimation of ICA. The number of components was estimated automatically for resting-state fMRI using the Laplace approximation to the Bayesian evidence for a probabilistic principal component model (Beckmann et al., 2005), which resulted in 18 independent components. The decomposition of task-positive fMRI was then restricted to an ICA dimensionality of 18 to avoid producing additional subnetworks (Smith et al., 2009).

Image pre-processing for dual-regression

Individual subject ICA fMRI analysis was carried out on baseline data of twelve Experiment 1 and eleven Experiment 2 data sets using Multivariate Exploratory Linear Optimized Decomposition into Independent Components (MELODIC) (Beckmann et al., 2005). Individual pre-statistical processing consisted of motion correction (MCFLIRT), brain extraction (BET), spatial smoothing using a Gaussian kernel of full-width at half maximum (FWHM) of 5 mm, and high-pass temporal filtering. Imaging volumes were registered to the individual's structural scan using boundary-based registration (BBR) (Greve and Fischl, 2009) and to standard space using FMRIB's Linear Image Registration Tool (FLIRT) with 12 degrees of freedom. Preprocessed functional data was temporally concatenated across subjects, with spatial smoothing using a Gaussian kernel of 5 mm FWHM, high-pass temporal filtering of 100 s, and registered to the standard brain using BBR.

Acquisition of effective connectivity measures by means of paired-pulse TMS

For Experiment 3, effective connectivity between PMv and M1 was assessed by means of paired-pulse TMS on a day separate from the two MRI image acquisition days (the baseline and post-TMS days) for ten participants from the Experiment 1 group. MEPs were recorded from right FDI muscle in response to either single-pulse TMS (spTMS; 20 trials) applied to left M1 or paired-pulse TMS (ppTMS) delivered over both left PMv and M1 [20 trials; 8 ms IPI]. Both spTMS and ppTMS trials were administered in pseudorandom order within the same block of trials. The effect of PMv stimulation on the effect of M1 stimulation was determined as the "ratio"; this is the relative difference between median ppTMS-evoked and median spTMS-evoked MEPs divided by the median spTMS-evoked MEP ([ppTMS - spTMS] / spTMS). Ratios were chosen since they indicate the effective influence of PMv on M1 relative to M1 motor excitability; the value is positive when PMv facilitates M1 and negative when PMv inhibits M1. Ratios do not indicate whether a relative enhancement of the effective influence is accompanied by an overall change in cortical excitability. The latter one can only be tested by comparing MEP amplitudes following stimulation of M1 or stimulation of PMv-M1.

For rest-state TMS, volunteers attended to cylinder illumination while simply maintaining a static hand posture. We used the same ITIs for rest blocks (mean $\pm$ SD, 6.23 $\pm$ 0.07 s) described in Methods of Chapter 2.

For the grasping-task experiment, volunteers sat in a darkened room and made right-hand reaching and grasping movements cued by illumination of one of two concentrically arranged cylinders (15 and 65 mm diameter) located 30 cm in front of the starting hand position (Buch et al., 2010). Each trial was initiated by pressing a touch bar with the right hand. Following an ITIs of 6.90 $\pm$ 0.79 s (mean $\pm$ SD) (same as described in Methods of Chapter 2), one cylinder was

illuminated. Volunteers responded by grasping it with their thumb and index finger before lifting it from its pedestal. Reaction and movement times were recorded. All trials were accompanied by either spTMS or pp TMS, with the pulse applied to M1 always occurring 100 ms after cylinder illumination, which was before movement onset.

Comparing effective and functional connectivity

In order to understand how effective connectivity (as assessed with paired-pulse TMS) and functional connectivity (derived from fMRI) relate to each other, it is necessary to correlate the two measures for the same connection, when they are taken within the same subject. For a direct comparison of the indices, we regressed the ratio of the median MEP amplitude for PMv-M1 against the z-transformed "partial correlation coefficient" for PMv-M1 using Pearson's correlation coefficient with a significance level of $P < 0.05$. The MEP ratio represented baseline PMv-M1 coupling within each participant, and was subsequently compared to (1) baseline functional connectivity and (2) to post-TMS functional connectivity.

4.4 RESULTS

4.4.1 Experiment 1: Paired stimulation of PMv-M1 at 8 ms IPI

Changes in functional coupling between the two stimulated areas

A qualitative sense of topographically distinct coupling patterns in the left hemisphere can be obtained by examining whole-brain co-activation maps, seeded in PMv, before and after repeated paired stimulation during task performance (Fig. 4.3 A and B) and in the resting-state (Fig. 4.3 D and E). In order to quantify these differences, in the first analysis, we examined the correlation between the fMRI-measured BOLD signal time near the two stimulated areas, left PMv and

M1, using the SBCA tool in the FMRIB Software Library (O'Reilly et al., 2010). Repeated paired TMS led to an increase in PMv-M1 BOLD coupling while participants performed the task (paired t test: t_{13} = -2.59, P < 0.05; Fig. 4.3 C). PMv-M1 BOLD coupling at rest was not modulated by the intervention (t_{14} = -0.07, P = 0.94; Fig. 4.3 F).

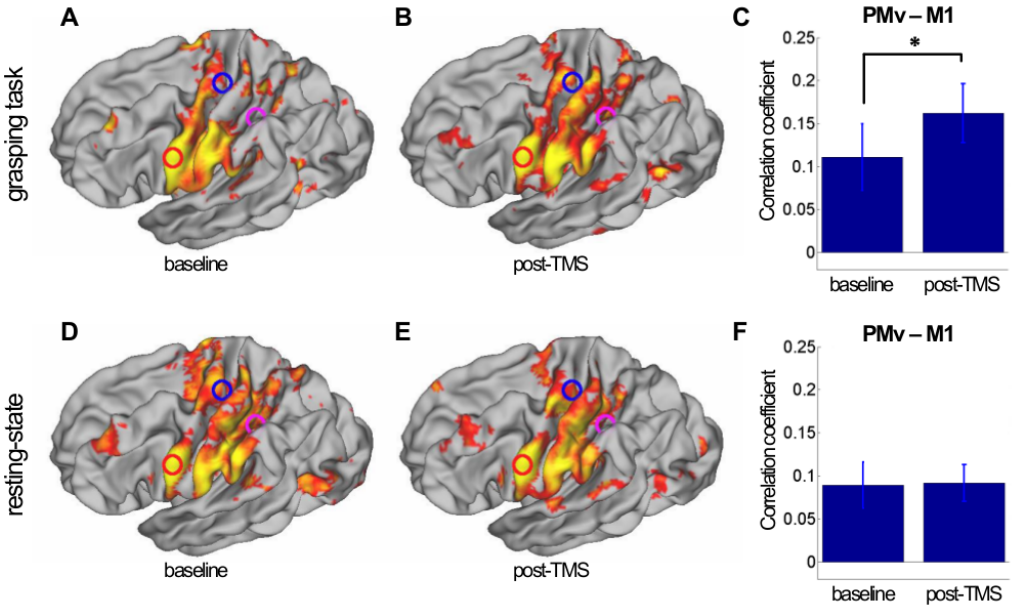

Figure 4.3: **Experiment 1: Seed-based correlation analysis.** Task-positive (A - B) and resting-state (D - E) fMRI showing PMv coupling with the whole brain at baseline and following plasticity induction. Standard-space group correlation maps seeded from PMv (red circle) with whole brain (thresholded at Z > 2.3, with a significance threshold of P < 0.05). The blue circle covers M1 from which correlation coefficients were extracted that are shown in (C) and (F). (C) Significant change in correlation coefficient for PMv-M1 functional connectivity from baseline to post-TMS for task-positive fMRI (n = 14). (F) No significant change in PMv-M1 functional connectivity during resting-state fMRI (n = 15). * P < 0.05 (significant effects). Error bars represent 1 s.e.m.

Distinct patterns of reorganization in dorsolateral and dorsomedial sensorimotor circuits

In line with our predictions that changes in effective coupling between two network nodes affects connectivity between distant nodes, we conducted indepen-

dent analyses of pairwise functional coupling in resting-state and task-positive fMRI. A partial correlation analysis investigates functional connectivity between two nodes by partialling out contributions that might arise due to effects of other network nodes. Notably, changes in pairwise coupling occur in the wider fronto-parietal network when investigated at rest. The parieto-frontal network includes anatomically distinct, parallel streams, a dorsomedial stream (V6A-PMd) and a dorsolateral stream (AIP-PMv), that are differentially involved in aspects of reaching and grasping (Verhagen et al., 2008).

Paired stimulation of one node of the dorsolateral stream, PMv, in synchrony with M1, selectively increased coupling at rest within the dorsolateral circuit - as indexed by the mean group partial correlation coefficient for the connection of PMv-AIP (paired t-test: t_{14} = -2.50, $P < 0.05$; Fig. 4.4 A). This increase was paralleled by decreased coupling within the dorsomedial circuit at rest (paired t-tests for PMd-V6A: $t_{14} = 2.22$, $P < 0.05$ and PMd-M1: $t_{14} = 2.84$, $P < 0.05$; Fig. 4.4 B and C). Fig. 4.4 D and E display a schematic of mean group connectivity weights across the fronto-parietal network before and after repeated paired TMS over PMv and M1 at an IPI of 8 ms. A relatively early visual area, V3A, is linked to both dorsomedial and dorsolateral circuits. Functional connectivity from V3A did not change with either V6A or AIP after TMS (paired t-test: $t_{14} \leq 1.45$, $P \geq 0.17$).

We subjected grasping task-related fMRI to the same analysis and confirmed that functional connectivity of PMv with M1 was significantly increased during post-TMS (t_{13} = -3.72, $P < 0.01$; baseline functional connectivity: 0.15 ± 0.19 and post-TMS functional connectivity 0.23 ± 0.22 (mean $\pm$ SD; not shown).

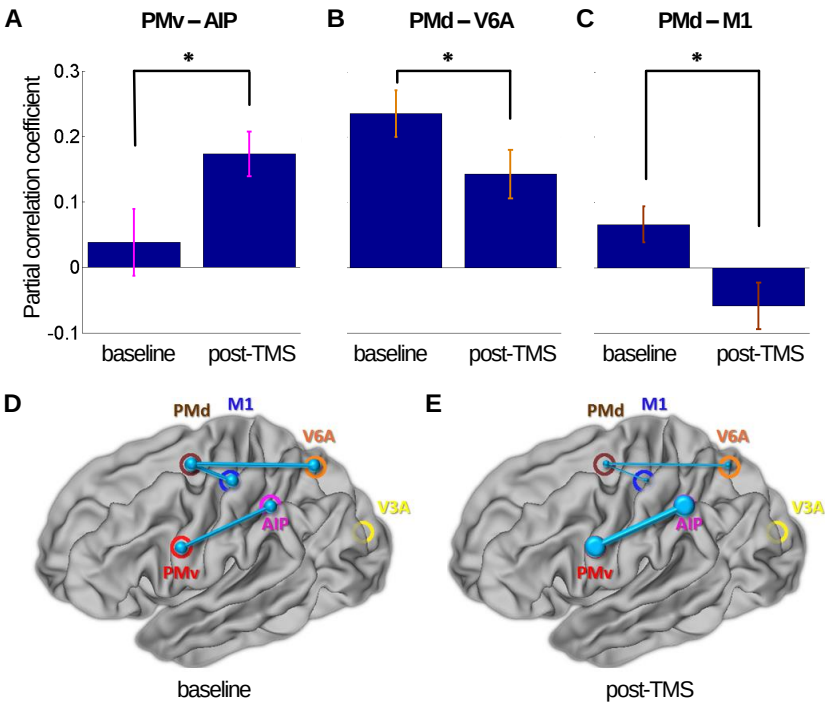

Figure 4.4: **Experiment 1: Partial correlation analysis for resting-state fMRI.** Mean group partial correlation coefficients for specific connections factoring out the contributions to those pairwise correlations that might arise due to effects of other network nodes (n = 15). Significant increase in PMv-AIP functional connectivity from baseline to post-TMS (A), with simultaneous significant decrease in coupling of PMd with M1 (B) and PMd with V6A (C). * $P < 0.05$ (significant effects). Error bars represent 1 s.e.m. (D - E) Schematic representation of mean group connectivity weights (cyan lines) at baseline and during post-TMS in standard space. (D) All weights are standardised to baseline partial connectivity of each connection. (E) Significant increase in PMv-AIP connectivity and decrease in PMd-M1 and PMd-V6A connectivity during plasticity expression proportional to change from baseline.

Reorganisation of sensorimotor network

SBCA and partial correlation analysis are hypothesis-driven analyses focussing on changes in functional connectivity within specific nodes of the reaching-and-grasping network. To assess the possibility that dynamic changes also occur in other neural networks, we conducted an exploratory analysis of resting- fMRI by means of a regression technique (dual-regression analysis) which allows for

voxel-wise comparisons of functional connectivity (Filippini et al., 2009). For dual-regression analysis, functional networks were defined based upon their temporally correlated low-frequency fluctuations of BOLD activity during the resting-state. Eighteen large-scale spatial components, representing group-averaged neuronal networks, were extracted from baseline resting-state fMRI of 23 participants (combining baseline fMRI from Experiment 1 and 2). To investigate differences within the coherence of large-scale networks itself and differential co-activation patterns at baseline and post-TMS, individual network maps were estimated before and after paTMS and compared to the group-averaged motor network.

Following TMS, a significantly increased degree of co-activation within the "sensorimotor network" (Smith et al., 2009, Power et al., 2011), which includes much of motor and premotor cortex and interconnected regions of parietal cortex (Mars et al., 2011, Tomassini et al., 2007), was found (Fig. 4.5 in orange-to-red). The significant increases in coherence within the sensorimotor network, that were identified with this alternative analytical approach, were most apparent in regions overlapping with or close by the parietal and premotor cortex (Fig. 4.5 in green). They were limited to the stimulated left hemisphere and included the precentral and postcentral gyrus (MNI coordinates [-36 -24 62] and [-40 -28 58]) and the junction of the postcentral and supramarginal gyrus [-50 -30 46]. Furthermore, the sensorimotor network was coactive with part of the IFJ region in ventral frontal cortex [-42 4 54] ($P = 0.047$; t-statistic images thresholded using cluster-based thresholding and corrected for multiple comparisons at $t > 1.5$; Fig. 4.5). As explained above, the more anterior TMS coil was placed over the border between 6v and IFJ (Neubert et al., 2014) (Fig. 4.1 B). The IFJ is a key prefrontal transition region involved in the high-level control of the motor system and it interacts prominently with PMv and the dorsolateral sensorimotor circuit as well as areas in inferior parietal cortex (Neubert et al., 2014).

