Fachbereich Erziehungswissenschaft und Psychologie der Freien Universität Berlin

Cerebral activation during visual stimulation of mirrored hand movements in normal subjects and stroke patients

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Personal contributions to the individual publications

Publication 1 - *"A comparison of neural mechanisms in mirror therapy and movement observation therapy"*

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- Co-authorship
- Leading planning, preparation and organization of the experiment
- Leading writing and bringing forward the ethics request at the Ethics Committee of the Charité - Universitätsmedizin Berlin
- Leading technical and conceptual preparation (programming of the investigator protocol with the software program *presentation* and preparation of the technical setup)
- Leading data acquisition, measurement and data management (during and after scanning procedure)
- Substantial involvement in data and statistical analysis
- Substantial involvement in writing and publishing the manuscript

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- Data acquisition, measurement and data management (during and after scanning procedure)
- Substantial involvement in data and statistical analysis
- Substantial involvement in discussion and presentation of results
- Substantial involvement in writing and publishing the manuscript

Publication 3 - *"Effect of a mirror-like illusion on activation in the precuneus assessed with functional near-infrared spectroscopy"*

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- Shared first-authorship with Jan Mehnert
- Leading planning, conceptualization, preparation and organization of the experiment
- Leading writing and bringing forward the ethics request at the Ethics Committee of the Charité - Universitätsmedizin Berlin
- Technical preparation (programming of the investigator protocol with software program *presentation* and preparation of the fNIRS setup)
- Subjects Recruitment and Main Investigator
- Leading data acquisition, measurement and data management
- Data preprocessing together with Jan Mehnert
- Main responsibility for statistical analysis and results presentation and discussion
- Leading responsibility in writing and publishing the manuscript

Publication 4 - *"Cerebral activation evoked by the mirror illusion of the hand in stroke patients compared to normal subjects"*

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- Co-authorship
- Leading planning, conceptualization, preparation and organization of the experiment

- Leading writing and bringing forward the ethics request at the Ethics Committee of the Charité - Universitätsmedizin Berlin
- Leading technical preparation (programming of the investigator protocol with the software program *presentation* and preparation of the technical setup)
- Recruitment of stroke patients and contact-person for information and measurement
- Leading data acquisition, measurement and data management (during and after scanning procedure)
- Substantial involvement in data and statistical analysis
- Substantial involvement in discussion and presentation of results
- Substantial involvement in writing and publishing the manuscript

Manuscript 5 - *"Potential determinants of efficacy of mirror therapy in stroke patients - a pilot study"*

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- First-authorship
- Planning, conceptualization, preparation and organization of the clinical trial
- Leading writing and bringing forward the ethics request at the Ethics Committee of the Charité - Universitätsmedizin Berlin
- Leading technical preparation and management and coordination of the clinical trial
- Recruitment of stroke patients and first information conversation
- First contact person for further information and in case of problems
- Main responsibility for data acquisition, measurement and data management
- Main responsibility for data pre-processing and statistical analysis
- Leading in writing and in submitting the manuscript

Declaration

I declare that my contribution was substantial and indispensable on every level of scientific work that led to the individual publications of this dissertation. My contributions in detail are presented above. I acknowledge that also the co-authors contributed substantially to the publication of the manuscripts. People that contributed to the publications that are not co-author are acknowledged separately in the particular publication. No other auxiliary means or assistance than the acknowledged was used for the completion of this dissertation. Based on this, I autonomously wrote this dissertation.

Furthermore, I declare that the Publications 1, 2 and 4 are also part of the dissertations of the co-authors Dr. Jing Wang and Dr. Claire Fritzsch at the Charité - Universitätsmedizin Berlin. Beyond that, Publication 3 is also part of the dissertation of the co-author Dr. Jan Mehnert at the Technische Universität Berlin.

However, in this compilation, the dissertation has not been submitted at the Freie Universität Berlin or other universities before.

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Dipl.-Psych. Maddalena Brunetti

Contents

Abbreviations

ACA	anterior cerebral artery
ADL	activities of daily living
ANOVA	analysis of variance
BA	Brodmann area
BOLD	blood oxygen level dependent
CIMT	Constrained Induced Movement Therapy
CNS	central nervous system
CRPS	complex regional pain syndrome
DA	discriminant analysis
DCM	dynamic causal modeling
DLP	dorsolateral pathway
EEG	Electroencephalography
FMA	Fugl-Meyer assessment
fMRI	functional magnetic resonance imaging
fNIRS	functional near-infrared spectroscopy
HBO	oxygenated hemoglobin
HBR	deoxygenated hemoglobin
IFG	inferior frontal gyrus
IPL	inferior parietal lobe
IQR	interquartile range
LH	left hand
LI	lateralization index
M1	primary motor cortex
MCA	middle cerebral artery
MCP	metacarpophalangeal
MEG	Magnetoencephalography
MEP	motor evoked potentials
MI	mirror index

MI-M1	mirror index on primary motor cortex
MI-PC	mirror index on precuneus
MIR	mirrored visual feedback
MNS	mirror neuron system
MT	mirror therapy
M1	primary motor cortex
NOR	normal visual feedback
OBS	observation
PC	precuneus
PCA	posterior cerebral artery
PET	Positron emission tomography
PNS	peripheral nervous system
PRR	parietal reach region
rCBF	regional cerebral blood flow
rTMS	repetitive transcranial magnetic stimulation
RH	right hand
ROI	region of interest
S1	primary somatosensory cortex
S2	secondary somatosensory area
SMA	supplementary motor area
SPL	superior parietal lobule
STG	superior temporal gyrus
tDCS	transcranial direct current stimulation
TMS	transcranial magnetic stimulation
Unc	uncorrected
V5 / V6 / V6A	visual area 5 / visual area 6 / visual area 6A
VMP	ventromedial pathway
VPC	ventral premotor cortex
VPL	ventral posterior lateral
VT	video therapy

1 Introduction

Stroke is the second leading cause for death worldwide (after ischemic heart disease) as per WHO and one of the leading causes for disability at advanced age (Feigin et al., 2014). Stroke is not limited to industrial countries how recent analysis demonstrated, but is a global problem, indeed stroke mortality and stroke burden measured by the disability-adjusted life years (DALY) is highest in low-income countries (Johnston, Mendis, & Mathers, 2009). If the observed trend from 1990 to 2010 in incidence, mortality, and DALYs continues, by 2030 there will be almost “12 million stroke deaths, 70 million stroke survivors, and more than 200 million DALYs” burden worldwide (Feigin et al., 2014). About one third of all stroke patients suffer from severe hemiparesis (disability to move one body side) of the upper limb (Jorgensen et al., 1995). In one study about first-ever unilateral stroke patients in the area of the middle cerebral artery (MCA) with following severe hemiparesis, even after intensive rehabilitation procedure 62% remained without any function and only about 38% regained some dexterity of the affected arm (complete recovery: 11.6%) (Kwakkel, Kollen, van der Grond, & Prevo, 2003). With regard to the individual suffering as well as to the raising costs that are caused by these low recovery rates, it is socially relevant to promote research in the field of neurological rehabilitation of severe hemiparesis and to search for alternatives to the conventional rehabilitation procedures. The thesis at hand aims for a better understanding of the underlying neurophysiological mechanisms of one alternative therapy, the so-called mirror therapy (MT). Although a lot of research on MT has been done in the past years, many questions about the underlying cerebral mechanism and about potential determinants of the efficacy of MT remain open.

2 Theoretical Background

Movement performances such as deliberately hand movements are highly complex operations. In his review on computational approaches to motor control Wolpert writes: “Even for the simplest task such as moving the hand to a target […] there are an infinite number of possible” combinations of hand paths, velocity and joint angles to reach the target. “Motor planning can therefore be considered as” a highly complex “computational process of selecting a single solution […] from the many alternatives […]” (Wolpert, 1997). Thus it is no wonder that the motoric system of the cerebral cortex that is responsible for movement planning, execution and control is complex and widely spread over the cerebral cortex.

In their meta-analysis on eight studies that measured regional cerebral blood flow (rCBF) during movement execution Grèzes and Decety (2001) found following structures to be involved: primary motor cortex (M1), premotor cortex, supplementary motor area (SMA), cingulate gyrus, cerebellum, inferior parietal lobe (IPL) and superior parietal lobe (SPL). Hence, it is no wonder that about one third of all stroke patients suffer from severe hemiparesis of the upper limb (Jorgensen et al., 1995).

As the thesis at hand aims at a better understanding of the neuronal mechanisms of MT, the thesis concentrates mainly on three cortical regions that seem to be involved in movements as well as in MT: M1, primary somatosensory cortex (S1) and precuneus (PC) as a part of SPL.

2.1 Primary motor cortex - M1

The primary motor cortex is situated on the posterior part of the precentral gyrus of the frontal lobule, just anterior of the central sulcus and corresponds to Brodmann area (BA) 4 (Brodmann, 1909; Pinel, 2001a). Penfield and Boldrey were the first to map M1. Electrical stimulation of the corresponding area on M1 elicited simple movements of certain body parts. In doing so, they found the somatotope organization of M1 and developed the motoric homunculus (Figure 1, right side) (Penfield & Boldrey, 1937). However, recent studies challenged the original point of view of one single somatotope body part area in the cortex controlling one specific body part. The areas controlling movements of the hand e.g. seem to be widely spread beyond M1 and also overlapping finger and wrist representations were found (Sanes, Donoghue, Thangaraj, Edelman, & Warach, 1995).

M1 is the main source of motoric fibers descending from the cerebral cortex to the different muscles of the body across the spinal cord. There are four different main pathways of descending fibers to the spinal cord: two ventromedial pathways (VMP) and two dorsolateral pathways (DLP). The VMPs are much more diffuse as they innervate interneurons on both sides in different sections of the spinal cord, i.e. that contralateral as well as ipsilateral body parts are controlled via the VMPs. Furthermore, the motor neurons of the VMPs innervate proximal muscle groups such as the shoulder muscles; meanwhile the motor neurons of the DLPs innervate distal muscle groups such as finger muscles, exclusively of contralateral body parts. Both pathways contain a direct and an indirect pathway. The direct DLP crosses to the contralateral side in the pyramids of the medulla oblongata (brainstem). The indirect DLP crosses to the contralateral side after forming synapses with the red nucleus of the midbrain and with the motoric nuclei in the brainstem and finally descending to the spinal cord. The indirect VMP forms synapses in the brainstem mainly with four structures: tectum (auditory and visual information about the spatial position), vestibular nucleus (equilibration information), reticular formation (information about complex movement programs such as

walking or swimming), motoric nuclei (controls face muscles) (Pinel, 2001a). Thus, motor control is mainly exerted by the contralateral hemisphere, but also by the uncrossed corticospinal tract, the ipsilateral proceeding VMP. This explains why a pre-existing hemiparesis can be worsened even by an ipsilateral stroke (Ago et al., 2003).

Although M1 is the main source of motoric fibers descending from the cerebral cortex and although lesions including the area of M1 can cause hemiparesis of the contralateral body side, lesions restricted to M1 do not cause severe hemiparesis (Pinel, 2001a). Probably, this is due to the fact that descending motoric fibers do not only have their origin in M1, but also in secondary motor areas, such as premotor cortex, SMA and motoric areas of the gyrus cingulate (Schwartz, 1994). Patients with a lesion in the area of M1 are not able to move fingers independently from each other. Furthermore, they suffer from astereognosis (disability to identify an object solely by fingering) (Pinel, 2001a; Schieber, 1990).

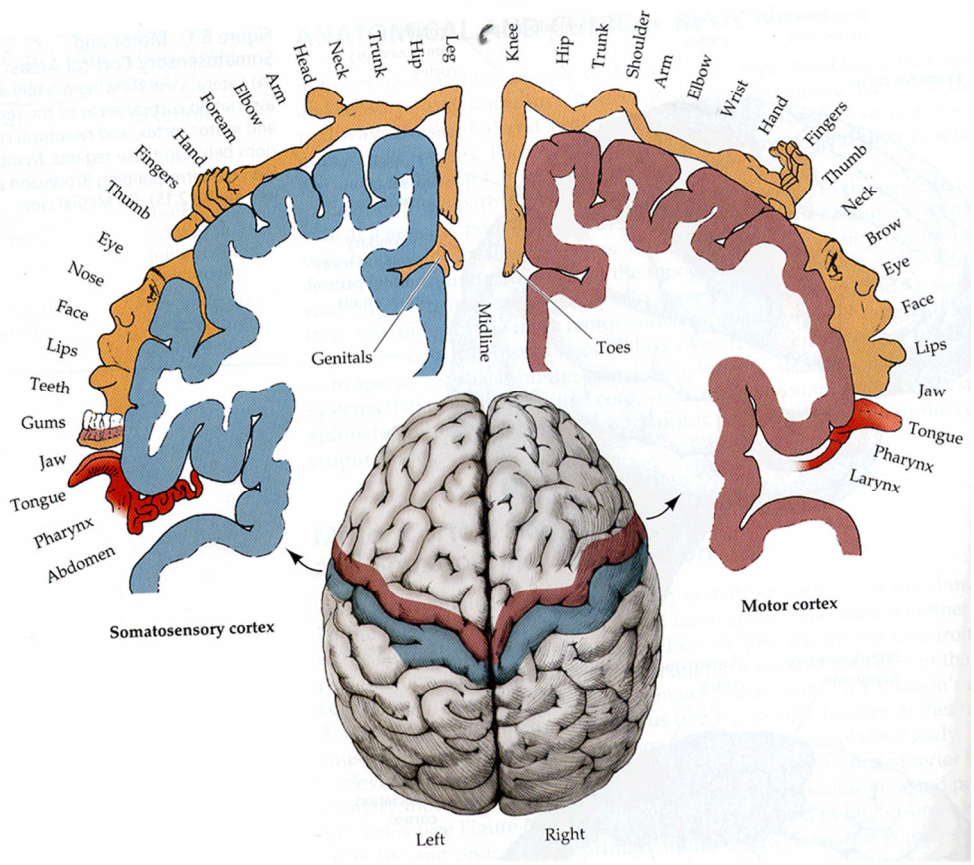


Figure 1: The somatosensory and the motor homunculus after Penfield and Rasmussen, 1950. Copied from galleryhip.com.

2.2 Primary somatosensory cortex - S1

The primary somatosensory cortex (S1) is situated on the postcentral gyrus of the parietal lobule, just posterior of the central sulcus and corresponds to BA 1, 2, 3a and 3b (Brodmann, 1909; Pinel, 2001b). In a similar manner to M1 mapping, Penfield and Boldrey mapped S1 by stimulating S1 areas of surgery patients and note the corresponding body part in which sensations were felt. They found a somatotope organization of S1 corresponding to the somatosensory homunculus displayed in Figure1, left side (Penfield & Boldrey, 1937). However, recent studies showed that S1 consists of four parallel layers (BA 1, 2, 3a and 3b) with different responsiveness to different kinds of sensory stimulations, such as tactile, temperature, pain, deep receptor or proprioceptive stimulation (Moore et al., 2000).

Neurons in S1 receive neuronal input signals from receptors - mainly situated in the skin - across the spinal ganglion in the spinal cord. From there the somatosensory pathway further ascends to the nucleus cuneatus in the medulla oblongata where it crosses to the contralateral side. Together with the trigeminus nerve[1], the somatosensory fibers project on the nucleus ventral posterior lateral (VPL) of the thalamus. VPL has axons projecting on S1, as well as on other somatosensory cortex areas, such as the secondary somatosensory area (S2) and posterior parietal cortex (Pinel, 2001b).

Impairments in patients with unilateral excision on S1 are not such as devastating as expected. Unilateral excision causes a rather minimally decreased capability to perceive slight touch and to identify an object solely by fingering (Corkin, Milner, & Rasmussen, 1970; Pinel, 2001b).

As M1 and S1 are both involved in motor control and execution (Pinel, 2001a), and as a clear distinction between these areas is claimed to be difficult (Diers, Christmann, Koeppe, Ruf, & Flor, 2010), they are sometimes merged as sensorimotor cortex (Hamzei et al., 2012).

[1] Trigeminus nerve transfers pain and temperature information from the face to the thalamus.

2.3 Precuneus - PC

2.3.1 Precuneus and its anatomy

PC is the medial portion of the posterior parietal cortex and adjoins on parieto-occipital fissure, cingulate sulcus and subparietal sulcus and corresponds to BA 7 with regard to its cytoarchitectonics (Brodmann, 1909; Cavanna & Trimble, 2006). This location in the depth of the longitudinal fissure made it traditionally difficult to measure precuneal activation with imaging techniques; however some recent functional imaging studies tracking PC are reviewed by Cavanna and Trimble (2006) amongst others. On the lateral surface of BA 7 lays SPL, hence overlapping with PC at least in its superior portion and sharing the same cytoarchitectonics (Cavanna & Trimble, 2006) (Figure 2). Despite this overlapping, PC corresponds rather to the medial and SPL rather to the lateral aspect of the upper parietal cortex, although in many neurophysiological studies PC is subsumed to SPL (Karnath & Perenin, 2005).