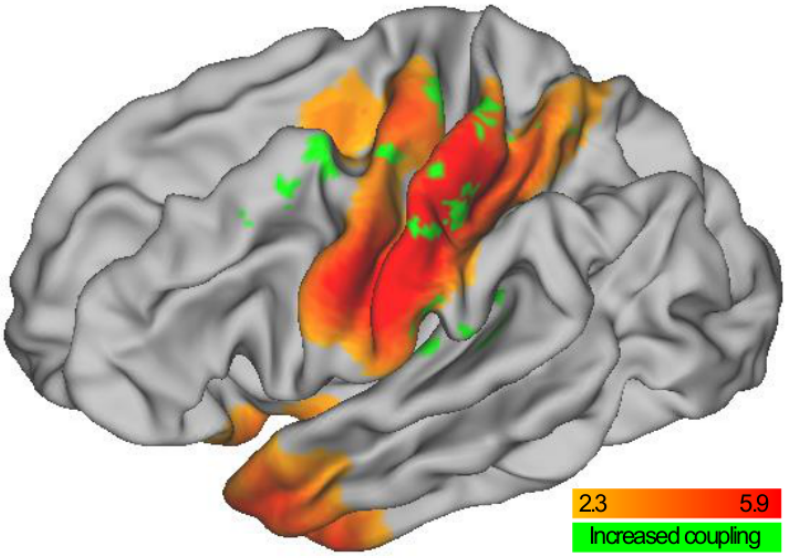

Figure 4.5: **Analysis of motor network co-activation following 8 ms IPI plasticity induction based on fMRI resting-state (n = 23).** Regions belonging to the motor network include bilateral precentral and postcentral gyri, junction of precentral gyrus and inferior frontal gyrus and superior frontal gyrus (the motor network component also covered temporal pole, temporal fusiform cortex and insular cortex) (orange-to-red spatial map represents z-scores from 2.3 to 5.9). Motor network comparison following plasticity induction revealed increased coherence of the motor network itself and co-activation within the left hemisphere only. Co-activation was found in dorsolateral prefrontal cortex, planum temporale and parietal operculum (green colour defines increase in coupling, $P < 0.05$).

4.4.2 Experiment 2: Paired stimulation of PMv-M1 at 500 ms IPI

To test our hypothesis that the effects of repeated paired TMS upon functional connectivity were selective to the 8 ms IPI (Fig. 4.3), which is believed to potentiate synaptic plasticity between PMv and M1, we compared functional connectivity changes as a result of specific IPIs (8 ms IPI versus 500 ms IPI).

No changes in functional coupling between the two stimulated areas

No changes in PMv-M1 coupling could be found either during task performance or at rest after paired TMS at 500 ms IPI (paired t-test for 500 ms IPI during task: $t_{13} = 0.94$, $P = 0.36$; Fig. 4.6 C; paired t-test for 500 ms IPI at rest: $t_{14} = 0.07$, $P = 0.95$;

Fig. 4.6 F). Moreover the PMv-M1 coupling change during task performance was significantly greater after 8 ms IPI paTMS (Experiment 1) than after 500 ms IPI paTMS (Experiment 2) (mixed-model ANOVA for PMv-M1 connection: TIME by PROTOCOL interaction: $F_{1,26} = 4.64$, $P = 0.041$).

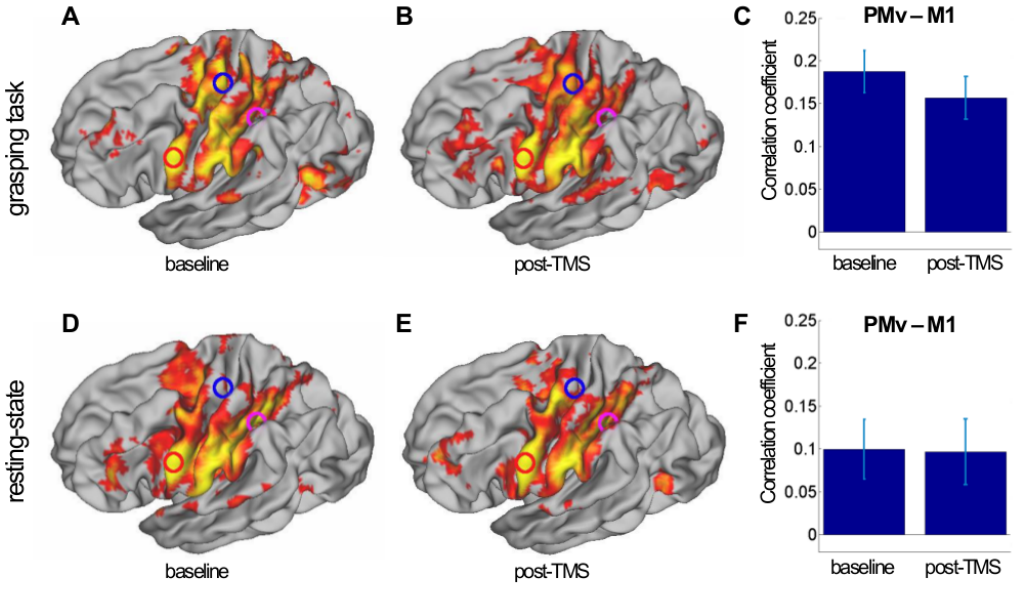

Figure 4.6: **Experiment 2: Seed-based correlation analysis:** Task-positive (A - B) and resting-state (D - E) fMRI showing PMv coupling with the whole brain at baseline and during plasticity induction. Standard-space group correlation maps seeded from PMv (red circle) with whole brain (thresholded at $Z > 2.3$, with a significance threshold of $P < 0.05$). The blue circle covers M1 from which correlation coefficients were extracted that are shown in (C) and (F). No significant change in correlation coefficient for PMv-M1 functional connectivity following 500 ms IPI intervention for either (C) task-positive fMRI ($n = 14$) or (F) resting-state fMRI ($n = 15$). Error bars represent 1 s.e.m.

No reorganization in dorsolateral and dorsomedial sensorimotor circuits

At rest, no changes in dorsolateral and dorsomedial sensorimotor circuit coupling could be found after 500 ms IPI paTMS. The functional coupling changes between PMv-AIP were significantly stronger after 8 ms IPI paTMS than after 500 ms IPI paTMS (mixed-model ANOVA: TIME by PROTOCOL interaction:

$F_{1,28} = 7.15$, $P < 0.05$) as were those between PMd-V6A (mixed-model ANOVA: TIME by PROTOCOL interaction: $F_{1,28} = 5.29$, $P < 0.05$) there was a tendency for the connection PMd-M1 (mixed-model ANOVA: TIME by PROTOCOL interaction: $F_{1,28} = 5.92$, $P < 0.05$). Neither of these effects seen for the 8 ms IPI intervention were present after 500 ms IPI paTMS (PMv-AIP: $t_{14} = 1.08$, $P = 0.30$, Fig. 4.7 A; PMd-V6A: $t_{14} = -1.24$, $P = 0.24$, Fig. 4.7 B; PMd-M1 $t_{14} = 0.47$, $P = 0.65$; Fig. 4.7 C).

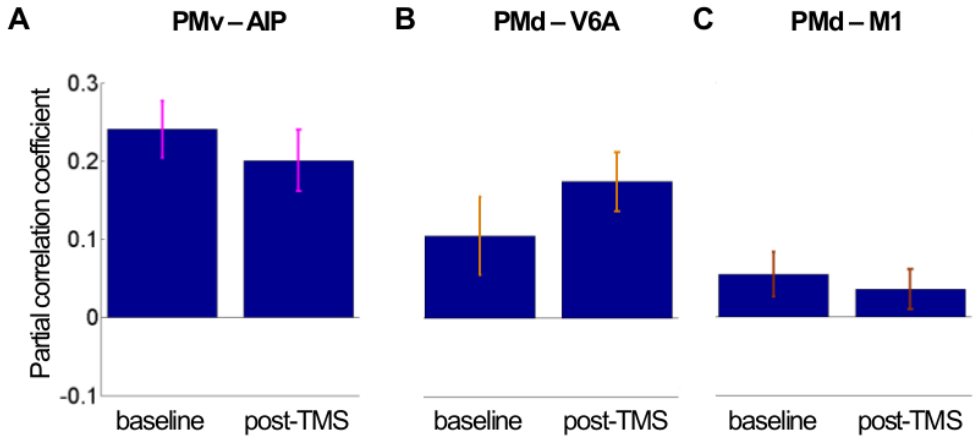

Figure 4.7: **Experiment 2: Partial correlation analysis for resting-state fMRI.** Mean group partial correlation coefficients for specific connections factoring out the contributions to those pairwise correlations that might arise due to effects of other network nodes ($n = 15$). No significant changes in functional connectivity from baseline to post-TMS for PMv-AIP (A), PMd-V6A (B) and PMd-M1 (C). Error bars represent 1 s.e.m.

No reorganisation of large-scale networks

Performance of the dual-regression analysis on resting-state fMRI data following application of 500 ms IPI repeated paired TMS identified no change in the large-scale sensorimotor network following plasticity induction ($P = 1.0$) or any other network ($P = 0.16$) (not shown).

Variability of connectivity at baseline

It could be argued that the observed changes in functional connectivity between Experiment 1 and Experiment 2 were not the result of TMS stimulation (compare baseline partial correlation coefficients in Figure 4.4 and Figure 4.7), but were rather due to the inherent variability of functional connectivity between two network nodes. To examine this possibility, we compared changes in functional connectivity across baseline scans of those seven participants who participated both in Experiment 1 and Experiment 2 using a within-subject repeated-measures ANOVA on the baseline functional connectivity (EXPERIMENT (baseline Experiment 1 / baseline Experiment 2) and AREA-PAIR (M1-PMv, PMv-AIP, AIP-V3A, M1-PMd, PMd-V6A, and V6A-V3A)). There were no significant difference between functional connectivity at baseline between the two experiments (no main effect of EXPERIMENT: $F_{1,6}$ =0.001, $P = 0.98$ and no interaction term of EXPERIMENT by AREA: $F_{5,6}$ =8.37, $P = 0.11$). Noticeable, but non-significant, differences in baseline functional connectivity across different scanning sessions, i.e. different days, have previously been reported (Eldaief et al., 2011). Importantly, the measure of interest in this study is the change in the partical correlation coefficient invoked by the different TMS interventions.

4.4.3 Experiment 3: Comparison of connectivity measures for PMv-M1 at 8 ms IPI

In Experiment 3 we investigated how effective and functional connectivity indices relate to each other within the same subjects, focusing on the connection of PMv and M1 within ten of the previously tested subjects. Specifically, we correlated the ratio of MEP amplitudes with functional coupling values derived from the partial correlation analysis and described the relationship of the two connectivity measures by means of a linear regression with Pearson's correlation

coefficient. The two connectivity indices (paired-pulse TMS and fMRI BOLD signal partial correlation) were compared independently in the two conditions; i.e. at rest and during performance of the grasping task.

During task performance, the two connectivity measures for the PMv-M1 pathway were positively correlated at baseline (Pearson's correlation coefficient: $R = 0.74$, $P < 0.01$; Fig. 4.8 A). A positive correlation indicates that the greater the facilitatory influence of PMv on M1, the greater the MRI-derived functional connectivity. When baseline effective connectivity was correlated against fMRI connectivity during post-TMS the correlation was again significant ($R = 0.87$, $P < 0.001$; Fig. 4.8 B).

During rest, the correlation of the two measures was not significant at baseline ($R = -0.21$, $P = 0.57$; Fig. 4.8 C). However, a significant correlation was observed for baseline TMS-derived effective connectivity and fMRI-derived post-TMS functional connectivity ($R = -0.68$, $P < 0.05$; Fig. 4.8 D).

Intriguingly, the correlation was negative at rest which implies that when effective connectivity has a greater negative value, i.e. PMv has a stronger inhibitory influence on M1, then functional connectivity measures have greater values too, but they have a different sign. Although neurophysiological measurements, such as those taken with paired pulse TMS reveal that PMv's influence over M1 is a net inhibitory one, the analysis of BOLD coupling suggested a positive relationship between the areas' activity levels underlining the caution with which inferences of facilitatory or inhibitory connectivity have to be made.

Taking together the observations for task-performance and resting-state, we show that stronger excitatory influences are represented by increased functional connectivity, and that stronger inhibitory influences are also represented by increased coupling. Baseline effective connectivity strength during rest (i.e. inhibitory) and during task (i.e. excitatory) is also correlated within individuals ($R = -0.63$, $P < 0.05$; Fig. 4.8 E).

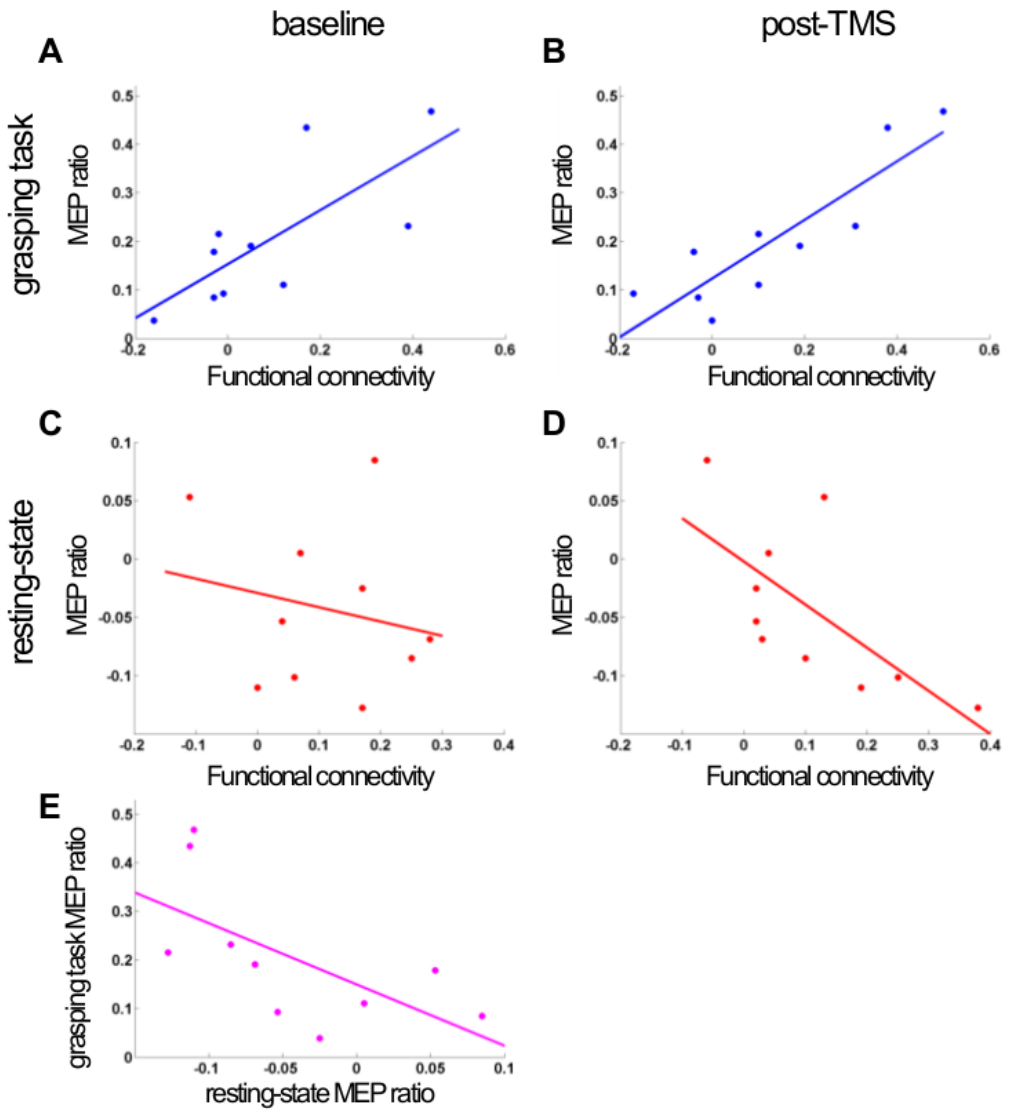

Figure 4.8: **Experiment 3: Correlation of PMv-M1 connectivity measures before and after 8 ms IPI intervention.** Correlation of functional connectivity (derived from partial correlation analysis) with baseline effective connectivity represented by MEP ratio (A - D). (A) Functional connectivity during performance of grasping task at baseline versus MEP ratio ($R = 0.74$, < 0.01). (B) Functional connectivity during performance of grasping task post-TMS versus MEP ratio ($R = 0.87$, $P < 0.001$). (C) Resting-state functional connectivity at baseline versus MEP ratio ($R = -0.21$, $P = 0.57$). (D) Resting-state functional connectivity during post-TMS versus MEP ratio ($R = -0.68$, $P < 0.05$). (E) MEP ratio during resting-state versus MEP ratio during task performance at baseline ($R = -0.63$, $P < 0.05$) ($n = 10$).