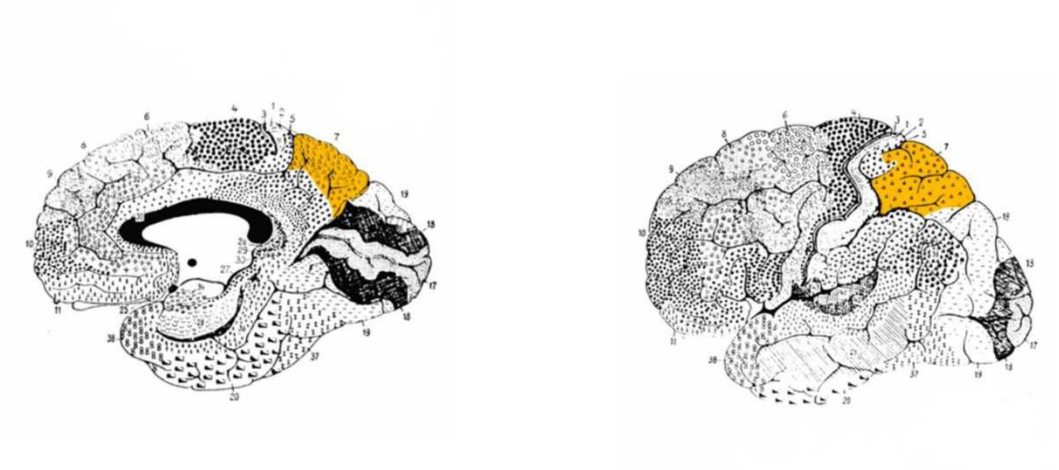


Figure 2: BA 7 (yellow) in medial view (left) and lateral view (right) (copied from Wikipedia and adapted).

2.3.2 Precuneus and its function

PC activity was shown to play a crucial role in processing of upper limb configurations during movement execution. A study of Fattori and colleagues (2009) on monkeys showed that neurons in V6A (situated in the medial parieto-occipital cortex and thus in the region of interest (ROI) of PC of the thesis at hand, cf. Figure 5) are modulated by the orientation of the hand, more precisely by changes of wrist and reach orientation. The most represented cells were those modulated by hand orientation both during preparatory and movement periods during the reach-to-grasp movements. As the experiment was conducted in complete darkness to exclude the involvement of visual feedback in neuronal cell modulation, it is concluded that V6A is involved during on-line control of hand movements on a higher-order representational level (Fattori et al., 2009). This was similarly confirmed for humans where V6A was found to be activated during reaching and pointing in complete darkness, pronouncing the motor-related response of V6A (Pitzalis et al., 2013). Earlier studies on corresponding brain area V6A found this region similarly to be involved in pointing (Connolly, Andersen, & Goodale, 2003) and reaching movements (Filimon, Nelson, Hagler, & Sereno, 2007). According to Pitzalis, V6A falls within a small part of the posterior part of the original parietal reach region (PRR) of the monkey (Connolly et al., 2003; Pitzalis et al., 2013). Furthermore, lateralized activation of PC was found during hand movements under normal and mirrored visual control in earlier functional magnetic resonance imaging (fMRI) studies (C. Dohle, Kleiser, Seitz, & Freund, 2004; C. Dohle et al., 2011). Thereby, different neuronal correlates for coordination mirroring and limb mirroring were found: only for limb mirroring, PC contralateral to the perceived limb was involved. It is suggested that PC might be the substrate of representation of a left or right arm, lateralized to the hemisphere contralateral to the perceived limb (C. Dohle et al., 2011).

Beyond that, there are several studies demonstrating PC involvement in self-referential mental representations and self-awareness (Cavanna & Trimble, 2006). One fMRI study comparing neural activation during processing of self-descriptive with non-self-descriptive

stimuli found PC bilaterally activated during the self-condition (Kircher et al., 2002). Furthermore, PC was previously found to play a crucial role also for memory retrieval, especially of episodic memory (Fletcher et al., 1995; Krause et al., 1999).

2.3.3 Lesions in the region of Precuneus

Lesions in the region of PC seem to cause Optic Ataxia (Perenin, 2006). Optic Ataxia is an impairment of grasping something with the left or right hand. This disturbance does not occur when the object is visually focused, but only when the object appears in the peripheral visual field and can thus not be focused. Optical Ataxia is often not noticed by patients and therapists, as this circumstance of grasping something that is not visually focused is very seldom in everyday life. Optic Ataxia occurs after left as well as after right hemisphere lesions (Perenin, 2006; Perenin & Vighetto, 1988; Rondot, de Recondo, & Dumas, 1977). In an analysis on 52 unilateral stroke patients with and without Optic Ataxia of Karnath and Perenin (2005) the lesions' overlapping region reached from the junction between the superior occipital cortex and IPL or SPL as far as PC near the parieto-occipital region. The center of lesions overlap was PC, but not for control patients (Karnath & Perenin, 2005). This was similarly found in other studies that found lesions in the posterior parietal cortex to cause grasping impairment without reaching deficits (Jeannerod, Decety, & Michel, 1994). It is crucial to notice that visual perceptive or oculomotor processes cannot account for Optic Ataxia, as they do not occur in every clinical case of Optic Ataxia (Lynch, 1980; Mountcastle, Lynch, Georgopoulos, Sakata, & Acuna, 1975; Perenin, 2006).

Another dysfunction that seems to be associated with lesions predominantly in the right posterior parietal cortex is asomatognosia (the inability to recognize own body parts) (Klawans, 1990; Pinel, 2001b). However, recent studies do not confirm the PC as solely lesion center of patients with asomatognosia, but found also other structures to be involved, such as occipito-temporal, lateral and posterior temporal cortex (Orjuela-Rojas et al., 2014),

posterior insula (Baier & Karnath, 2008) or premotor cortex (Arzy, Overney, Landis, & Blanke, 2006).

2.4 Interconnections between M1, S1 and PC

The areas M1, S1 and PC are interconnected through axonal projections (Figure 3). The areas of S1 project to PC (BA 7) and BA 5, as well as to M1 (BA 4) and S2. M1 has projections to PC, as well as to BA 5 and S1 (Kolb & Whishaw, 1996c). Although PC does not have direct projections to M1, it has reciprocal cortical connections with SMA and with the dorsal premotor cortex (BA 6) (Cavanna & Trimble, 2006), which in turn have reciprocal connections with M1 (Kolb & Whishaw, 1996c). Thus, the movement control system seems to be hierarchical, whereat the information pathway goes from somatosensory across parietal to the frontal cortex and from there to M1. Meanwhile, it seems to be parallel, too: M1, SMA and premotor cortex have all independently descending neuronal fiber tracks to the spinal cord for movement control and are very similar concerning their organization. Furthermore, even sensory cortex areas, such as S1, PC and BA 5 have neurons with descending axons to the spinal cord for movement control (Kolb & Whishaw, 1996c).

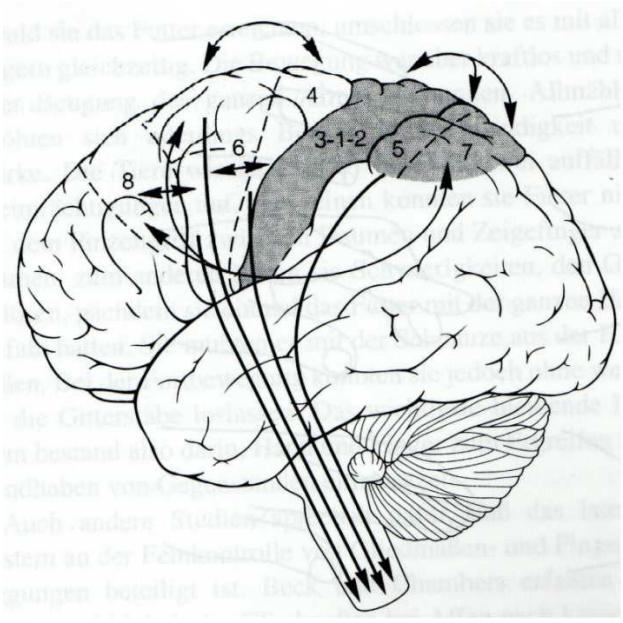


Figure 3: Reciprocal interconnections between the sensory (BA 1, 2, 3, 5 and 7) and the motoric system (BA 4, 6 and 8) and different descending fiber tracks to the spinal cord (Kolb & Wishaw, 1996, p. 99).

2.5 How the brain reorganizes after lesions

The blood oxygenation supply of the brain is provided by two cervical arteries: The internal carotid artery further branches out into the anterior cerebral artery (ACA) and the middle cerebral artery (MCA), meanwhile the vertebral artery branches out into basilar artery, posterior cerebral artery (PCA) and several smaller arteries supplying the cerebellum with blood. The MCA supplies the frontal and middle areas of the cortex including frontal, temporal and parietal lobe in large part mainly on the lateral site. Thus, an ischemic (blockage of the artery) or a hemorrhagic (bleeding of the artery) stroke leading to a lack of blood supply or to an increased pressure on the brain tissue and hence to a neuronal die off in the area of the MCA can cause vast damages in the movement control system, such as hemiparesis and somatosensory deficits (Kolb & Whishaw, 1996b). Hemiparesis includes atonic muscles, loss of all reflexes and loss of volitional movements. The stages of recovery after severe hemiparesis resemble the stages of grasping movements' development observed in children. First, tendons and stretching reflexes recur, followed by a rigor. Afterwards, grasping reflexes caused by body posture and proprioception and volitional grasping of objects is observed. Finally, grasping reflexes caused by tactile stimulation recurs, followed by mainly grasping under volitional motor control. The recurrence of volitional motor control develops further until independent finger movements are possible. About 30 % of all examined patients in a study attained this stadium of full recovery (Kolb & Whishaw, 1996a; Twitchell, 1951).