4.5 DISCUSSION

Here, I employed a paTMS protocol that selectively increases connectivity in a cortico-cortical pathway in line with Hebbian principles of synaptic plasticity (Buch, Johnen et al., 2011; Chapter 2). Our previous study suggested that pathway-specific spike-timing dependent plasticity (STDP) can enhance the context-dependent causal influence of one neural node on another - PMv and M1. The current results show that it is possible to causally *manipulate* functional coupling in the stimulated pathway. Moreover, I will discuss the results in the context of dynamic network reorganisation. Brain stimulation has previously been used to alter network dynamics as measured with resting-state MRI (Eldaief et al., 2011). Eldaief et al. suggest that the manipulation of activity in *one node* leads to changes in connectivity in a wider resting-state network. In contrast, the current study addresses the question of how alterations of effective connectivity in *one connection* - precisely, in one specific direction - relates to functional coupling as measured with fMRI. In graph theoretical terms, it assesses the causal influence of one node over another node via one specific "edge".

Furthermore, I presented evidence for a direct relationship between fMRI-based indices of functional connectivity and paired-pulse TMS measures of effective connectivity, and to this end, I will offer a possible framework to explain both excitatory and inhibitory connectivity by drawing on anatomical and neurophysiological knowledge about the stimulated pathway between premotor cortex and M1. Taken together, the current sets of results suggest that functional connectivity is in part reflective of synaptic efficacy.

Synaptic efficacy

A first set of analyses (Experiment 1 and Experiment 2) was aimed at understanding the relationship between alteration of effective connectivity within a

specific pathway and the representation thereof in MRI-derived functional coupling. In my previous study (Buch, Johnen et al., 2011; Chapter 2), paTMS was demonstrated to be capable of selectively increasing or decreasing the causal influence of one node upon the other depending on synchronous and asynchronous activity in interconnected neurons. Experiment 1 with an 8 ms IPI is designed to modify synaptic efficacy by inducing presynaptic activity prior to postsynaptic activity within a critical window of tens of milliseconds. In contrast, the 500 ms IPI chosen for Experiment 2 is too long to expect co-activation of the two areas. Many brain stimulation experiments have been designed with the goal of selectively manipulating coupling between neural network nodes in a targeted fashion. The current results complement my neurophysiological findings of increased coupling between targeted network nodes when the pathway is endogenously activated by task demand (Buch, Johnen et al., 2011; Chapter 2): paTMS also increased correlations in functional connectivity BOLD signal over the stimulated network nodes. This finding might speak towards the possibility that increased correlations in functional connectivity describe strengthened synaptic efficacy.

There is some consensus emerging about the direct relationship between resting-state fMRI measured coupling and structural connectivity (O'Reilly et al., 2013), although this relationship is far from being straightforward (Damoiseaux and Greicius, 2009). The manipulation used here is unlikely to induce macrostructural alterations, such as increases in grey matter volume or changes in white matter organisation (Draganski et al., 2004, Scholz et al., 2009), but is more likely to induce changes in synaptic efficacy of the stimulated pathway (Johansen-Berg et al., 2012). Those microstructural changes have been linked to learning on the minute and hour time scale (Sagi et al., 2012).

Considering the observations that connectivity patterns and strength of cou-

pling change at intermediate time periods (i.e. seconds) (Honey et al., 2009, Smith et al., 2012), one way of interpreting these findings is that a specific pattern "consolidates" itself and is picked up as increased correlations in BOLD signal fluctuations. It is possible to think that stimulation induced increases of connectivity are driven by greater synchronisation of neural activity and / or by elevated mean activity of the interconnected neuronal populations. Based on a neural network model, Chawla and colleagues argue that increased synchronisation of neural activity cannot be distinguished from increases in mean firing-rate (Chawla et al., 2000). The concept of an increase in mean neuronal activity is directly linked to an increase in signal-to-noise ratio which has previously been suspected to be a driver of modifications in connectivity (Polana et al., 2012). The current experimental design accounted for a potential TMS-induced higher level of activity in PMv and M1: in Experiment 2 the same number of paired TMS stimuli were applied, but at a distinct IPI of 500 ms. This way PMv and M1 received equal amounts of electrical energy. Frequencies of 3 Hz rTMS applied for approximately 10 seconds can evoke a local hemodynamic response below the stimulation coil and in interconnected areas that can be visualised by concurrent fMRI (Bestmann et al., 2005). Yet, there is no evidence that changes in BOLD amplitude last for up to 5 minutes (which would be the time point at which image acquisition would have started in the present study). In addition, should any lasting increase in BOLD activity have occurred, one would expect to see greater correlation between the network nodes in both stimulation conditions (8 ms IPI and 500 ms IPI). The lack of change in functional coupling in Experiment 2 speaks towards the notion that increases in signal-to-noise ratio were not producing the effects observed following plasticity induction.

More generally, the present results support the idea that effective connectivity is the most likely direct determinant of functional connectivity (Passingham et al., 2002). Changes to synaptic efficacy and / or alterations in

neural population synchrony offer a potential explanation for the changes in brain dynamics that have been observed (Friston et al., 1995, Friston et al., 2000).

Network reorganisation

Dynamic network reorganisation is a significant feature of brain function allowing for acquisition of new skills, refining existing abilities and response to brain injury. By focussing on the observations made during resting-state fMRI, I would like to discuss some aspects related to network reconfiguration. Resting-state analysis revealed qualitatively and quantitatively distinct modifications in functional coupling in response to paTMS. Whereas paTMS over PMv and M1 selectively targeted a corticocortical pathway, the network nodes are strongly coupled to a network crucially involved in information processing related to visuomotor transformations for successful reaching and grasping (Jeannerod et al., 1995, Fogassi et al., 2001, Davare et al., 2006, Grol et al., 2007). Intriguingly, the modification of cortical excitability was expressed as a greater correlation in BOLD signal in the dorsolateral circuit (PMv-AIP) with simultaneous decreases of coupling in the dorsomedial stream (PMd-M1 and PMd-V6A). To explain this shift of connectivity weights within the wider, interconnected network, it is crucial to understand that any net TMS-induced effect (this can refer to neural activity or motor output) is produced by neural activity in remote regions that are activated directly - via orthodromic or antidromic conduction - or trans-synaptically (Bestmann et al., 2005). With respect to decreased connectivity between PMd and M1 it is plausible that it is caused by synaptic long-term depression (LTD), which in an experimental context can be induced by presynaptic low-frequency stimulation paired with a small postsynaptic depolarisation. PMd neurons might be endogenously active and their activation might coincide with postsynaptic depolarisations evoked by antidromically conducted M1 depolarisations following M1 stimulation. However, decreases in functional

connectivity between M1 and PMd were not found in Experiment 2 and therefore, synaptic down-weighting (Abbott and Nelson, 2000) cannot fully explain the effects observed. Another framework offering a potential explanation for the observed effects, particularly the distinct changes in coupling strength in dorsolateral and dorsomedial parieto-frontal circuits, would be a mechanism of competition between dorsomedial and dorsolateral streams, potentially mediated by mutual inhibition. By increasing activity in one circuit, the inhibitory impact on the other would be augmented. Similar increased coupling within the "sensorimotor network" at rest was also revealed by an exploratory analysis (dual-regression of resting-state fMRI). The "sensorimotor network" was also more coupled to premotor regions, namely area IFJ (Neubert et al., 2014) and area 8A (Sallet et al., 2013), that are closely interconnected with the stimulated area PMv and implied in more complex motor behaviours such as task switching and visual attention, respectively.

Comparison of TMS-derived and fMRI-derived connectivity

Effective connectivity between areas in the motor system has been investigated by means of paired-pulse TMS. The connection of PMv and M1 exhibits two types of effective connectivity dependent upon context. In Experiment 3 we probed the relationship of a context-dependent influence of PMv over M1 as indexed by paired-pulse TMS with coupling of the same two regions as indexed by fMRI BOLD signal. During the performance of a prehension movement, a greater facilitatory influence of PMv on M1 (as probed with paired-pulse TMS) is related to stronger BOLD signal correlation between the interconnected regions; this is before and after application of the 8 ms IPI paTMS protocol. During resting-state, the fMRI-based index of functional connectivity is not correlated with the TMS-based measure of effective connectivity. However, I found that after induction of increased coupling between PMv and M1, the enhanced

causal influence of PMv on M1 leads to significant correlations between functional and effective connectivity measures during the resting-state too. A potential explanation for this pattern of correlations is that: At rest, the influence of other interconnected areas on the connection of PMv-M1 impacts on the fMRI-based measure of functional coupling, but this is not the case in the paired-pulse TMS-based measure. However, after plasticity induction, the coupling between PMv-M1 is much more directly a consequence of the synaptic efficacy between those two stimulated areas. To this effect, functional connectivity during performance of a task, which relies upon PMv-M1 connectivity, appears to already reflect effective connectivity during pre-TMS.

Intriguingly, paired-pulse TMS measures are positively related to MRI functional coupling indices in the task-state, but are *negatively* correlated when subjects are resting (during post-TMS). The impact of PMv activity on M1 can either be a net excitatory or inhibitory effect. One might hypothesize that an inhibitory influence of one brain area upon another is represented by negative correlations in the BOLD signal. However, we find that increased functional connectivity is related to increased, effective *inhibitory* coupling at rest (as well as increased functional connectivity being correlated with increased, effective excitatory coupling). I would like to propose a model based upon anatomical and neurophysiological evidence: During performance of a precision grip, PMv facilitates M1 cortico-spinal motor output (Davare et al., 2008, Buch et al., 2010). When the connection is probed during rest or when one has to refrain from executing a previously planned movement, PMv invokes an inhibitory influence on cortico-spinal motor output (Davare et al., 2008, Neubert et al., 2010, Buch et al., 2010, Buch et al., 2011). This cognitive state-related change in excitability within the pathway appears to be in line with our understanding of anatomical connectivity derived from an electrophysiological stimulation study in macaques. Excitatory premotor neurons predominantly project onto inhibitory, GABAergic in-

terneurons in M1, whereas some of the projections synapse directly onto excitatory neurons in M1 (Tokuno and Nambu, 2000). Contextual demands shift connective weights within this circuit between inhibitory and excitatory influences. In agreement with the understanding - that functional connectivity measures are capable of capturing aspects of the causal influence of one area of another area - the current study provides novel evidence that measures of functional connectivity might be insensitive to the neurophysiological nature of the influence, i.e. whether it is a net excitatory or inhibitory influence.

The concept of selectively enhancing a specific, causal pathway effect and thereby augmenting their respective behavioural correlates is at the core of developing targeted neurotherapies. The present results support the idea that we can causally and selectively manipulate functional coupling within a cortico-cortical pathway by means of paTMS (Buch, Johnen et al. 2011). Whereas we do not link behaviour with the observed neuronal changes, we provide evidence that altered coupling strength varied as a function of whether or not functional connectivity was measured at rest or during a simple motor task that activates the pathway under investigation. For clinical applications of the paTMS protocol to selectively strengthen neural circuits it is crucial to understand the relationship of neural changes and behaviour. Moreover, the current work indicates that there is a direct relationship between fMRI-based indices of functional connectivity and paired-pulse TMS measures of effective connectivity.

5

Modulating connectivity of ventral premotor and primary motor cortex during grasping in chronic subcortical stroke

5.1 ABSTRACT

The ventral premotor cortex (PMv) is critical for performance of goal-oriented actions and transformation of visual information into motor commands for hand-object interactions. Whereas the dorsal premotor cortex (PMd) has been implicated in supporting recovered motor function after stroke, little is known about the functional contributions of PMv to recovered dexterity.

In order to elucidate the functional role of PMv in the chronic subcortical stroke brain, we studied the influence of ipsilesional and contralesional PMv on primary motor cortex (M1) during grasping. We conducted three experiments in which we assessed PMv-M1 connectivity using paired-pulse transcranial magnetic stimulation (TMS). We specifically tested whether we can selectively enhance PMv-M1 pathway connectivity by means of paired associative TMS (paTMS) (Buch, Johnen et al., 2011), within

one hemisphere (Experiment 1) and across hemispheres (Experiment 2). Experiment 3 served as a control experiment which should not induce pathway-strengthening.

During object grasp, PMv facilitates M1 in healthy adults (Davare et al., 2008, Davare et al., 2009, Buch et al., 2010). Here we report that the causal influence from ipsilesional PMv on ipsilesional M1 (M1 contralateral to affected hand) is critically reduced in subcortical stroke patients in comparison to healthy controls. This reduced effective interaction between intrahemispheric PMv-M1 could be selectively enhanced by applying paTMS over ipsilesional PMv and ipsilesional M1 at an interpulse interval that induces Hebbian plasticity in this direct corticocortical pathway. This finding may be interpreted as a "normalisation" of neurophysiological interactions.

It is uncertain whether the increase in ipsilesional PMv-M1 connectivity is behaviourally relevant in stroke patients. All patients improved upper limb function during the course of each of the three experiments; the improvement is likely related to repeated performance of the grasping task since it is also seen in the control experiment. Intriguingly, we found that those stroke patients, in whom PMv-M1 interactions were not facilitatory at the start of the study, achieved the greatest motor improvements at the end of the study. Our TMS-induced "normalisation" of the ipsilesional PMv-M1 connectivity also correlated positively with an overall functional improvement as assessed by a clinical test. This relationship may imply that there is a link between "responsiveness" to plasticity induction and motor learning in subcortical stroke patients.

5.2 INTRODUCTION

After ischaemic insult surviving neural networks reorganise spontaneously with the aim to compensate for the lesion and to generate meaningful motor output again. Long-term motor outcome is significantly influenced by the integrity of corticospinal tracts (Stinear et al., 2007). When motor output from primary motor cortex (M1) is significantly reduced due to subcortical white-matter lesions, cortical areas of similar functional characteristics as M1, e.g. so-

matotopic organisation and direct cortico-spinal projections, take over function from M1 and are critically involved in generating adaptive motor behaviour via alternative routes (Ward et al., 2003a, Byrnes et al., 2001). Functional brain imaging studies (Weiller et al., 1992, Gerloff et al., 2006, Ward et al., 2003a, Ward et al., 2003b, Lotze et al., 2006) and transcranial magnetic stimulation (TMS) (Johansen-Berg et al., 2002, Bestmann et al., 2005, O'Shea et al., 2007b) have advanced our understanding of specific contributions of secondary motor areas to motor recovery in chronic stroke patients.

The premotor cortex shows has direct corticospinal tracts that descend in parallel with corticospinal projections originating in M1 (Dum and Strick, 1991, He et al., 1993, Hoyer and Celnik, 2011). A number of studies have suggested that dorsal premotor cortex (PMd) plays a functionally relevant role in optimising control of the remaining motor output after stroke (Ward et al., 2006). Disruption of PMd activity by means of TMS significantly impairs movement execution in chronic stroke patients, and the effects of disruption are greater in more severely impaired patients (Johansen-Berg et al., 2002, Fridman et al., 2004). Better motor recovery relies on ipsilesional reorganisation, whereas poorer recovery is associated with functional involvement of contralesional motor areas (Ward and Cohen, 2004). Recruitment of contralesional areas may simply reflect the brain's attempt to generate motor output to spinal cord motoneurons via any residual pathway. PMd in the unaffected hemisphere is particularly relied upon for upper limb function in patients with poorer outcome, as indexed by a greater disruptive effect of TMS over contralesional PMd in poorly recovered patients (Johansen-Berg et al., 2002). In contrast to the compensatory involvement of secondary motor areas, over-activity in contralesional M1 has been associated with disturbances of network equilibrium following stroke. In patients with larger motor deficits, interhemispheric inhibition from contralesional M1 over ipsilesional M1 persists upon movement onset. In healthy individuals such inhibitory

effects disappear and are overtaken by facilitatory ones at a point prior to movement initiation (Murase et al., 2004). The negative modulatory effect of intact M1 on ipsilesional M1 is believed to contribute to impaired movements of the stroke-affected hand.

Despite spontaneous and therapy-induced cortical reorganisation, paretic hand function does not fully recover in about two-thirds of stroke survivors (Kolominsky-Rabas et al., 2001). Active motor training programmes have been shown to be effective at improving upper extremity function, even during the chronic phase of stroke. However, physiotherapy remains time-consuming and expensive (Kwakkel et al., 1999); therefore adjuvant treatment approaches employing non-invasive brain stimulation techniques are highly desirable. TMS not only provides a powerful means to investigate the contributions of different motor areas to motor recovery, but it may also be a way to selectively modulate neural activity. Its neurorehabilitative potential and its effects on cortical reorganisation have recently been investigated in stroke patients (Takeuchi et al., 2005, Hummel et al., 2005, Nowak et al., 2008, Grefkes and Fink, 2012). So far, neuromodulatory interventions have targeted ipsilesional and contralesional M1, with the aim to correct abnormal interhemispheric inhibition. Some patients have benefited from a suppression of contralesional M1 over-activity by means of low-frequency repetitive TMS (rTMS) (Nowak et al., 2008). Similarly, other patients have benefitted from increasing ipsilesional M1 activity, induced either by high-frequency rTMS (Ameli et al., 2009) or anodal transcranial direct current stimulation (tDCS) (Bastani and Jaberzadeh, 2012, Butler et al., 2013).

However, treatment efficacy of non-invasive brain stimulation approaches has varied (Grefkes and Fink, 2012). One reason why efficacy has varied is that we have insufficient knowledge of the different types of stroke, post-stroke states and individual "recovery biographies". A better knowledge of these could

inform improved neuromodulatory protocols that work synergistically with ongoing processes of adaptive network organisation and recovery specific to individual patients. Such novel protocols may involve, for example, secondary motor areas which have been implicated in motor recovery in stroke patients, such as PMd, PMv, and supplementary motor area (SMA) (Rehme et al., 2012).

PMv is a critical part of a fronto-parietal network that controls prehension and is involved in learning sensorimotor transformations for visually-guided actions (Deiber et al., 1997, Kantak et al., 2012). It is densely interconnected with M1 and has direct corticospinal projections to the cervical enlargement, and via these connections PMv may be able to control individualised finger movements. Extensive rewiring involving PMv has been associated with recovery of dexterity in several animal models following neural damage (Frost et al., 2003, Dancause et al., 2005, Nishimura et al., 2007). Relative expansion of PMv as well as the formation of new corticocortical connections from PMv to somatosensory cortex (S1) have been described in response to lesions of the M1 hand representation (Frost et al., 2003, Dancause et al., 2005).

The present study investigated the functional importance of ipsilesional and contralesional PMv on grasping function in subcortical stroke patients. Furthermore, it aimed to explore the level of cortical plasticity in the PMv-M1 pathway within one hemisphere and across hemispheres (with M1 being stimulated within the hemisphere contralateral to the hand involved in the grasping task, i.e. in patients the stroke-affected hemisphere) using our paired associative TMS (paTMS) protocol. We specifically wanted to test if it is possible to enhance corticospinal motor output (Buch, Johnen et al., 2011) and improve grasping movements in patients by increasing the effective influence of PMv on M1.

5.3 METHODS

Volunteers

Six patients (age: 61.8 ± 12.9 years (mean $\pm$ SD); 2 female) and ten healthy age-matched control participants (age: 64.3 ± 8.9 years (mean $\pm$ SD); 3 female) were recruited with Local Ethics Committee approval (NRES Committee South Central - Oxford C Ref: 09/H0606/45) and gave their written informed consent to participate, in accordance with the Declaration of Helsinki (Rickham, 1964).

We also reanalysed data from younger healthy participants to examine if changes in corticospinal excitability following plasticity induction are dependent on the participant's age. We used data that was acquired in young volunteers, i.e. Experiment 3 in Results 2.4.4 and Experiment 2 in Results 3.4.1 (baseline and "Expression" only). The mean group age within this group of twenty-three young individuals was 26.8 ± 7.1 years (mean $\pm$ SD; 11 female) and is referred to as the "young" group below.

Patients had mild to medium weakness of one hand and were at least 6 months post first ischaemic or haemorrhagic stroke, had no lesions in M1, and had no previous history, signs, or symptoms of other neurological conditions. Clinical characteristics of patients studied are described in Table 5.1. Neither patients nor healthy volunteers were on CNS-active medications.

Summary of experimental design

Healthy controls as well as patients participated in three experiments; the order of experiments was counterbalanced. Testing sessions were on average 18 ± 9 weeks apart for healthy controls and 2 ± 1 weeks apart for patients (mean $\pm$ SD). In each experimental session volunteers performed two blocks of a visually-guided reaching-and-grasping task whilst motor cortical excitability was probed with TMS applied to M1 contralateral to the active hand (each block was composed of 50 trials). The two blocks were separated by application of paTMS

Sex	Handedness	Age	Time since stroke (months)	Type of stroke	Lesion side	ARAT
F	Right	43	20	posterior limb of internal capsule, thalamus	Right	46
M	Right	60	109	posterior limb of internal capsule, putamen	Left	38
M	Right	79	232	posterior limb of internal capsule, putamen, thalamus, superior longitudinal fasciculus	Right	21
M	Right	72	14	posterior limb of internal capsule, putamen	Right	17
M	Right	69	75	posterior limb of internal capsule	Right	57
F	Right	48	167	medulla oblongata	Left	49

Table 5.1: Patient details

(15 minutes) and referred to as the baseline block and the post-TMS block (Fig. 5.1). Stroke patients were all right-handed and performed the movement with their impaired hand (right: n = 2 and left: n = 4). Right-handed healthy controls either used their dominant or their non-dominant hand for the task (n = 9 and n = 1, respectively). I am aiming to involve more right-handed healthy controls who will perform the grasping task with their non-dominant, left hand to better match the stroke patient group. Whereas PMd is known to have a left-hemispheric dominance, for example, in action selection (O'Shea et al., 2007b), no such laterality is known for PMv.

One of the following three stimulation protocols was applied during paired-associative transcranial magnetic stimulation (paTMS) (Fig. 5.1): Experiment 1 with paTMS over ipsilesional PMv and ipsilesional M1 with 8 ms interpulse interval (IPI) (intrahemispheric stimulation: M1 and PMv stimulation contralateral to active hand, i.e. affected hand in patients); Experiment 2 with paTMS over contralesional PMv and ipsilesional M1 with 8 ms IPI (interhemispheric stimulation: M1 stimulation contralateral to active hand (i.e. affected hand in patients), PMv stimulation ipsilateral to active hand); and Experiment 3 with

paTMS over ipsilesional PMv and ipsilesional M1 with 500 ms IPI (intrahemispheric stimulation: M1 and PMv stimulation contralateral to active hand, i.e. affected hand in patients). Changes in corticospinal excitability were probed during the baseline block and the post-TMS block, always in the pathway that was stimulated during paTMS. In all cases the effect of PMv stimulation on the effect of M1 stimulation was determined by comparing motor-evoked potentials (MEPs) recorded from the first dorsal interosseus (FDI) and abductor pollicis brevis (APB) muscle of the task-performing hand, in response to either single-pulse TMS (spTMS) applied to M1 alone or ppTMS delivered over both PMv followed by M1 (ppTMS; 8 ms IPI). Whereas we hoped to obtain MEPs in both FDI and APB, we found that we tended to have data for either one or the other muscle. Therefore, we focussed our analysis on MEPs from the FDI muscle for which we had obtained reliable MEPs in most participants; MEPs from APB were only used in three occasions in healthy controls and four occasions in patients. Patients performed the Action Research Arm Test (ARAT) (Lyle, 1981) before and after each experimental session.

Task blocks

Baseline and post-TMS blocks consisted of 50 grasping trials, from which the first ten trials were not included in any further analysis. MEPs from 20 spTMS trials and 20 ppTMS trials were included in the analysis. Both spTMS and ppTMS trials were administered in pseudo-random order within the same block of trials (Buch et al., 2010). Precise IPI timing is critical if both PMv and M1 pulses are to produce coincident influences on corticospinal activity; both resting-state and task-state interactions emerge at 6 - 8 ms IPIs (Davare et al., 2008, Davare et al., 2009, Buch et al., 2010, Neubert et al., 2010). In Experiment 1 and Experiment 3 the influence of PMv on M1 within the hemisphere contralateral to the active hand was assessed; Experiment 2 assesses the

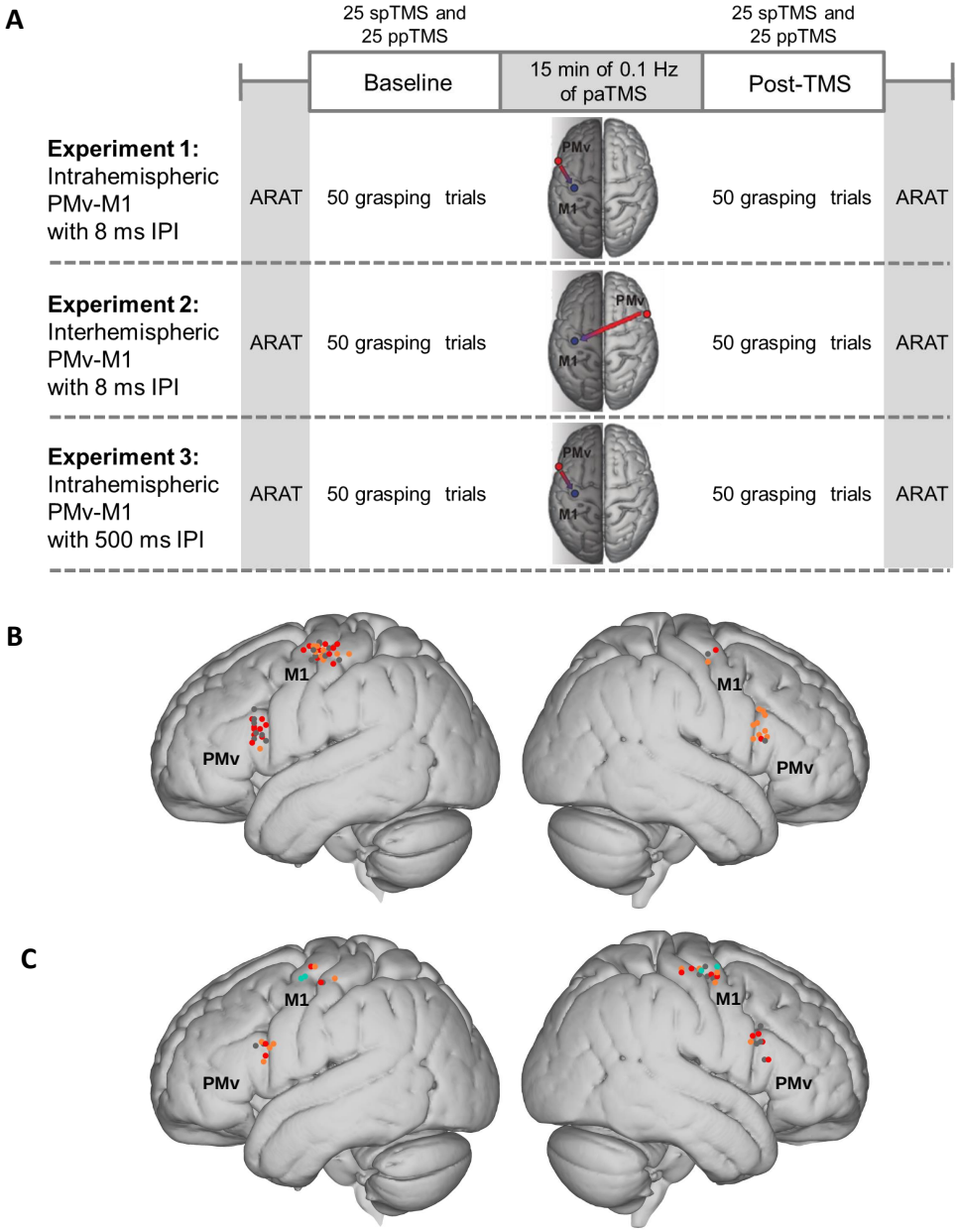

Figure 5.1: (A) Experimental design and set-up for all experiments. Measurements of corticospinal excitability were taken while the subjects were performing reaching-and-grasping movements (either with spTMS over M1 or ppTMS over PMv and then M1 (IPI of 8 ms)) before and after 15 minutes of 0.1 Hz of paTMS (baseline and post-TMS, respectively). The lesioned hemisphere is plotted on the left. Patients also performed the ARAT before and after TMS. Individual stimulation sites on MNI brain for healthy controls (B) and stroke patients (C). The left hemisphere is shown on the left, the right hemisphere on the right. Stimulation sites for Experiment 1 are shown in *red*, for Experiment 2 in *orange* and for Experiment 3 in *grey*. The M1 stimulation "hot-spot" for FDI for the intact hemisphere in patients is shown in *mint*.

influence of PMv on M1 across hemispheres, with M1 being stimulated in the hemisphere contralateral to the active hand, i.e. affected hand in patients.