2.5.1 Cellular processes - degeneration and regeneration

The underlying cellular processes for functional loss and recovery after hemiparesis and after brain lesions in general are neuronal degeneration and regeneration of neurons. There are

different kinds of degeneration. Anterograde degeneration means degeneration of the axon that was separated from the cell body and does always occurs. Retrograde degeneration is not compelling and means the degeneration of the proximal part of the axon and the cell body. For some, but not for all neurons retrograde degeneration can be reversible, such as for moto neurons. Beyond that, also neurons that are connected with a degenerated neuron across synapses can die off. When neurons that were innervated from a degenerated neuron die off, the process is called anterograde transneuronal degeneration and is caused by a loss of neuronal input signals that was essential for survival of this neuron. On the other hand, a neuron that is trophic dependent of the degenerated neuron to which it is sending his signals might consequently also degenerate (retrograde transneuronal degeneration) (Pinel, 2001c). Another example of neuronal degeneration is excitotoxicity. Neurons usually do not die off immediately because of oxygen deprivation, such as during an ischemic stroke. However, oxygen deprivation leads to an excessive release of the excitatory neurotransmitter glutamate causing a neuronal die off by overstimulation of the neuron. Excitotoxicity seems to be the underlying mechanism for the neuronal die off during and immediately after ischemic stroke (Camacho & Massieu, 2006; Kolb & Whishaw, 1996a).

Hence, no brain lesion leads to a merely locally restricted damage. A small lesion e.g. in the frontal cortex that seems to be locally restricted might nevertheless lead to retrograde, anterograde and transneuronal degeneration and cause vast damages in the thalamus, basal ganglia, brainstem and the whole cortex. Furthermore, degeneration processes might last for several years and even accelerate the aging process (Kolb & Whishaw, 1996a).

Neuronal regeneration processes, i.e. neuronal regrow after damages caused by lesions is something that does not occur in the central nervous system (CNS) of adult mammals, but only in the peripheral nervous system (PNS). This is due to the fact that the axons in the PNS are myelinated by Schwann cells that produce neurotrophic substances (proteins) and cell adhesion molecules (nerve growth factor, NGF) that are crucial for neuronal growth. In contrast, the axons in the CNS are myelinated by oligodendroglia without NGF. Motor axons and other neurons in the PNS might e.g. regenerate through collateral sprouting. Collateral

sprouting occurs when a neuron adjacent to a degenerated neuron develops new axons to a third neuron that was previously innervated by the degenerated neuron and forms new synapses to innervate this abandoned neuron (Pinel, 2001c).

2.5.2 Cortical functional reorganization processes

Although regeneration does not or rarely occur in the adult CNS, the brain seems to maintain the capability to change and reorganize lifelong. This capability is called cortical reorganization or neuroplasticity (Taub, Uswatte, & Elbert, 2002).

2.5.2.1 Animal experimental studies

Studies on monkeys have shown that acquiring a new manual skill leads to changes in the motoric representation of the hand in the primary motor cortex (Nudo, Milliken, Jenkins, & Merzenich, 1996). This was similarly shown for the somatosensory cortex in monkeys after intensive skin stimulations of one or more fingers. Several positional and topographic changes in the representational map of the somatosensory cortex were recorded, but the largest was recorded in relation to the stimulated finger areas (Jenkins, Merzenich, Ochs, Allard, & Guic-Robles, 1990). Thus, behavioral changes or experience seem to change the functional organization of the brain and hence foster neuroplasticity.

Similarly processes of use-dependent neuroplasticity were also observed in early studies on monkeys with functional loss due to excision. The cortical reorganization was found to be correlated to functional recovery. Nudo and his colleagues mapped the hand representation in the motor cortex in monkeys before and after an excision of a vast part of the cortical finger and hand area, which lead to a reduced usage of the contralateral hand. Anew mapping of the motor cortex revealed that there was no more cortical representation of this

hand and finger area. Even the finger and hand areas that were not removed by the excision disappeared. This was different for monkeys that were forced to use their affected hand by tying up the non-affected arm: Although these monkeys did not regain hand and finger representations in the excised area, they maintained the not removed cortical hand and finger areas and regained the lost hand and finger functions. Probably, their regained function was provided by the representations of the not damaged finger areas (Kolb & Whishaw, 1996a; Nudo, Jenkins, Merzenich, Prejean, & Grenda, 1992).

A similar experiment with ischemic stroke induced lesions in the cortical hand area of monkeys showed that daily training of the affected hand facilitated intact brain tissues adjacent to the lesioned structures to reorganize in order to take over the hand representation that was damaged. Meanwhile, no training lead to a further reduction of the functional cortical representation area of the affected body part (Nudo, 1997). The capability of functional cortical reorganization after CNS lesions in mammals was also demonstrated after digit amputation (Merzenich et al., 1984), and after deafferation (Kaas, Merzenich, & Killackey, 1983; Wall & Egger, 1971).

2.5.2.2 Human experimental studies

Similarly, the capability of use- or experience dependent cortical reorganization was observed also in healthy humans. It was shown that the cortical representation of the left hand's digits in the somatosensory cortex of professional string players was enlarged compared to controls. This effect was smallest for the thumb that is less used during string playing (Elbert, Pantev, Wienbruch, Rockstroh, & Taub, 1995). The same effect was shown for the long-term neuroplasticity in the primary motor cortex. Vaalto and colleagues examined string players, figure skaters and controls and found out that the hand's and leg's representation in M1 altered depending on the individual muscle's role in the skilled movements (Vaalto et al., 2013).

It was done a lot of research about neuroplasticity. The bulk of scientific studies on stroke patients prove that cortical reorganization of survived brain tissues after stroke might be the basis for functional rehabilitation also in humans, corresponding to the findings of animal studies (Hodics, Cohen, & Cramer, 2006). Some examples are shortly summarized below.

The applicability of what Nudo tested on monkeys for humans, was first shown by Taub and colleagues and is known as constrained induced movement therapy (CIMT) (Taub et al., 1993). Examinations with stroke patients showed that the forced use of the affected limb by tying up the non-affected one leads to functional recovery and overcomes the “learned non-use” of the affected limb (Taub, Crago, & Uswatte, 1998). Motor improvements due to CIMT were shown even in chronic stroke patients, transferred to life situations and even after a follow-up testing of two years (Taub et al., 1993). Mapping of the motor cortex with TMS before and after treatment with CIMT demonstrated that cortical reorganization is the basis of the functional improvements after CIMT in chronic stroke patients. Motor cortex representations of the affected limb was thereupon enlarged and excitability was increased (Liepert, Bauder, et al., 2000; Liepert et al., 1998). Other studies confirm these findings for subacute stroke patients even after a single session of physiotherapy (Liepert, Graef, Uhde, Leidner, & Weiller, 2000). Cortical reorganization in subacute stroke patients after receiving daily physical and occupational therapy was even shown before functional motor recovery arose. Additional activation due to passive elbow movements in stroke patients after therapy compared to normal subjects was found in the contralateral SPL and in the ipsilateral sensorimotor cortex (Nelles et al., 1999).

Moreover, cortical reorganization seems to be the basis also for spontaneous recovery of lost functions that can occur after brain lesions. This kind of capability of recovery after brain damages due to neuroplasticity can be further enhanced and fostered by appropriate therapy strategies (Taub et al., 2002).

2.5.2.3 Underlying mechanisms for cortical reorganization

Several mechanisms and patterns underlying cortical reorganization after brain lesions were discussed until now and are shortly summarized in this chapter.

One of the most important mechanisms is disinhibition. Experimental data indicate that there seem to be cortical areas that develop a specific function only after the inhibiting or competing neuronal system was omitted. Consequently, different brain areas seem to overlap regarding their innervation. Disinhibition enables functional transfer to previously inhibited neurons after functional loss due to a brain lesion (Kolb & Whishaw, 1996a). Several examinations prove that disinhibition might be a crucial mechanism for motor functional recovery due to cortical reorganization after stroke in patients with severe hemiparesis (Liepert, Storch, Fritsch, & Weiller, 2000).

Two more underlying mechanisms were discovered by a combined fMRI and transcranial magnetic stimulation (TMS) study on stroke patients with hemiparesis obtaining CIMT. One is a potential increase in synaptic efficiency. Thereby, less sensorimotor activation was observed together with higher intracortical excitability. Meanwhile, this enhanced synaptic efficiency was not observed in patients with lesioned hand area on M1 or damaged descending motor tracts. For these patients the cortical reorganization was based on expanded synaptic interaction resulting in increased neuronal activity together with decreased intracortical excitability. Cortical reorganization in these patients included additional areas of the sensorimotor cortex (Hamzei, Liepert, Dettmers, Weiller, & Rijntjes, 2006). This example clearly illustrates that functional recovery after stroke might be based on different kinds of cortical reorganization.

Another mechanism of reorganization that was suggested due to observations in case studies is that the corresponding areas in the non-affected hemisphere could partially take over the motor control functions. This might be provided by an intensifying of the uncrossed corticospinal tract and functional reorganizing of the ipsilateral, i.e. non-affected hemisphere. Patients with pre-existing hemiparesis caused by a stroke induced lesion deteriorated in their

motor functions after a further ipsilateral stroke (Ago et al., 2003; Song, Lee, Park, Yoon, & Roh, 2005; Yamamoto, Takasawa, Kajiyama, Baron, & Yamaguchi, 2007).

On the other side, it is also well known that interhemispheric activity imbalance in favor of the contralesional hemisphere is related to poor motor recovery in patients with hemiparesis after stroke. An over activation of the non-affected hemisphere seems to inhibit the affected one and thereby impede functional reorganization by transcallosal inhibition (Calautti et al., 2007). This hypothesis about an excessive interhemispheric inhibition of the affected hemisphere by the non-affected one leading to poor motor outcome seems to be well-established by now and was shown even in chronic stroke patients with hemiparesis (Murase, Duque, Mazzocchio, & Cohen, 2004).

This is corroborated by clinical studies that implemented this knowledge for functional motor recovery. Inhibition of the non-affected M1 via repetitive transcranial magnetic stimulation (rTMS) thereby switching off the excessive interhemispheric inhibition of the affected M1 promoted motor recovery of the affected hand (Grefkes et al., 2010; Mansur et al., 2005; Nowak, Grefkes, Ameli, & Fink, 2009). Beyond that, a review of several studies about functional and effective connectivity confirms pathological intra- and interhemispheric activations as a crucial underlying mechanism for motor impairment after stroke and confirms the beneficial effect on motor recovery by reversing this interhemispheric inhibition (Grefkes & Fink, 2011).

Thus, in the thesis at hand it is presumed the latter model of competing hemispheres for motor and somatosensory activation as a more common underlying mechanism for motor recovery after stroke. Future research might clarify how the interplay between the two hemispheres precisely contribute to motor recovery after hemiparesis and how the other mechanisms presented in this chapter might be crucial under certain conditions for motor recovery after stroke.

2.6 Conventional and alternative therapy procedures

The majority of conventional therapy strategies that are applied during physiotherapy, occupational therapy or training of activities of daily living (ADL) are based on actively or passively conducted movements of the affected limb. However, most of the actively conducted training strategies, such as repetitive arm training (Butefisch, Hummelsheim, Denzler, & Mauritz, 1995), the above introduced CIMT (Taub et al., 1998) or robot-assisted arm training (Mehrholz, Platz, Kugler, & Pohl, 2008) always require some degree of independent movement capability of the affected upper limb. For CIMT e.g. the inclusion criteria for application are a capability of wrist extension ability of more than 20 degrees and a metacarpophalangeal (MCP) joint extension ability of more than 10 degrees (Taub et al., 1998). Therefore, the conventional active training strategies are inapplicable for stroke patients with severe upper limb paresis such as hemiplegia[2] - the most severe form of hemiparesis - that do not fulfill such criteria (Wang et al., 2013). Moreover, even training strategies that are based on passively conducted movements disadvantage patients with severe hemiparesis, as they often suffer additional sensory deficits. Sensory deficits are associated with bad motor recovery as they impede appropriate sensory stimulation during passively conducted movements. However, appropriate sensory stimulation seems to be crucial for cortical reorganization in the process of motor recovery (Broeks, Lankhorst, Rumping, & Prevo, 1999).

Another passive therapy strategy that was recently examined is the application of non-invasive brain stimulation via transcranial direct current stimulation (tDCS). TDCS as compared with sham stimulation was shown to foster functional recovery of the affected hand in chronic stroke patients (Hummel et al., 2005). However, another study could not replicate the beneficial effects of tDCS when additionally applied on subacute patients with severe hemiparesis (Hesse et al., 2011). Indeed, it seems as if the beneficial effect for motor

[2] Complete paralysis of one body side

recovery of tDCS is limited to a subgroup of patients with a subcortical infarct (Boggio et al., 2007; Hesse et al., 2007) and with a certain maintained capability of grasping and releasing objects with the affected hand (Boggio et al., 2007; Hesse et al., 2011; Hummel et al., 2005).

Probably, the lack on appropriate therapy strategies for hemiplegic patients is the reason for low rates of complete recovery even after intensive rehabilitation procedure, reaching from 11.6 % to 30 % (Kwakkel et al., 2003; Twitchell, 1951). Others reported a fair to good motor recovery of 50 %, meanwhile sensory recovery was found in only about 26 % of the patients (Broeks et al., 1999).

Hence, alternatives to conventional therapies also applicable for patients with severe hemiparesis have been developed and investigated for several years, such as e.g. motor imagery and mental practice, or rehabilitation procedures implementing visual input. Mirror therapy and video therapy are two examples that use visual stimulation for motoric rehabilitation.

2.6.1 Motor imagery or mental practice

Motor imagery or mental practice can be defined as “mental rehearsal of simple or complex motor acts that is not accompanied by overt body movements” (Porro et al., 1996). Porro and colleagues (1996) compared cerebral activations during motor execution with motor imagery via fMRI. They found M1 and S1 to be activated during both tasks, although to a less extend during motor imagery as compared with motor execution. They point out that on the long term, motor imagery and mental practice can indeed enhance neuroplasticity, as e.g. demonstrated in an earlier study (Pascual-Leone et al., 1995). Furthermore, several studies showed that motor imagery and execution activate similar brain areas. A meta-analysis found much overlap between motor imagery and execution in the SMA, dorsal premotor cortex, supramarginal gyrus and SPL. Mental simulation is additionally associated with ventral premotor cortex and movement observation with temporal pathways (Grèzes & Decety,

2001). Motor imagery or mental practice was shown to improve motor recovery of the upper limb after stroke, but only in combination with occupational therapy (Nilsen, Gillen, DiRusso, & Gordon, 2012). However, there is hardly any evidence that motor imagery on its own can lead to motor recovery (Ietswaart et al., 2011).

2.6.2 Mirror therapy and video therapy

In MT a mirror is placed in the mid-sagittal plane in front of the patient on a table, while the patient is sitting on a chair. The affected arm is placed behind the mirror, the non-affected in front of the mirror while the patient looks into the mirror. Thus, the patient sees the reflection of the movements of the non-affected limb as if they were movements from the affected one. E.g., if the left arm is the non-affected one and reflected in the mirror, it looks as though it were the right one and vice versa. Beyond that, the reflection of the non-affected arm appears where the patient is used to see the affected non-moving limb, creating a so called mirror illusion (Figure 4). This mirror illusion is meant to be strongest, when the affected arm behind the mirror is plegic, i.e. without any capability to move (C. Dohle, Pullen, et al., 2009). MT was firstly used for patients with an amputated limb and was found to relieve their phantom limb pain (Ramachandran & Rogers-Ramachandran, 1996).

Figure 4: Mirror therapy at the MEDIAN Klinik Berlin-Kladow. The therapist gives verbal commands about which movements are to be conducted by the patient.

MT relieves pain also in patients with complex regional pain syndrome (CRPS) (G. L. Moseley, 2004), promotes motor recovery after stroke and activities of daily living (Thieme, Mehrholz, Pohl, Behrens, & Dohle, 2012) and pain even in chronic stroke patients (Bowering et al., 2013). Also an improvement of visuospatial neglect following stroke was found (C. Dohle, Püllen, et al., 2009; Thieme et al., 2013). It is noticeable that Dohle and colleagues suggested to separate the patients in MT responders and non-responders due to the high inter-variability of the motor outcome in their randomized clinical trial (C. Dohle, Püllen, et al., 2009). Also between different studies, outcome after MT is not always equal (Thieme et al., 2012). However, up to now, nobody succeeded in identifying determinants for MT efficacy. This is one of the aims of the thesis at hand.

The fundamental idea of MT in the implementation for motor recovery after stroke is to develop a more appropriate therapy strategy for patients with hemiplegia. Furthermore, MT is meant to be most effective for hemiplegic patients, as their mirror illusion is strongest. Hence, the inclusion criteria for MT are at the same time the exclusion criteria of CIMT: wrist extension ability of less than 20 degrees and a MCP joint extension ability of less than 10 degrees (C. Dohle, Morkisch, Lommack, & Kadow, 2011; C. Dohle, Pullen, et al., 2009; Taub et al., 1998).