Volunteers were seated at a table, their head supported by a chin-rest, and their arms comfortably placed on the table in order to make reaching-and-grasping movements towards one of two concentrically arranged cylinders (25 and 50 mm diameter) placed in front of them, cued by illumination of one of two concentrically arranged cylinders on a computer screen.

The distance of cylinders from the starting hand position was determined for each individual subject so that each person, including patients, could perform the movement easily; the distance, however, was then kept constant for each of the three experimental sessions for a given subject. Each trial was initiated by pressing a touch-bar. Following a variable delay 2.5 to 5.0 s (3.7 ± 0.8 s, mean$\pm$ SD) one cylinder on the screen was illuminated. Volunteers responded by grasping the corresponding cylinder with their thumb and index finger, lifting it from its pedestal and returning it. All trials were accompanied by either spTMS or ppTMS, with the pulse applied to M1 always occurring 75 ms after movement onset. Because reaction times in patients vary more than in healthy controls, stimulation was applied during the perimovement period during which PMv facilitates M1 corticospinal activity (Buch et al., 2010). For the duration of each experiment, TMS coils were fixed in place tangentially to the scull by means of adjustable metal arms and monitored throughout the experiment.

TMS and electromyography recordings

TMS was applied using two Magstim200 (The Magstim Company Ltd) stimulators each connected to a 50 mm figure-of-eight coil. The M1 "hot-spot" was the scalp location where stimulation evoked the largest MEP amplitude in the FDI (the M1 investigated was in the hemisphere contralateral to the task-executing

hand.) The "hot-spot" scalp location was projected onto high-resolution T1-weighted anatomical MRIs of each volunteer's brain using frameless stereotactic neuronavigation (Brainsight, Rogue Research Inc.). The mean Montreal Neurological Institute (MNI) location for the left and right M1 "hot-spots" are shown in Table 5.2 and Figure 5.1 B and C.

The PMv coil location was determined anatomically with a marker being placed on the cortical surface of each individual's MRI and adjusted with respect to individual sulcal landmarks to a location immediately anterior to the inferior precentral sulcus. Mean stimulation sites for PMv are shown in Table 5.2 and Figure 5.1 B and C. Cortical stimulation sites were similar to those reported previously in Chapter 2 and 4.

	Healthy controls	Stroke patients	Intact hemisphere M1 in stroke patients
left M1	-37 -16 59	-32 -14 59	-33 -6 58
right M1	38 -9 57	33 -12 58	33 -9 60
left PMv (ipsilateral to left M1)	-55 17 20	-53 15 22	
left PMv (ipsilateral to right M1)	54 17 22	54 15 23	
right PMv (ipsilateral to right M1)	58 19 14	52 17 23	
right PMv (ipsilateral to left M1)	-60 16 10	-54 12 22	

Table 5.2: Mean TMS stimulation sites in MNI space ([x y z] coordinates).

PMv stimulation intensity was proportional to 115 % of M1 resting motor threshold (RMT) (Rossini et al., 1994). In patients, RMT was determined for the FDI "hot-spot" of the unaffected hemisphere on the first experimental session and was 39 ± 7 % (mean $\pm$ SD %) stimulator output. Individual stimulation intensities were kept constant over the course of all three experimental sessions as determined on the first session. In patients PMv was stimulated with 43 ± 8 % stimulator output, and in healthy individuals PMv was stimu-

lated with 42 ± 13 % stimulator output (healthy control RMT: 37 ± 12 % stimulator output). M1 stimulation intensity during experiments was set to elicit baseline single-pulse MEPs of ~ 0.6 mV (60 ± 6 % in patients (affected hemisphere) and 44 ± 12 % stimulator output in healthy controls).

FDI and APB electromyography (EMG) was recorded with bipolar surface Ag-AgCl electrode montages. Responses were band-pass filtered between 10-1000 Hz, with additional 50 Hz notch filtering, sampled at 5000 Hz, and recorded using a CED 1902 amplifier, a CED micro1401-3 A/D converter, and PC running Spike2 (Cambridge Electronic Design, Cambridge, UK).

Analysis focused on peak-to-peak amplitudes of MEPs in response to either spTMS (20 trials) applied to M1 or ppTMS delivered over both PMv and M1 (20 trials). MEPs recorded during the first ten trials were not included in the analysis to allow for titration of stimulation intensity.

Paired associative TMS (paTMS)

paTMS was applied for 15 minutes at a frequency of 0.1 Hz (90 total stimulus pairings). For Experiment 1 and Experiment 2 repeated paired TMS was applied at an IPI of 8 ms. The IPI of 8 ms has been shown to modulate intrahemispheric PMv-M1 pathway connectivity in younger, healthy controls (Buch, Johnen et al., 2001; Chapter 2 and Chapter 4). In Experiment 3 we employed a 500 ms IPI, an interval many times longer than the longest one at which PMv-M1 interactions have been shown to occur and which has been shown to not alter pathway connectivity in healthy participants (Chapter 4). Volunteers kept their hand relaxed throughout the paTMS period.

Comparing effects of paTMS over intrahemispheric PMv-M1 on motor cortical excitability in different age groups

Because the MEP amplitudes in the "age-matched" group were matched in size to the group mean amplitude measured in stroke patients, the group mean MEP amplitudes with ~ 0.6 mV were smaller than those of 1.0 mV from the "young" group (i.e. baseline measurements from Experiment 3 in Results 2.4.4 and from Experiment 2 in Results 3.4.1. To compare the effects of paTMS over intrahemispheric PMv-M1 (Experiment 1) on corticospinal motor output between the "young" and "age-matched" experimental groups, we normalised individual median MEP amplitudes within the three independent experiments. Individual median MEP amplitudes (baseline spTMS, baseline ppTMS, post-TMS spTMS and post-TMS ppTMS) were divided by the group mean baseline spTMS MEP amplitude and expressed as a "percentage of the baseline spTMS".

Pathway connectivity strength indexed by paired-pulse TMS ratio

The effective influence of PMv on M1 motor output can be expressed as the "ratio" of the median MEP amplitude, i.e. MEP amplitude following ppTMS (PMv-M1) minus MEP amplitude following spTMS (M1 alone) relative to MEP amplitude following spTMS (= [(ppTMS-spTMS)/spTMS]). The ratio describes the effect PMv has on M1 and is positive when PMv has a facilitatory effect over M1, and it is negative when the impact of PMv on M1 is inhibitory. The ratio does not directly describe changes in M1 excitability; however, it could be affected by changes in M1 excitability. If M1 excitability decreased as assessed by an spTMS MEP, but there was no change to the ppTMS MEP itself, the resulting ratio would present as increased and therefore the result might be interpreted as an increase in PMv's effective influence over M1. However, in the following we tested that increases in PMv-M1 connectivity were not driven by changes in M1 alone. We investigated the ratio effect in stroke patients as we hypothesised

that the relative influence of PMv over M1 was selectively increased following pathway plasticity induction and not affected by the type of ceiling-effect seen in healthy controls (compare Experiment 3 in Results 2.4.4).

Action Research Arm Test (ARAT)

At the start and at the end of each experimental session, patients completed the ARAT which assesses grasp, grip, pinch, and gross arm movements and has a maximum score of 57. An "overall improvement" in motor function is represented by the difference of the ARAT score at the beginning of the first experimental session and at the end of the last (third) experimental session.

Statistical tests

Effects of individual paTMS protocols on motor cortical excitability in healthy controls were tested with a repeated-measures ANOVA with within-subject factors TIME (baseline / post-TMS) and TMS (spTMS / ppTMS) on the dependent variable median MEP amplitude.

Changes in PMv-M1 pathway connectivity, as induced by application of paTMS protocols, were compared between healthy controls and stroke patients using a mixed-model ANOVA on the MEP ratio with between-subjects factor GROUP ("stroke patients" / "healthy controls") and within-subjects factor TIME (baseline / post-TMS) and EXPERIMENT (Experiment 1 / Experiment 2 / Experiment 3).

Modifications in PMv-M1 pathway connectivity were then analysed separately for each experiment using an independent one-tailed t-test with non-equal variances on "ratio difference" ("post-TMS ratio" minus "baseline ratio"); a positive number indicates an increased positive influence of PMv on M1 following paTMS. Significant results were subsequently analysed with paired-samples t-test on "baseline ratio" versus "post-TMS ratio".

For stroke patients, we analysed changes in motor function as indexed by the ARAT score by comparing ARAT scores prior to and following application of either intervention (Experiment 1, 2, and 3). We conducted a repeated-measures ANOVA on the ARAT score with within-subjects factors TIME (baseline / post-TMS) and EXPERIMENT (Experiment 1 / Experiment 2 / Experiment 3). Such analyses were not possible in control subjects because control subjects all tend to obtain the maximum score on the ARAT and there is little variation in performance over time.

Pearson's correlation coefficients were calculated to assess correlations between the MEP ratios or differences in MEP ratios (baseline vs. post-TMS) and overall improvements of motor function (for detail see Results 5.3).

5.4 RESULTS

5.4.1 Paired associative TMS increases motor cortical excitability in healthy controls

Experiment 1: Increased motor cortical excitability independent of age in healthy controls

Application of paTMS over intrahemispheric PMv-M1 with an 8 ms IPI (Experiment 1) has been shown to induce cortical plasticity in the PMv-M1 connection in line with Hebbian principles (Buch, Johnen et al. 2011). In our first analysis we examined whether age had an impact on plasticity expression. For this purpose we combined data that was acquired in young volunteers following application of the same stimulation protocol (i.e. Experiment 3 in Results 2.4.4 and Experiment 2 in Results 3.4.1) with data acquired for healthy volunteers who are matched in age to the stroke patients.

Plasticity induction over PMv-M1 significantly increased corticospinal excitability in both spTMS and ppTMS trials independent of age as revealed by a

repeated-measures ANOVA with within-subject factors TIME (baseline / post-TMS) and TMS (spTMS / ppTMS) and AGE as a covariate (main effect of TIME: $F_{1,31} = 7.14$, $P = 0.012$; Fig. 5.2). There was no interaction between TIME and AGE: $F_{1,31} = 0.79$, $P = 0.38$. These results indicate that age has no impact on the brain's response to plasticity induction by means of paTMS.

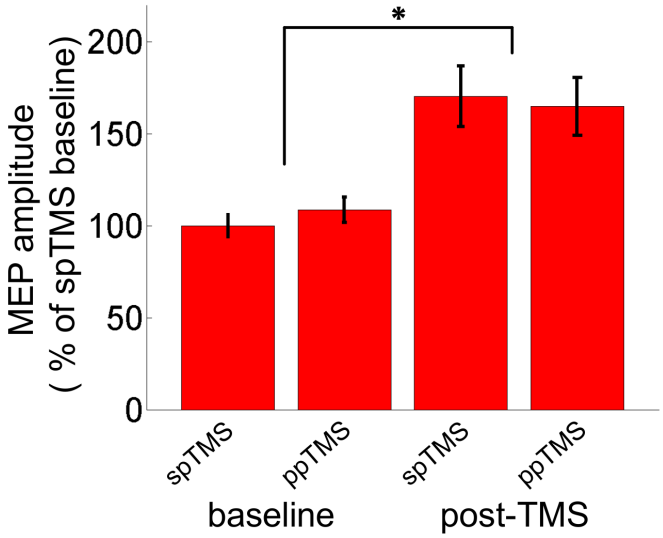

Figure 5.2: **Experiment 1: paTMS over intrahemispheric PMv-M1 increases corticospinal motor output in all individuals independent of age.** The figures show group mean of median MEP amplitudes (averaged across "young" and "age-matched" healthy controls) as measured with either spTMS over M1 or ppTMS over PMv and then M1 (with an 8 ms IPI) at baseline and after TMS (post-TMS) and is expressed as a percentage of the "baseline spTMS" group mean MEP amplitude. Measurements were taken while the subjects were performing a reaching-and-grasping movement. Error bars represent 1 s.e.m.

Experiment 2: Increased motor cortical excitability in healthy controls

In the current experiment, we also included a variation of the previous paTMS protocol: we investigated whether it was possible to induce plasticity within the interhemispheric PMv-M1 pathway. Figure 5.3 illustrates that corticospinal excitability was increased significantly following paTMS over the PMv-M1 pathway with an IPI of 8 ms (PMv ipsilateral and M1 contralateral to active hand)

(Buch et al., 2010) in age-matched healthy controls (repeated-measures ANOVA with within-subject factors TIME (baseline / post-TMS) and TMS (spTMS / ppTMS): main effect of TIME: $F_{1,9} = 5.11$, $P = 0.05$; Fig. 5.3). The increase in spTMS-evoked MEP amplitude was significant following paTMS ($t_9 = -2.27$, $P^- 0.049$), whereas the ppTMS measure showed a tendency for increased excitability ($t_9 = -1.90$, $P^- 0.091$). The results therefore demonstrate that it is, indeed, possible to selectively potentiate neurophysiological connectivity within an interhemispheric pathway using paTMS at intervals that lead to synchronous activity in interconnected areas.