Another therapy using visual input is the so called video therapy (VT). In VT the patient observes a third person on video accomplishing different activities, which has to be imitated exactly afterwards by the patient. At least the observation part is applicable for hemiplegic patients. Meanwhile, the imitation part is inapplicable. Nevertheless, VT was shown to have a beneficial effect on motor recovery for moderate hemiparesis after stroke (Ertelt et al., 2007), for subacute (Page, Levine, Sisto, & Johnston, 2001) and even for chronic stroke patients (Mulder, 2007; Page, Levine, & Leonard, 2005) and on activities of daily living of Parkinson patients as demonstrated by a pilot study (G. Buccino et al., 2011).

2.6.3 Cerebral correlates of MT

The neuronal mechanisms of MT are still not fully understood. Several studies examined the effect of the mirror illusion as the basis of MT on cerebral activation, but there are divergent results. Others examined the long-term effects on neuroplasticity due to the implementation of MT.

2.6.3.1 Mirror neuron system

As VT and MT have the application of visual input for therapeutic use in common, MT is often mentioned together with VT when trying to give an explanation about the neuronal mechanisms. VT is known to activate the mirror neuron system (MNS). fMRI studies on normal subjects revealed the bilateral activated MNS as the underlying neuronal mechanism in motoric learning through imitation (G. Buccino et al., 2004) and through mere observation (G. Buccino et al., 2001). Furthermore, the involvement of the MNS in functional motor recovery was demonstrated in several studies (Iacoboni & Mazziotta, 2007; Rizzolatti, Fabbri-Destro, & Cattaneo, 2009). Consequently, some authors have suggested the MNS to be the neuronal basis for the effect of MT, too (Rizzolatti et al., 2009).

Mirror neurons (MN) are defined as neurons that fire not only when action is executed, but also when the same action is merely observed, thus “mirroring” the executed action of another person in the own brain (Iacoboni & Mazziotta, 2007). In humans, MN were found in ventral premotor cortex (VPC), inferior parietal lobule (IPL) and in the pars opercularis, i.e. the caudal part of inferior frontal gyrus (IFG) (Rizzolatti et al., 2009). In their review about the MNS, Iacoboni and Mazziotta (2007) summarize that the MNS encodes more than the observed actions per se. Also the goal of the imitated action, imitation and learning through imitation are encoded. To sum up, they define MN as “special class of premotor neurons” and highlight the crucial role the MNS plays for social cognition and empathy, language and motor behavior (Iacoboni & Mazziotta, 2007).

However, to the best knowledge of the author, none of the examinations on MT found the MNS to be involved. A systematic review of the effect of mirrored visual feedback on brain activity cites two out of 33 included studies ostensibly detecting the MNS (Deconinck et al., 2014). One of them found additional activation during mirrored visual feedback compared with normal visual feedback in the superior temporal gyrus (STG) (Matthys et al., 2009). However, STG does not belong to the MNS that consists of the above mentioned structures, namely VPC, IPL and IFG (Rizzolatti et al., 2009). In the other study the VPC is mentioned. However, the experimental task during the fMRI measurement was a mere action observation task of a third person's hand compared with an imitation task. The effect of the mirror illusion on cerebral activation was not examined in this study. Besides, enhanced coupling between premotor cortex, SMA and sensorimotor cortex was observed, but only due to extensive MT based training of skilled hand movements as revealed by effective and functional connectivity analysis (Hamzei et al., 2012).

Beyond that, MT does not afford any kind of social interaction or social perception, as only own movements in an egocentric (first-person) perspective are visually perceived, never movements of another person. Thus, neither with regard to the cerebral activation studies, nor with regard to conceptualization the MNS is suggested to be involved in MT. Furthermore, up to now, nobody directly compared the neuronal correlates of MT and VT. This was done via fMRI in Publication 1 of the thesis at hand.

2.6.3.2 Primary motor and somatosensory cortex

With regard to an activation of M1 due to the mirror illusion, there are divergent results. M1 activation due to the mirror illusion was examined with diverse measurement techniques, experimental designs and setups, varying in e.g. unilateral versus bilateral hand movements, visual feedback of one versus two hands, implementation of a mirror box, a real mirror or a video-chain and control conditions (Deconinck et al., 2014). This diversity might explain the

divergent results concerning M1 modulation due to mirrored visual feedback to a certain extend.

In any case, while some researchers found that the mirrored visual feedback of visually guided hand movements compared with normal visual feedback directly enhances activity of M1 (Diers et al., 2010; Tominaga et al., 2009), others could not replicate these findings (C. Dohle et al., 2004; C. Dohle et al., 2011; Matthys et al., 2009; Michielsen, Smits, et al., 2011). Other studies report enhanced M1 excitability measured via TMS and the resulting motor evoked potentials (MEP) due to the mirror illusion. However, a more detailed analysis of the compared conditions reveals that the enhanced M1 excitability could only be found when the mirrored condition was compared to a neutral stimulus or to a static hand. When the mirrored condition was compared to the same condition with normal visual feedback, enhanced M1 excitability was not found (Fukumura, Sugawara, Tanabe, Ushiba, & Tomita, 2007; Funase, Tabira, Higashi, Liang, & Kasai, 2007; Garry, Loftus, & Summers, 2005; Kang, Ku, Kim, & Park, 2011; Kang et al., 2012). This indicates that M1 excitability seems to be modulated by visual input of own body parts, in this case by seeing the hand. However, it does not argue for a modulation of M1 excitability due to the mirror illusion. Furthermore, it seems to be clear is that the mirror illusion does not reverse the lateralization of M1 activity from contralateral to ipsilateral (Praamstra, Torney, Rawle, & Miall, 2011) as it was suggested (Touzalin-Chretien & Dufour, 2008).

Whereas, the effects of MT on S1 are less extensive examined and hardly compared to the effects on M1. Clinical data argue for different activation patterns of M1 and S1 due to mirror illusion: While motor functional improvement of the upper limb is achieved only after extensive MT training (Thieme et al., 2012), sensory improvements were already observed after single MT trials (G. Moseley & Wiech, 2009; G. L. Moseley, 2004). This suggests an immediate modulation of S1 activation by the mirror illusion, leading to quick changes in neuroplasticity of S1 and surrounding areas. Meanwhile, M1 activation might not be modulated immediately, but only via neuronal projections leading to a more slowly cortical reorganization. To the best knowledge of the author, Diers and Wasaka were the only ones

who directly analyzed S1 activity during visually guided hand movements under mirrored conditions. Diers and colleagues found additional activation in both areas, M1 and S1, contralateral to the limb seen in the mirror, i.e. ipsilateral to the moving hand, when comparing the mirror condition with the normal visual feedback condition (Diers et al., 2010). However, this study bears some methodical problems. Firstly, the conditions mirror and normal visual feedback were not directly contrasted with each other, i.e. no T-contrasts were calculated. Secondly, different z-layers are consulted for comparison of mirror and normal condition. And finally, by application of a real mirror in the scanner to create the mirror illusion the visual input of two hands is provided. This degrades comparability of the two conditions as in the normal condition the visual input of only one hand is provided. In the second study, Wasaka and colleagues' experimental task consisted in a symmetric and asymmetric task with a mirror or without a mirror between both hands. They found significantly higher activation in the contralateral S2 in the mirror compared with no mirror condition, but only during the asymmetric task that provided unexpected visual feedback. This was not found for S1 (Wasaka & Kakigi, 2012). However, it remains questionable if this setup corresponds to the mirror illusion that is elicited during MT, as during MT asymmetric movements between affected and non-affected limb are not intended as they are known to cause unpleasant or even painful sensations (Altschuler et al., 1999; Bieniok, Govers, & Dohle, 2009).

Clinical data strongly argue for a direct modulation of S1 areas due to the mirror illusion, meanwhile evidence from imaging studies is insufficient for any final conclusion. Further investigations need to be done about S1 concerning the effect of the mirror illusion (Publication 2).

2.6.3.3 Effects on neuroplasticity due to MT

In contrast to the results on immediate M1 and S1 modulation due to the mirror illusion, the results regarding neuroplasticity due to MT training on healthy subjects or MT on patients with hemiparesis are more homogeneous. A recent review reveals that several studies were able to demonstrate the effects of intensive MT training on cortical reorganization of the primary motor or sensorimotor cortex which correlates with motor functional improvements (Deconinck et al., 2014). Different underlying mechanisms for cortical reorganization of M1 due to MT are discussed.

TMS studies demonstrated that excitability of M1 increases in the affected and decreases in the non-affected hemisphere after MT training in comparison with control training (Lappchen et al., 2012; Nojima et al., 2012). This effect is suggested to be based on disinhibition, including intracortical inhibition (ICI) and facilitation (ICF) and not on interhemispheric inhibition (Lappchen et al., 2012). On the other hand, others found a shift in interhemispheric activity imbalance of M1 towards the affected hemisphere in chronic stroke patients after six weeks MT when pre- and post fMRI measurements were compared (Michielsen, Selles, et al., 2011). Beyond that, fMRI based analysis on functional connectivity found increased interconnections between both premotor regions and the ipsilateral SMA. In turn, SMA was found to develop increased interconnectivity with the ipsilateral sensorimotor cortex due to MT training of healthy subjects (Hamzei et al., 2012). Finally, also an examination on three patients - not included in the above mentioned review - confirms enhanced ipsilesional M1 activation due to intensive MT in phantom limb patients. This increase was correlated to relieve in phantom limb pain (Giraux & Sirigu, 2003).

Hence, these studies corroborate the above mentioned suggestion that neuronal modulation of M1 is only to be expected based on long-term neuroplasticity effects due to intensive MT training.

2.6.3.4 Precuneus

Another cortical structure that was suggested to play a crucial role during mirror illusion of MT is PC. An fMRI study on normal subjects found lateralized higher order visual areas, especially precuneal activity due to the mirror illusion. The experimental setup for generation of the mirror illusion was based on a video-chain that allowed for systematical manipulation of the visual feedback of the visually guided hand movements. I.e. mirrored or non-mirrored visual feedback of the hand movements was provided. Thereby, the hand movements of the subjects were filmed and projected on-line on special goggles (C. Dohle et al., 2004).

Beyond that, Dohle and colleagues were the first to separately examine limb and coordination transformation during MT by means of virtual reality in their Positron emission tomography (PET) study. When we see our limb movements through a sagittal placed mirror such as in MT, not only the limb per se is mirrored (limb transformation), but also the space-coordinates are mirrored, i.e. moving to the left looks like moving to the right and vice versa (coordination transformation). Different neuronal correlates were found: for limb transformation, the posterior portion of the PC contralateral to the perceived limb was involved, but not for coordination transformation, which elicited an activation of the insular cortex contralateral to the actually moving arm. It is suggested that visual processing of own arm movements relies on lateralized PC activation contralateral to the perceived limb (C. Dohle et al., 2011). Based on these results, MT is suggested to function via an lateralized activation of the visual body representation, having its neuronal substrate in the area of PC (Cavanna & Trimble, 2006; C. Dohle et al., 2011; Fattori et al., 2009).

Furthermore, another fMRI study on chronic stroke patients also found PC additionally to posterior cingulate cortex activation due to mirror illusion, however only during bimanual conducted movements (Michielsen, Smits, et al., 2011).

FMRI analyses on functional connectivity indirectly support the involvement of PC in MT, too. As already mentioned above, Hamzei and colleagues found increased interconnections between both premotor regions and the ipsilateral SMA, which in turn developed increased

interconnectivity with the ipsilateral sensorimotor cortex due to MT training (Hamzei et al., 2012). Meanwhile, PC is known to have reciprocal connections with the dorsal premotor area and with SMA (Cavanna & Trimble, 2006), which in turn have projections on M1. This circuit is one of the crucial neuronal mechanisms for motor learning (Kolb & Whishaw, 1996c). Thus, PC might act as intermediary cortical structure that receives and processes the visual input of the mirrored hand during MT and transfers it to premotor cortex and SMA as relevant structures for motoric learning.

Further indirect support derives from studies examining PC function. As already illuminated above, PC plays a decisive role for self-related body representations (Cavanna & Trimble, 2006) and is involved especially in processing of hand and wrist orientation during different hand movements (Fattori et al., 2009; Pitzalis et al., 2013). Processing of hand and wrist features and the activation of self-related body representation are immanent to MT (C. Dohle et al., 2011).

Thus, the thesis at hand suggests that PC, might not only be directly modulated by the mirror illusion, but might even play a prominent role in the facilitation of cortical reorganization in the motor cortex as basis of functional motor recovery of patients with severe hemiparesis after stroke.

2.7 Aims

The cumulative thesis at hand aims to examine the underlying neuronal mechanisms of MT more in detail. Especially, the cerebral activation during visually guided hand-movements - comparing normal and mirrored visual feedback - is experimentally examined. This is examined in healthy subjects on a group-level as well as in stroke patients and on a single-subject level. Furthermore, it is aimed to investigate what determines MT responders and non-responders.

The thesis at hand tries to give an answer to following questions:

- What changes in the brain, when we suddenly see our moving hand mirrored?
- Is the mirror neuron system involved in the effect of MT?
- Do MT and VT have similar or different neuronal correlates?
- How does activation in M1 and S1 due to mirror illusion differ?
- Is the neuronal activation during MT in stroke patients similar as in normal subjects?
- Could the beneficial effect of MT be determined by precuneal activity?
- How do MT responders and non-responders differ?
- What are the cortical projections that might mediate between visual stimulation and cortical reorganization in the motor cortex due to MT?

In order to achieve these aims several studies were conducted in the context of the thesis at hand. In Publication 1, MT and VT were directly compared. In Publication 2, the different effects on M1 and S1 due to the mirror illusion were examined and compared in detail by means of laterality indexes. In Publication 3, the implementation of functional near-infrared spectroscopy (fNIRS) for experimental investigation of M1 and PC activity due to mirror illusion is established, especially with the intention to prepare for the patients' measurements in Manuscript 5. Beyond group-analysis level, an exploratory examination was conducted to

determine if the activation pattern observed in normal subjects is likewise observable in stroke patients or if it differs on a single-subject level (Publication 4). Finally, on the basis of the fNIRS results of Publication 3, a neurophysiological index is calculated and compared with other factors in their discriminant potential between MT responders and non-responders. It is examined if this index differs between MT responders and non-responders (Manuscript 5, under revision).

3 Methods and Results

The methods and results of the four publications and the manuscript under revision are shortly summarized in this chapter. For more details concerning the studies see the full publications and the manuscript provided in the appendix.

3.1 Imaging techniques - fMRI versus fNIRS

The neuroimaging methods of choice used in the thesis at hand were functional near-infrared-spectroscopy (fNIRS) as well as functional magnetic resonance imaging (fMRI). Before presenting and comparing the fMRI and fNIRS results, several general methodical commonalities and differences must be considered. More specific differences concerning the individual publications are debated below in the discussion.

In general, both techniques strongly depend on focal concentration changes in the cerebro-vascular system. While fMRI measures the blood oxygen level dependent (BOLD) signal, which is highly dependent on the concentration change of the deoxygenated chromophore of the hemoglobin, fNIRS measures both, oxygenated and deoxygenated hemoglobin changes (HBO and HBR, respectively) (Obrig & Villringer, 2003; Steinbrink et al., 2006). This is important to keep in mind, as it could partly explain the differences between Publication 4 and Manuscript 5: Here the calculated fNIRS index refers to the HBO change, which is not directly comparable to fMRI's BOLD contrast.

Furthermore, the spatial resolution is higher in fMRI (~ 2 mm) compared to fNIRS (~ 10-30 mm) (Steinbrink et al., 2006). Nevertheless, using a multi-distance channel approach (Habermehl et al., 2012; Koch et al., 2010; Yamada, Umeyama, & Matsuda, 2009; Zeff,

White, Dehghani, Schlaggar, & Culver, 2007) can enhance fNIRS spatial resolution to a sub-centimeter range. However, such an approach needs dense fiber arrangements. This would be accompanied by longer preparation times and is therefore not suitable for patients. Last but not least fNIRS can only measure cortical structures on superficial layers of the brain as its depth penetration is strongly limited (Obrig, 2014).

A disadvantage concerning fNIRS is that similar to Electroencephalography (EEG), but in contrast to fMRI, no anatomical brain scan can be done, making adaptation to possible inter-individual differences in brain anatomy impossible. In the fNIRS studies at hand inter-individual variability of brain anatomy was only roughly considered by head sizes and corresponding EEG cap size. On the other hand, the normalization step in the fMRI group-analysis leads to a similar problem by fitting the individual brains to a standard brain.

In any case, the paradigm for the fNIRS measurements was carefully chosen (e.g. long and jittered inter-stimulus intervals) to prevent extra-cerebral, especially cardiovascular, contamination and the data was corrected for movement artifacts and filtered (Kirilina et al., 2012; Takahashi et al., 2011) to attenuate drifts in the signal.

And finally, differences in the experimental setups must be taken into account when comparing the results: In the fMRI patients lay wearing googles, while they sit in front of the screen in the fNIRS. The latter corresponds rather to the MT setup which is conducted in the sitting position, and advantages fNIRS studies as experiments can be undertaken in a more natural setting.