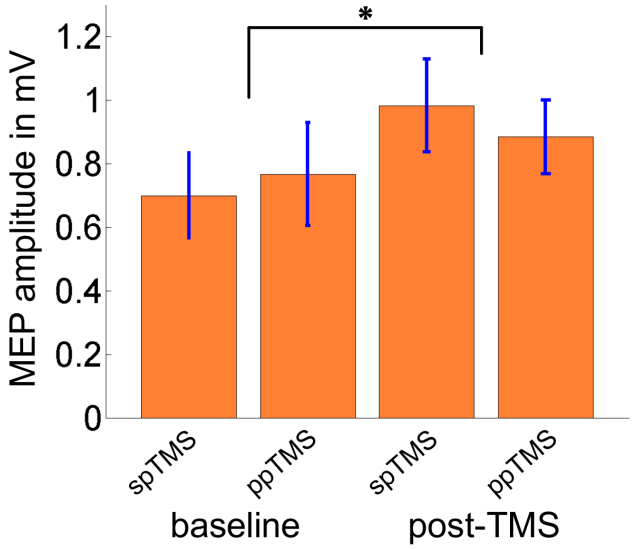

Figure 5.3: **Experiment 2: paTMS over contralateral PMv-M1 increases corticospinal motor output in age-matched healthy controls.** The figure shows group mean of median MEP amplitudes as measured with either spTMS over M1 or ppTMS over contralateral PMv and then M1 (with an 8 ms IPI) at baseline and after TMS (post-TMS). Measurements were taken while the subjects were performing a reaching-and-grasping movement. Error bars represent 1 s.e.m.

Comparison of Experiment 1 and 2: Increased motor cortical excitability in healthy controls following within and across hemispheres paTMS

A direct comparison of Experiment 1 and Experiment 2 with a three-factor ANOVA confirmed that paTMS induces increased motor excitability across experiments (significant main effect for TIME: main effect of TIME: $F_{1,41} = 9.33$, $P = 0.004$); the plasticity induction protocols did not modulate excitability differently (no interaction of TIME by EXPERIMENT: $F_{1,41} = 2.25$, $P = 0.14$).

Experiment 3: No change in motor cortical excitability in healthy controls

When the intrahemispheric PMv-M1 connection was repeatedly stimulated with an IPI of 500 ms, an interval many times longer than the longest one at which PMv-M1 interactions have been shown to occur (Davare et al., 2009, Neubert et al., 2011), no change in corticospinal excitability was observed (re-

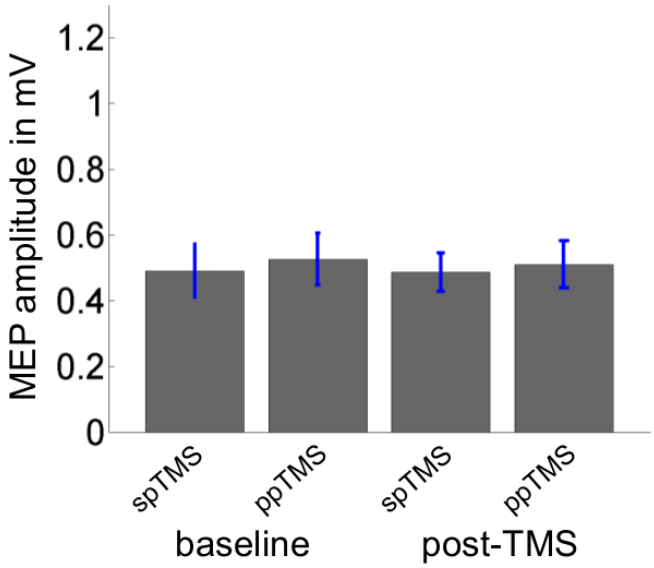

Figure 5.4: **Experiment 3: paTMS over intrahemispheric PMv-M1 does not increase corticospinal motor output in age-matched healthy controls.** The figure shows group mean of median MEP amplitudes as measured with either spTMS over M1 or ppTMS over PMv-M1 contralateral to active hand (with an 500 ms IPI) at baseline and after TMS (post-TMS). Measurements were taken while subjects were performing a reaching-and-grasping movement. Error bars represent 1 s.e.m.

peated-measures ANOVA with within-subject factors TIME (baseline / post-TMS) and TMS (spTMS / ppTMS): no main effect of TIME: $F_{1,9} = 0.03$, $P = 0.87$).

5.4.2 Comparison of healthy controls and stroke patients

Grasp-specific facilitation of PMv over M1 significantly reduced in stroke patients

Movement of the affected hand is associated with altered patterns of neural activity in chronic stroke patients, and better functional recovery has been linked to "normalisation" of pathological activation patterns over time (Grefkes and Ward, 2014). In the following we probed whether there were any differences in intrahemispheric PMv-M1 pathway connectivity when stroke patients performed grasping movements with their impaired hand compared with healthy controls. In healthy adults, execution of a precision grip towards objects is related to a net facilitatory influence of PMv over M1 (Davare et al., 2008, Buch et al., 2010). For stroke patients and age-matched healthy controls we had two baseline MEP ratios (acquired during Experiment 1 and Experiment 3) and used the averaged "baseline ratio" in the following analysis.

The facilitatory influence of ipsilesional PMv on M1 during performance of a grasping task was significantly different at baseline, i.e. prior to any attempt of plasticity induction, in stroke patients and healthy controls (independent two-samples t-test: $t_{9.2} = -2.79$, $P = 0.01$; Fig. 5.5).

A one-sample t-test confirmed a significant facilitatory influence of PMv over M1 in healthy participants ($t_9 = 3.98$, $P = 0.003$) and the lack thereof in stroke patients ($t_5 = 0.46$, $P = 0.67$). This finding suggests that reduction of ipsilesional PMv's influence on M1 partially contributes to the clinical motor disability seen in the patient group. We then went on to investigate if it is possible to modulate connectivity strength in the pathway in stroke patients by applying our paTMS protocols.

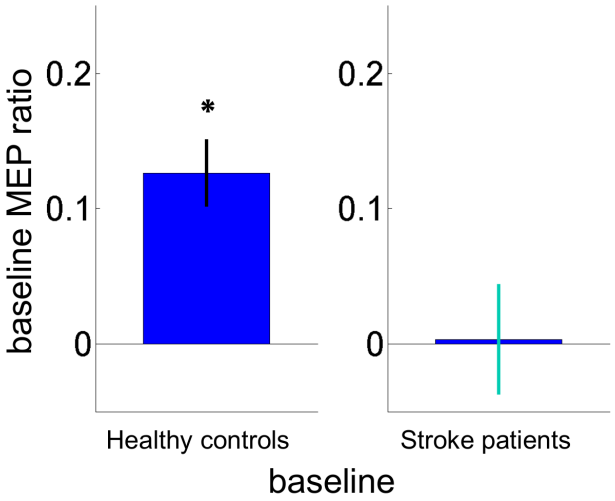

Figure 5.5: **Within hemisphere facilitatory influence of PMv on M1 contralateral to active hand is reduced within affected hemisphere in stroke patients.** Figure shows group mean of median MEP ratio at baseline as assessed with 8 ms IPI for ten age-matched healthy controls and six stroke patients. Measurements were taken while the subjects were performing a reaching-and-grasping movement. Error bars represent 1 s.e.m.

Paired associative TMS modulates corticospinal excitability differently in stroke patients and age-matched healthy controls

As a first step, we investigated whether there was a difference in how application of the three different paTMS protocols altered cortical excitability in stroke patients and age-matched healthy controls. For this purpose, we conducted a mixed-model three-way ANOVA on the dependent variable median MEP amplitude (within-subject factors EXPERIMENT (Experiment 1 / Experiment 2 / Experiment 3), TIME (baseline / post-induction) and TMS (spTMS / ppTMS) and between-subject factor GROUP (healthy controls / patients). The analysis revealed that modulation of connectivity by means of paTMS altered excitability differently in healthy controls and patients (interaction of TIME by GROUP: $F_{2,14} = 4.98$, $P = 0.043$; no other main or interaction effect was significant: $F \leq 4.02$, $P \geq 0.07$).

Whereas motor cortical excitability was generally increased following

paTMS with an 8 ms IPI in age-matched healthy controls, this effect may be different in stroke patients with motor disabilities. This hypothesis is grounded on the understanding that a reduction of dexterity in stroke patients is related to the extent of corticospinal tract disruption (Stinear et al., 2007, Schulz et al., 2012), and that the reduction in TMS-evoked corticospinal motor output of M1 correlates with the extent of corticospinal tract damage (Ward et al., 2007). Ultimately, this means that higher stimulation intensities have to be applied to evoke a given change in MEP amplitude in patients. In the current protocol the stimulator output is not increased following paTMS and therefore, spTMS over M1 may not result in increased motor output. However, as the protocol strengthens the input from PMv into M1, it may be that the facilitatory influence of PMv on M1 invokes changes to motor cortical excitability as probed with ppTMS.

Subsequently, knowing that both Experiment 1 and Experiment 2 induce increases in motor cortical excitability in healthy controls (see Results 5.4.1 above), we tested whether there was a difference in how stroke patients respond to those excitability-modulating interventions with an 8 ms IPI. We conducted a direct comparison of motor excitability using a mixed-model ANOVA with within-subject factors EXPERIMENT (Experiment 1 / Experiment 2), TIME (baseline / post-induction) and TMS (spTMS / ppTMS) between healthy controls and patients (GROUP). The analysis revealed that paTMS modulated motor excitability (main effect of TIME: $F_{1,30} = 5.68$, $P = 0.032$), but does so differently in healthy controls and patients (interaction effect of TIME by GROUP: $F_{1,30} = 6.56$, $P = 0.023$). In stroke patients, paTMS did not lead to increases in motor excitability as revealed by a two-factor ANOVA with within-subject factors EXPERIMENT (Experiment 1 / Experiment 2) and TIME (baseline / post-induction) (no main effect of TIME: $F_{1,5} = 0.05$, $P = 0.84$). However, there was a main effect of PULSE ($F_{1,5} = 6.78$, $P = 0.048$) indicating that PMv has a significant, directional influence on M1 in patients.

We further investigated the nature of this effective influence of PMv on M1 by analysing the MEP ratio. The ratio is of particular interest as it is thought to reflect the neurophysiological, causal interactions between two brain areas and therefore, a measure that can selectively show changes in pathway efficacy. Here, we demonstrated that stroke patients and age-matched healthy controls responded differently to paTMS protocols with respect to their PMv-M1 connectivity strength (mixed-model repeated-measures ANOVA with the dependent variable MEP ratio: interaction effect of GROUP by TIME: $F_{1,14} = 5.86$, $P = 0.03$; no interaction of GROUP by EXPERIMENT: $F_{2,13} = 0.73$, $P = 0.50$; no interaction of GROUP by EXPERIMENT by TIME: $F_{2,13} = 0.19$, $P = 0.83$). Therefore, we wished to further investigate how each paTMS protocol modulates PMv-M1 effective connectivity differently in stroke patients and healthy controls and therefore focussed the following analyses on the change in PMv-M1 connectivity.

Experiment 1: Selective enhancement of facilitatory influence of ipsilesional PMv on ipsilesional M1 in stroke patients

Application of the paTMS protocol over the intrahemispheric PMv-M1 pathway (Experiment 1) had a different effect on PMv-M1 connectivity in stroke patients and age-matched healthy controls (independent one-tailed t-test with non-equal variances on "ratio difference": $t_{10.4} = -1.97$, $P = 0.038$, Fig. 5.6). A one-sample t-test on the "ratio difference" revealed that the facilitatory effect of PMv on M1 was significantly increased in stroke patients (patients: $t_5 = 3.56$, $P = 0.008$, Fig. 5.6 D; healthy controls: $t_9 = -1.02$, $P = 0.17$; Fig. 5.6 C).

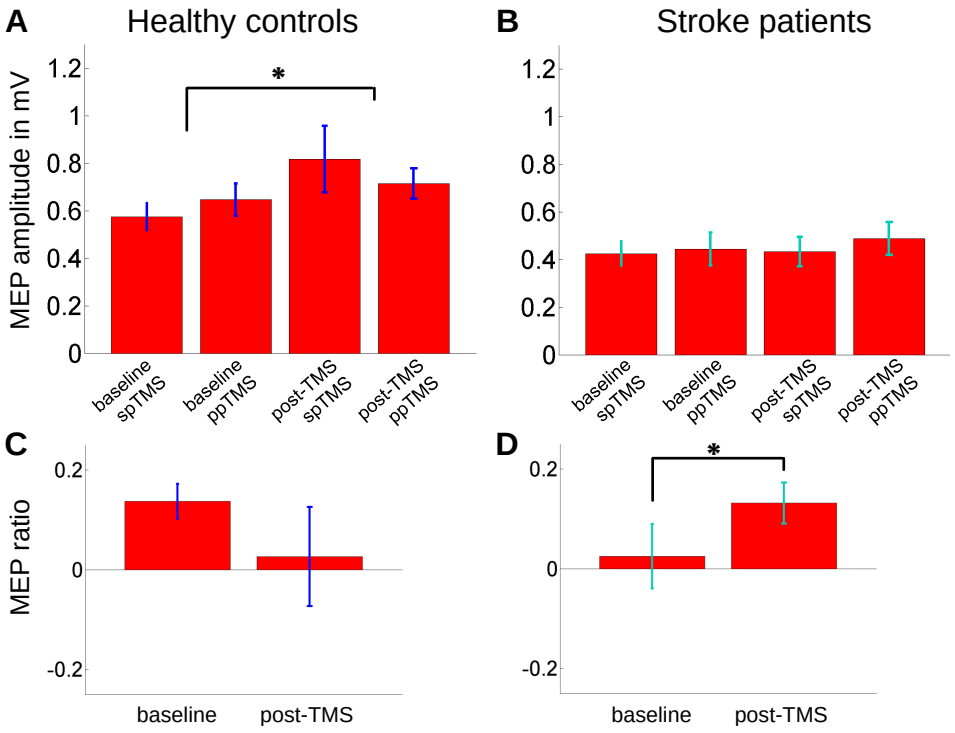

Figure 5.6: **Experiment 1: Intrahemispheric PMv-M1 paTMS enhances facilitatory effect of PMv on M1 motor output during performance of grasping task in stroke patients within lesioned hemisphere.** (A - B) The figure shows group mean of median MEP amplitudes for (A) healthy controls and (B) patients. (C - D) Group mean of median MEP ratio (effect of PMv on M1) for baseline and post-TMS time point. In healthy controls (C) the effect of PMv on M1 did not significantly change following plasticity induction. (D) In patients, corticospinal activity of M1 is significantly driven by input from ipsilesional PMv following plasticity induction. Error bars represent 1 s.e.m.

Experiment 2: No differential modulation of influence of PMv on M1 across hemispheres

In contrast, application of the paTMS protocol over the interhemispheric PMv-M1 pathway (Experiment 2; in the patients this corresponds to contralesional PMv and ipsilesional M1) did not modulate the pathway differentially in subcortical stroke patients and age-matched controls (independent one-tailed t-test with non-equal variances on the "ratio difference": $t_{13.8} = -1.02$, $P = 0.16$; Fig. 5.7).

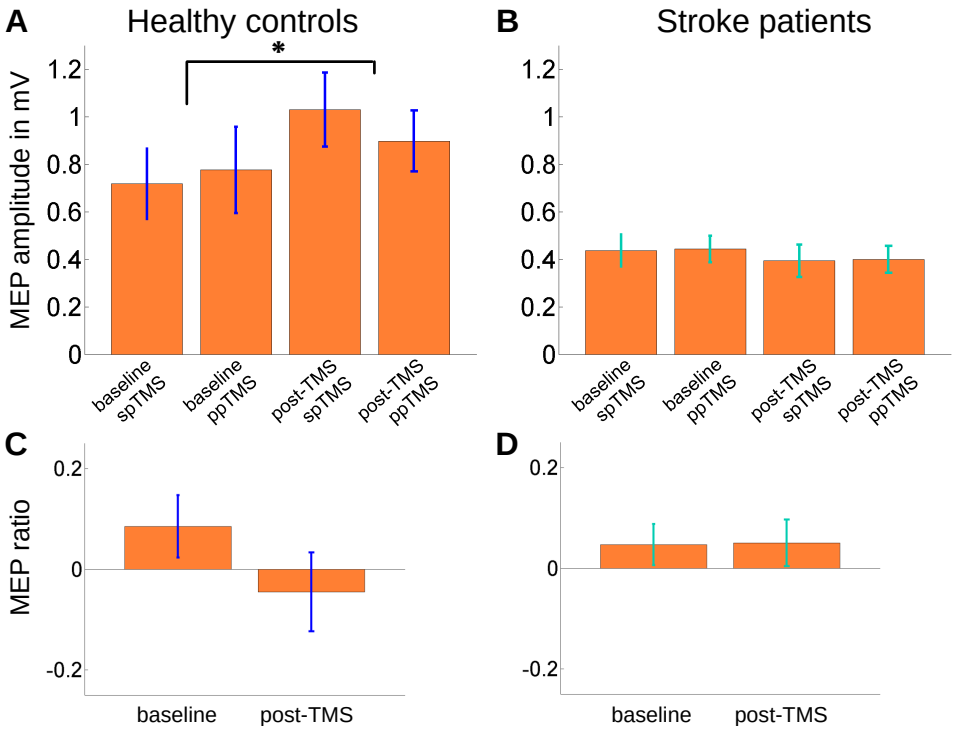

Figure 5.7: **Experiment 2: Interhemispheric paTMS with 8 ms IPI does not differentially alter connectivity in interhemispheric PMv-M1 pathway in healthy controls and stroke patients.** (A - B) The figure shows group mean of median MEP amplitudes for (A) healthy controls and (B) patients. (C - D) Group mean of median MEP ratio (effect of PMv on M1) for baseline and post-TMS time point. The effect of contralateral PMv on M1 was not significantly modulated in either in healthy controls (C) or stroke patients (contralesional PMv - ipsilesional M1) (D). Error bars represent 1 s.e.m.

Experiment 3: No differential modulation of influence of PMv on M1 within hemisphere

Application of paTMS with 500 ms IPI over intrahemispheric PMv-M1 pathway did not have a differential effect on pathway connectivity in stroke patients and healthy controls (Experiment 3) (independent one-tailed t-test with non-equal variances on "ratio difference" between patients and healthy controls: $t_{13.8} = -1.37$, $P = 0.10$; Fig. 5.8).

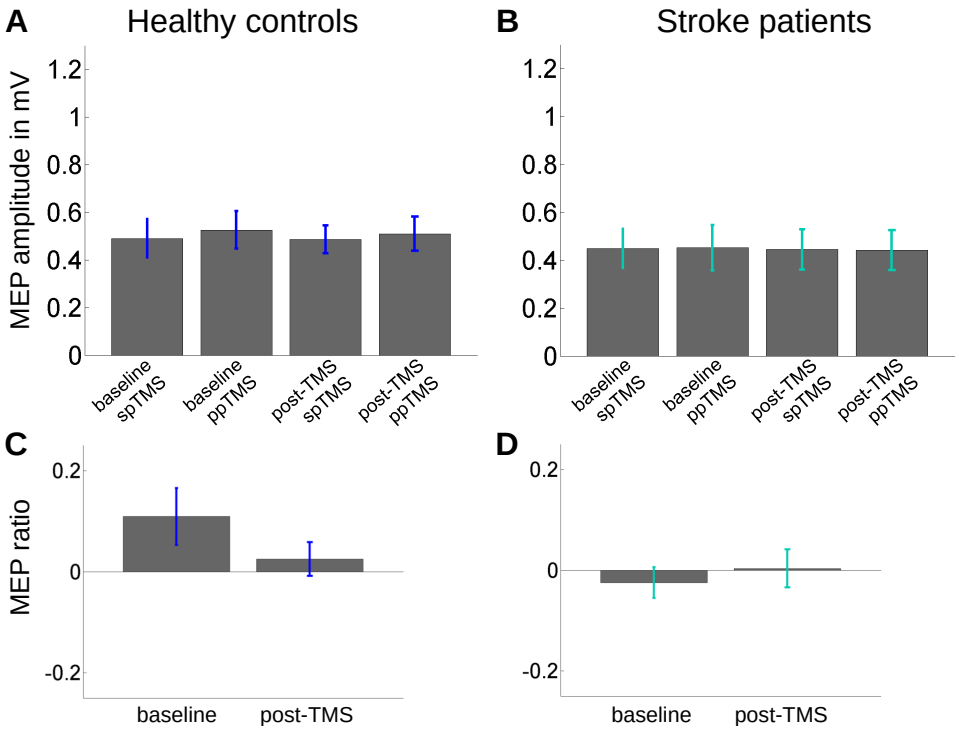

Figure 5.8: **Experiment 3: paTMS with 500 ms IPI does not differentially alter intrahemispheric PMv-M1 connectivity in healthy controls and stroke patients.** (A - B) The figure shows group mean of median MEP amplitudes for (A) healthy controls and (B) patients. (C - D) Group mean of median MEP ratio (effect of PMv on M1) for baseline and post-TMS time point. The effect of PMv on M1 within the stimulated hemisphere was not significantly modulated in either in healthy controls (C) or stroke patients (contralateral to active (affected) hand) (D). Error bars represent 1 s.e.m.

5.4.3 Behavioural improvements in patients

Improved motor performance in stroke patients

Whether the experimental intervention has any functional significance is best assessed by testing for an observable behavioural effect. Over the course of the three experimental sessions (i.e. from baseline at the first session to post-TMS after the last session), stroke patients improved their motor function as assessed by the ARAT score (main effect of TIME: $F_{1,4} = 26.64$, $P = 0.007$). A significant improvement was also present within each experimental session, but the improvement did not vary across the different experimental sessions (main

effect of TIME: $F_{1,4} = 13.64$, $P = 0.014$, no interaction of EXPERIMENT by TIME: $F_{1,4} = 0.81$, $P = 0.51$). In other words, ARAT improvement appeared to be the consequence of a general practice effect related to performance of the reaching and grasping movements within each experiment.

Experiment 1: Increased ipsilesional PMv-M1 pathway strength is correlated with achieved overall motor improvement

We wished to understand whether the neurophysiological finding of a reduced facilitatory effect of ipsilesional PMv on ipsilesional M1 (Fig. 5.5) is predictive of the patient's potential to improve their hand function. We found that there was a negative relationship between the overall improvement of upper limb motor function (difference in ARAT score from start of first to end of last experimental session) and the individual baseline PMv-M1 effect (R = -0.95, P = 0.004; Fig. 5.9 A), which suggests that patients with little PMv-M1 facilitatory effect will subsequently show the greatest behavioural benefits from the general motor training that the task provided. We tested whether individual baseline connectivity of PMv-M1 was correlated with the baseline ARAT score and found no significant relationship here (R = 0.40, P = 0.43). Taken together, the findings imply that low baseline PMv-M1 connectivity predicts the scope for functional improvement, but does not predict the functional ability at the time.

Application of 8 ms IPI paTMS over ipsilesional PMv-M1 may help to "normalise" the reduced impact of PMv over M1 in subcortical stroke patients (Fig. 5.6 B). We therefore tested if the extent of this increased effect of ipsilesional PMv on M1 also correlated with the overall improvement in motor performance. A greater responsiveness to plasticity induction, i.e. increased, facilitatory influence of PMv on M1 in Experiment 1, correlated positively with total improvement in arm-and-hand function (R = 0.85, P = 0.03; Fig. 5.9 B). Any changes in the impact of PMv on M1 following 8 ms IPI plasticity induction could also be

the consequence of improved grasping function due to within-session training effects that occurred during any particular testing session. This, however, seems unlikely; there was no relationship between the change in ratio and improved grasping function within the same session (R = 0.53, P = 0.28).

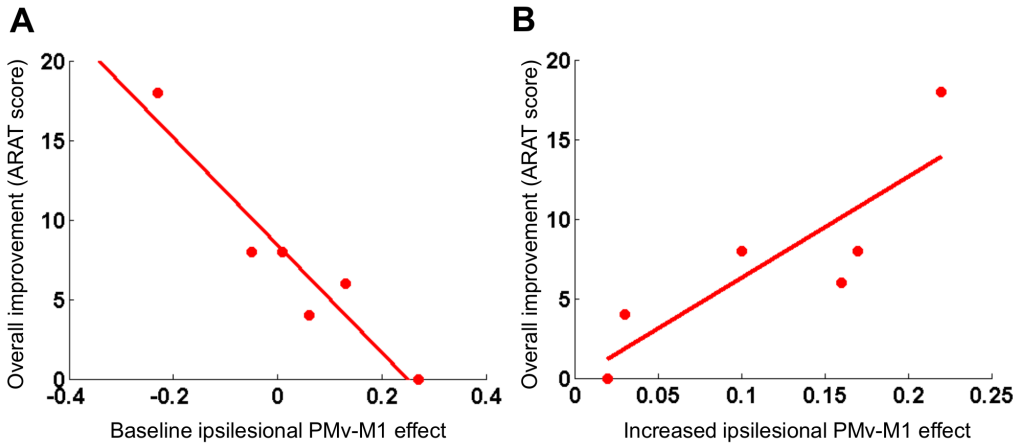

Figure 5.9: **Relationship between improvement of hand function and ipsilesional PMv and M1 interactions in subcortical stroke.** Overall motor improvement correlates negatively with baseline facilitatory effect of PMv on M1 (A), but correlates positively with responsiveness to plasticity induction with 8 ms IPI over ipsilesional PMv-M1 (Experiment 1; difference in MEP ratio from baseline to post-TMS) (B). Overall improvement in motor performance is represented by the difference of the ARAT scores at the start of the first experimental session and after the last experimental session for each patient.

One might also think that the correlations of increased neurophysiological measures with behavioural improvements are attributable to a statistical regression to the mean, whereby there is a tendency for a the variables most distinct from the mean to return to "normal". However if this was the case, the correlation should also be present in Experiment 3 (paTMS over intrahemispheric PMv-M1 at 500 ms). We demonstrated that total motor improvement did not correlate with changes in PMv-M1 coupling following application of paTMS over intrahemispheric PMv-M1 at 500 ms (Experiment 3; R = -0.51, P = 0.30). There neither was a relationship between total motor improvement and the changes evoked by paTMS over interhemispheric PMv-M1 at 8 ms (Experiment 2; R = 0.48, P =

0.33). Taken together, these findings may indicate that patients who have greater scope to improve their motor function also respond more readily to plasticity induction over ipsilesional PMv-M1 as induced by 8 ms IPI paTMS and their effective influence of PMv on M1 does not simply regress to the mean, but only increases when pathway-strengthening is conducted over the ipsilesional PMv-M1 pathway.

5.5 DISCUSSION

PMv is a critical node in the fronto-parietal network that controls hand movements for prehension and object manipulation. In light of the fact that grasping function is often critically disrupted in subcortical stroke patients, we aimed to explore whether PMv's functional contributions to dexterity are distinct from those observed in healthy individuals. Whereas there are different lines of evidence that ipsilesional as well as contralesional PMd supports recovered motor function after stroke, little is known about the role of PMv in motor recovery. Work in primates has demonstrated that PMv is critical for recovery of dexterity (Frost et al., 2003, Dancause et al., 2005, Nishimura et al., 2007). The altered influence of PMv has been related to a relative expansion of PMv and novel corticocortical projections from PMv to S1 following lesions of the M1 hand area (Frost et al., 2003, Dancause et al., 2005). Whereas the M1 area is not directly lesioned in the case of subcortical stroke, pyramidal cells in the M1 hand area may be compromised as a consequence of ascending Wallerian degeneration. In accordance with the findings of increased connectivity between PMv and S1 (Dancause et al., 2005), it may be that PMv also forms novel connections with neurons in the sensorimotor cortex in humans with subcortical stroke. Evidence of increased activity in bilateral PMv during hand movements made by human patients in the chronic post-stroke period comes from a meta-analysis of func-

tional MRI studies (Rehme et al., 2012). Motor recovery is critically supported by PMd (Johansen-Berg et al., 2002, Fridman et al., 2004, Bestmann et al., 2010, Schulz et al., 2012), and given that the two premotor cortices show organisational similarities, such as somatotopic organisation and direct corticospinal projections that are descending in parallel with corticospinal projections originating in M1 (Dum and Strick, 1991, He et al., 1993, Hoyer and Celnik, 2011), PMv may play an alternative supportive role in recovery of dexterity.

By means of paired-pulse TMS we assessed the contribution of ipsilesional as well as contralesional PMv on M1 in a natural grasping task. Whereas contralesional PMd shows greater facilitation of M1 after cerebral insult (Bestmann et al., 2010), we found evidence of a different pattern of functional connectivity for the PMv-M1 pathway. The effective facilitatory influence of ipsilesional PMv over ipsilesional M1 during reach-and-grasp movement was reduced in subcortical stroke patients as compared to healthy controls. Intriguingly, a reduced facilitatory effect of ipsilesional PMv on ipsilesional M1 implied that the patient had a great capacity to improve their motor function. This finding may suggest that part of the functional impairment is due to decreased PMv input to M1. Our neurophysiological findings of a reduced influence of ipsilesional PMv on M1 appear to be contrary to functional imaging evidence of increased PMv activity during hand movement in recovered stroke patients (Rehme et al., 2012); however, increased fMRI activity does not assess a net effect of one cortical area upon another as does paired-pulse TMS. It is possible that activity in PMv represents increased local neural activation, as opposed to representing increased input into M1. The connection between PMv-M1 may have undergone synaptic depression due to its history of under-use when patients have refrained from executing complex grasping movements with their affected hand. It is possible that increased PMv activity is related to new corticocortical connections, for example projections to S1 corticospinal neurons as

demonstrated in squirrel monkeys following a focal lesion to the M1 hand representation. In the current study, it is unlikely that we assessed motor output generated from S1 since the M1 stimulation "hot-spot" was not shifted posteriorly relative to the stimulation "hot-spot" of the intact hemisphere (see Fig. 5.1 C). Moreover, it is also possible that PMv activity in imaging studies represents descending activity from PMv via the direct corticospinal tract which would not be assessed by paired-pulse PMv-M1 TMS protocols of the sort that we used here.

Within the first months of stroke, motor deficits improve via a mechanism referred to as "spontaneous biological recovery" (Cramer, 2008, Prabhakaran et al., 2008), which is contrasted with later processes of "compensation" in relation to motor learning (Kitago and Krakauer, 2013). The early process occurs in a sensitive period and leads to general motor recovery as opposed to training-associated, task-specific motor recovery (Zeiler and Krakauer, 2013). It has been suggested that plasticity levels are similar in the chronic post-stroke brain to those in the uninjured brain (Zeiler and Krakauer, 2013). Our current results however show that neuroplasticity, i.e. the brain's response to paTMS, is qualitatively different in chronic subcortical stroke patients and healthy, age-matched controls. Our results in stroke patients provide a classic example of Hebbian plasticity in response to paired associative stimulation over intrahemispheric PMv-M1 whereby the connection between area A and B is selectively enhanced, and the output of area B itself remains constant. Healthy participants, on the contrary, responded to paired associative stimulation within and across hemisphere(s) with a general increase in corticospinal excitability (Experiment 1 and Experiment 2; see Fig. 5.5). This effect was induced by stimulating the intrahemispheric PMv-M1 and the interhemispheric PMv-M1 connection with an IPI of 8 ms. This finding may appear puzzling because the anatomical route across the midline is longer and therefore may have a longer conduction time.

However, it is possible that myelination of the interhemispheric connection is higher and thus allows for increased transmission velocity. In this way both PMv areas may be simultaneously active during interactions with M1. There is evidence emerging that oligodendrocyte control of myelination is sensitive to network synchronization and may optimise function in an activity-dependent manner (Fields, 2010). It should, however, also be remembered that, because we did not probe a variety of IPIs, we cannot be certain that there are not small differences in the most effective IPIs for within and across hemisphere influences of PMv on M1.

One possible explanation for distinct patterns of neuroplasticity in subcortical stroke patients may be related to a different history of use of the PMv-M1 connection. Whereas object grasping is easy to perform with either hand in healthy individuals and does not require any motor learning, this is likely to be different for patients with impaired hand function who use their affected hand to a limited degree and instead use their unaffected hand to execute grasping movements. Accordingly, it is possible that increased PMv-M1 connectivity as seen in Experiment 1 is solely the expression of training-induced plasticity within the experimental session, evoked by repeated performance of the task (100 trials in total). It is, for example, possible to think that stroke patients' reaction times or movements became faster in the post-TMS block and that the timing of M1 TMS pulse was assessing different aspects of the grasping movement. However, if it was the case that increased pathway coupling was only the result of training, we would expect to see the same pattern of plasticity following application of paired TMS at an IPI of 500 ms (Experiment 3) which does not induce plasticity (Chapter 4.4.2). Therefore, it is likely that increased pathway connectivity is being evoked by inducing concurrent pre- and post-synaptic activity at PMv-M1 synapses, thereby increasing the pathway's efficacy. However, it remains the case that the effects of protocol-induced pathway strengthening

might have combined with movement practice effects.

We cannot conclusively say that increased pathway connectivity between ipsilesional PMv-M1 is relevant for an increased performance of the reaching-and-grasping task since motor improvements were similar over the course of each session. The finding of a positive relationship of the "normalisation" of connectivity and a patient's scope to improve their hand-arm function over the course of all experimental sessions however hints at functional relevance. The relationship of "responsiveness" to plasticity induction and motor learning may suggest that there is (1) endogenous variability in how plastic a brain is or (2) that some patients have not exhausted their potential for motor recovery. To understand whether an increased influence of ipsilesional PMv on M1 positively affects execution of grasping movements, I will analyse if online-grasping was altered differently in Experiment 1 compared to Experiment 3. In the current study, I also used an ultrasound-based motion analysis system to record the position of thumb, index finger and wrist in space; the data still requires analysing. It may be that the change in neurophysiological coupling is related to a reduced degree of movement variability - for example, in the grasp aperture, faster movements or a smoother transition from the transport-phase to the grasping-phase (Raghavan et al., 2010).

One other aspect that I aim to investigate is potential differences in motor retention. The period following training of a newly learnt visuomotor task has been associated with changes in M1 activity (Galea et al., 2011), and it may be that motor retention following "grasping-training" is enhanced more when TMS-induced PMv-M1 pathway plasticity induction occurred simultaneously. For this analysis, a larger stroke patient group and a fourth session, in which motor function is assessed, is required.

One striking finding is, that a relatively small group of six stroke patients responded highly consistently to plasticity induction (Experiment 1); more so than

did healthy controls. The differential level of variability may be a consequence of the limited degrees of freedom to respond to induced changes in excitability in the stroke brain. Importantly, we found no age-related reconfiguration of brain networks in the current population of age-matched healthy controls. Age-related decline in motor performance has been related to altered interactions within motor networks (Talelli et al., 2008, Ward et al., 2008).

The current study set out to investigate an alternative neuromodulatory treatment approach for stroke patients which would involve selectively strengthening connectivity between PMv-M1 and thereby promoting prehension movements which critically rely on PMv-M1 interactions. Currently most non-invasive brain stimulation protocols in motor rehabilitation target M1 and are aimed at either increasing ipsilesional M1 activity or rebalancing interhemispheric inhibition (Nowak et al., 2008, Ameli et al., 2009, Grefkes et al., 2010, Bastani and Jaberzadeh, 2012, Butler et al., 2013). The current protocol is based upon the notion that secondary motor areas are involved in supporting motor recovery, and the finding that PMv-M1 coupling is significantly reduced in subcortical stroke patients suggests that a "normalisation" of this interaction may benefit grasp execution. TMS may be suited to provide the "activation energy" to shift the network balance towards a normal movement-related facilitatory interaction between PMv and M1 which may allow for better motor learning. The current results also corroborate the finding that motor training, i.e. the enforced execution of one-hundred object-grasps, is effective at improving function of the affected upper limb even in the chronic post-stroke period (Kwakkel et al., 1999).

6

Conclusions

6.1 SUMMARY and CONCLUSIONS

Associative neuroplasticity has been linked to learning processes and shaping of neural networks. In Chapter 2, I presented a novel cortico-cortical paired associative stimulation (PAS) protocol that appears to selectively strengthen the stimulated pathway in line with principles of spike timing-dependent plasticity (STDP). Pathway plasticity in the stimulated connection of ventral premotor cortex (PMv) and primary motor cortex (M1) is expressed partly as a function of the endogenous activity induced by the current task state: inhibitory interactions are increased at rest and facilitatory interactions are enhanced during performance of a visuomotor task. In Chapter 3, I furthered my understanding of the plasticity induction protocol by showing that altered pathway excitability lasts for at least one hour and only starts to diminish at three hours. Chapter 4 investigated the neuroimaging correlates of plasticity induction within a larger network of interconnected fronto-parietal regions involved in prehension behaviour. Neuroimaging with functional magnetic imaging (fMRI) confirmed that PAS potentiates functional coupling in the PMv-M1 pathway, and furthermore that it has an impact on parallel pathways involved in reaching and grasping. I compared neurophysiological and neuroimaging indices of pathway interactions. There was a linear relationship between the strength of functional

connectivity and the strength of effective connectivity. The sign of an effective influence of one area upon another can be assessed by paired-pulse transcranial magnetic stimulation (TMS). Both inhibitory and facilitatory physiological influences of PMv onto M1 are reflected in positive functional coupling measures. This suggests that conclusions about the physiological nature of an interaction between two brain areas - whether it is facilitatory or inhibitory - cannot easily be drawn solely based on functional coupling measures.

In Chapter 5, I tested the potential of cortico-cortical PAS protocol as a neurorehabilitative tool in subcortical stroke patients with upper limb impairments. Patients presented with reduced PMv-M1 pathway connectivity in comparison to controls. Whereas patients showed a different pattern of neuroplasticity in comparison to controls, exogenous pathway strengthening was also induced in stroke patients. Differences in responsiveness to plasticity induction between patients could be directly linked to improvement of motor function.

The unifying theme of the projects in this thesis is that they employ non-invasive brain stimulation to explore associative plasticity induction in a targeted, specific anatomical pathway critical for prehension behaviour, both in the healthy human brain and in the chronic subcortical stroke brain. They investigate how the technique affects functional network interactions in healthy controls and describe the neuroimaging signature of altered network node coupling. Furthermore, they provide a novel non-invasive brain stimulation protocol that can successfully induce targeted pathway plasticity in subcortical stroke patients. All of this information is crucial to developing a greater understanding of pathological network interactions following stroke and hopefully to seeing the method applied as a therapeutic tool which can effectively restore behavioural impairments by promoting plasticity to shape neural networks in an adaptive manner.

6.2 FUTURE DIRECTIONS

To date, some progress has been made in understanding altered network configurations following stroke and the effects of specific non-invasive brain stimulation protocols on enhancing recovery of motor function. However, whereas there is a great heterogeneity of stroke patients - particularly with respect to stroke location, time since stroke, severity of motor impairment as well as genetics - neuromodulation attempts have all focussed on application of single-site neuromodulation. Strengthening connections may be more important than just modulating activity in one area if only because, firstly, all behaviour is produced by interactions of network nodes and, secondly, many strokes affect white matter and the location of white matter lesions is often the best predictor of symptoms and symptom severity. Therefore, novel stimulation protocols which can manipulate targeted pathway-specific interactions are required. Non-invasive brain stimulation is likely to alter neural activity patterns that may subsequently result in changes of brain anatomy. An interesting possible future avenue of research might be to attempt seeing if such changes can be measured using imaging protocols that have previously identified grey matter and white matter changes in other learning contexts (Scholz et al., 2009).

The prevailing hypothesis which has been addressed by all neuromodulatory approaches within the last five years is that stroke-induced network pathology is related to disturbed interhemispheric inhibition. Both repetitive TMS and transcranial direct current stimulation (tDCS) are aimed at correcting this network pathology by enhancing activity in ipsilesional M1 or by suppressing over-activity in the contralesional M1, and thereby improving motor function of the affected limb (Grefkes and Fink, 2012).

Neuroimaging and neurophysiological studies have provided us with increased understanding about brain lesion-induced reorganisation of the motor network, and it is now crucial to gain further insight into which interactions are

adaptive and maladaptive. There is evidence that - at the stage from acute phase to the chronic phase post-stroke - enhanced coupling between ipsilesional secondary motor areas and ipsilesional M1 is the best predictor of good recovery (Rehme et al., 2011). Indeed, the current study provides evidence for reduced functional interactions of ipsilesional PMv to ipsilesional M1 connectivity in the chronic stroke brain which could be corrected by paired associative plasticity induction. The findings suggest that it might be beneficial to generate novel hypotheses about the heterogeneity of post-stroke network interactions, which will allow for hypothesis-driven testing of pathway-neuromodulation. It may be of particular importance to test at different phases after infarct: patients may benefit from correction of pathway connectivity more at an early stage rather than during the chronic stage.

Combined regimes of neuromodulatory interventions (both repetitive TMS (rTMS) and tDCS approaches) and physical training - aimed at improving hand dexterity and strength - have increasingly been tested within the last three years (Avenanti et al., 2012, Talelli et al., 2012, Zimerman et al., 2012, Stagg et al., 2012). Recent work suggests that this combination over multiple sessions may induce long-term functional improvements of dexterity (Allman et al., submitted). However, the treatment effects vary significantly, and whereas motor function improved in some patients, neuromodulation did not show superiority to sham stimulation in other patients (Grefkes and Fink, 2012). In summary, better stratification of the stroke patient population is required as well as the development of additional targeted stimulation protocols that can be used as adjuvants to physical therapy.

Simultaneously, continued investigation of corticospinal projections that might be crucial for generation of motor behaviour in the more severely impaired patients is required. Medial and lateral premotor areas also have access to corticospinal tract neurons and their corticospinal pathways descend

both crossed and uncrossed (Dum and Strick, 1996, Grefkes and Ward, 2014). Crossed fibres from ipsilesional dorsal premotor cortex (PMd) have been shown to support dexterity in recovered patients (Schulz et al., 2012). Whereas there is evidence that contralesional PMd also supports dexterity in severely impaired patients (Johansen-Berg et al., 2002), the spinal path mediating such effects is less well understood. The anatomical route supporting motor recovery might rely on premotor cortex projections to the red nucleus and the pontomedullary reticular formation, from which the rubrospinal and reticulospinal tracts arise respectively (Schieber, 2007). A recent finding demonstrated that projections from the reticulospinal tract to forearm flexors and intrinsic hand muscle were strongly enhanced in primates following lesions to the pyramidal tract and that this enhancement was related to recovery of hand motor function (Zaaimi et al., 2012). In summary, these findings have launched a new debate whether spinal tracts emerging from medial brain stem nuclei are aiding functional recovery in human stroke patients.

Advancing our understanding of the anatomical substrate of recovery may ultimately allow for application of neural prosthetics in the central nervous system. Within the last five years, one attempt was made to use targeted direct electrical stimulation (DES) as a neurostimulation therapy in stroke patients. This randomised clinical trial attempted to facilitate rehabilitation for recovery of upper limb function in stroke patients by applying epidural DES to the M1 representation controlling hand and wrist movements concurrently with intensive rehabilitation therapy (Harvey et al., 2009). The study, however, was discontinued since cortical DES did not induce behavioural improvements. A post-hoc analysis revealed that the stimulation implant should have been placed more carefully into M1, for example by using a functional localiser which had been used in the prospective study preceding the clinical trial (Brown et al., 2006). Whereas no further research into application of invasive neurorehabilitation was carried out

in the last years, research into neuroprosthetics as well as DES-based investigation of motor control has moved on. There is direct evidence for Hebbian plasticity induction in local motor circuits in freely-moving primates, which altered motor output for more than one week (Jackson et al., 2006). The neural implant used in this experiment induced spiking-activity in response to intrinsic spiking-activity, and was implanted into the cerebral grey matter rather than epidurally. In humans, a recent study employing DES over the lateral frontal cortex demonstrated selective enhancement of higher motor function, namely proactive stopping (Wessel et al., 2013). In the light that DES can enhance the causal influence of one brain area over another at a given instant and that paired associative TMS can selectively modulate connectivity within a cortico-cortical pathway, it is not unreasonable to think that a form of paired electrical stimulation of two interconnected network nodes may prove useful for therapeutic applications based on modulation of aberrant network activity. It is plausible that DES can induce any of these different forms of plasticity with far better spatial and temporal resolution, and thus may have greater therapeutic potential in appropriate patient populations.

At this point, more studies should focus on describing different post-stroke network reconfigurations and should test novel non-invasive brain stimulation protocols in a hypothesis-driven fashion. This combination may allow for a better stratification of the heterogeneous stroke patient population. Targeted pathway-specific protocols might be particularly suitable to induce directed changes in connectivity.

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