For implementation of imaging techniques in the daily rehabilitation routine, e.g. for diagnostics or monitoring, fNIRS is much more appropriate than fMRI, due to its low costs, easiness to apply, higher availability in clinical settings and portability (Kato, Izumiyama, Koizumi, Takahashi, & Itoyama, 2002; Muehlschlegel et al., 2009; Strangman, Goldstein, Rauch, & Stein, 2006). Beyond that, it is perfectly suited for patients, because there are no contraindications for patients - compared to fMRI (e.g. aneurysm clip, heart pacemaker), so that it can be applied for every patient. For these reasons, fNIRS was the method of choice in

the clinical study (Manuscript 5). Before implemented on patients, the fNIRS setup was examined on healthy subjects (Publication 3).

The corresponding EEG positions for the fNIRS optodes (sources and detectors) for the PC-ROI and M1-ROI were selected according to a recent study on cortical projections on EEG sensors and anatomical structures (Koessler et al., 2009). According to Koessler (2009) the part of PC belonging to BA 7 corresponds to the EEG positions P1, P2 and Pz. These were the positions of the optodes in the fNIRS paradigm, together with POz which corresponds to occipital lobule (BA 19) (Koessler et al., 2009). However, it is not the cortex situated directly *below* the optodes that is measured by fNIRS, but the tissue *between* two optodes, the so-called channels (Figure 5). Respectively, the part of M1 (BA 4) corresponds to the EEG positions C1, Cz and C2 (Koessler et al., 2009). As the corresponding hand areas for finger and thumb representations are located more lateral, the EEG positions C2 and C4 were chosen as sources for the M1-ROI (Pinel, 2001a).

It is important to notice that it might be difficult to measure the parts of PC located in the deeper longitudinal fissure. Because, as already mentioned above, fNIRS can only measure cortical structures on superficial layers of the brain (Obrig, 2014). Hence, the PC-ROI referred to in the fNIRS measurements of the thesis at hand rather corresponds to an overlapping region between the superior parts of PC, the medial part of SPL and parts of the anterior medial parieto-occipital cortex (Figure 5).

Finally, the data of the present thesis support the higher practicability of fNIRS in comparison with fMRI: While only five patients could be measured by fMRI, due to medical contraindications, all included patients (13 including the subsequent drop-outs) were measured with fNIRS without any constraints.

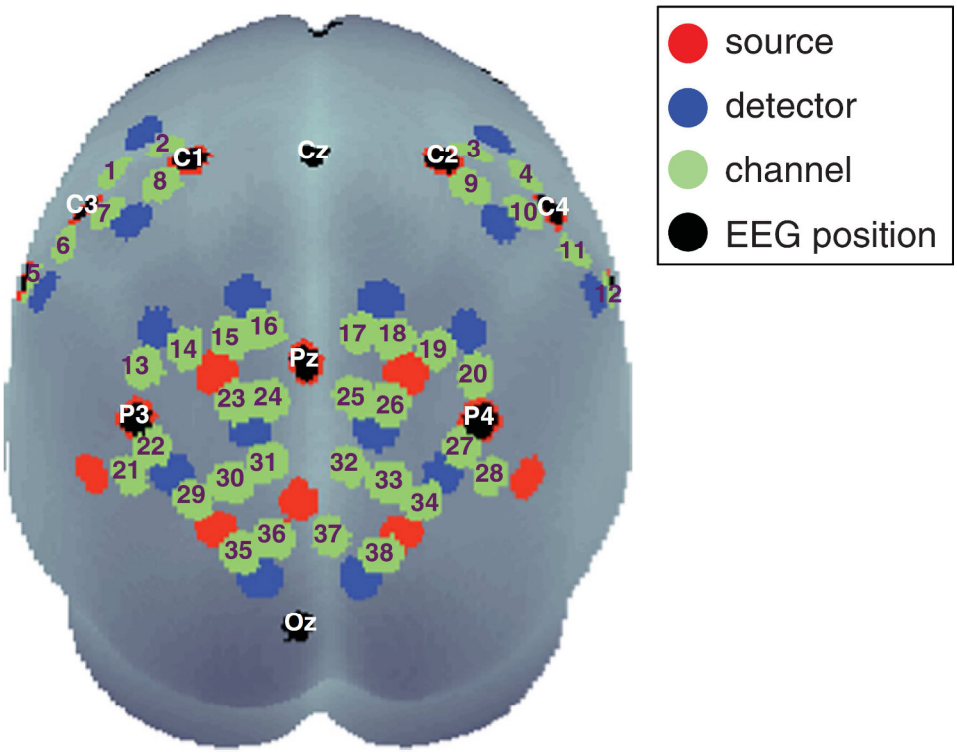


Figure 5: Schematic illustration of the optodes (sources and detectors) and the measured channels in between in the fNIRS paradigm. Channel numbers of PC-ROI: 23, 24, 31 (left); 25, 26, 32 (right). M1-ROI: 1, 2, 7, 8 (left); 3, 4, 9, 10 (right).

3.2 Definitions

The thesis at hand assessed healthy subjects as well as sub-acute stroke patients. Thus, it is important to note that in healthy subjects related to the moved hand under *normal visual conditions* the ipsilateral hemisphere corresponds to the ipsilesional and the contralateral hemisphere to the contralesional hemisphere of the patients. This is different during MT or mirror stimulation, where the left hand is perceived as the right hand and vice versa. Under *mirrored visual conditions* contralateral to the *perceived limb* means ipsilesional, because it is always the non-affected limb that is reflected in the mirror. The following Figure 6 illustrates this.

Furthermore, the terms mirror illusion, mirror-like illusion, mirror stimulation and mirrored visual feedback refer all to the same phenomenon as the underlying basis of the MT: the phenomenon of seeing the moving hand mirrored such as if it would be the other hand. That means that the left hand in the mirror looks like the right hand and vice versa. In the following presented studies the mirror stimulation implemented by means of an on-line video-chain was used as the operationalization basis of MT.

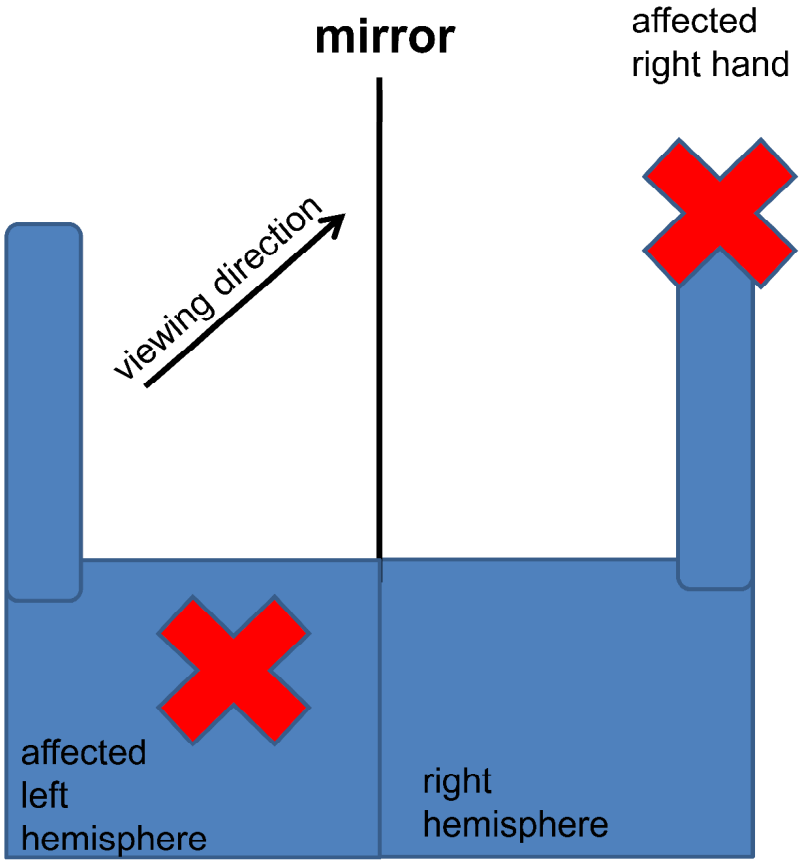


Figure 6: Schematic illustration of MT. In this example, the left hemisphere is affected. The left hand is moved and mirrored, so it looks like the right hand (=mirror stimulation). Contralateral to the *perceived* hand under mirrored conditions thus means the affected hemisphere (=ipsilesional).

3.3 Healthy subjects - group analyses

3.3.1 Publication 1 - *"A comparison of neural mechanisms in mirror therapy and movement observation therapy"*

3.3.1.1 Methods

The first publication aimed to compare the neuronal mechanism of MT with the neuronal mechanism of VT via fMRI in healthy, right-handed subjects (n=15). Following concrete hypotheses were tested:

1. The effects of MT and VT are based on different cerebral activation patterns.
2. Lateralized precuneal activity is modulated by mirrored visual feedback in healthy subjects and is not due to mere hemifield stimulation.
3. Mirrored visual feedback does not immediately modulate M1 activity.

The subjects had to conduct two different tasks in the scanner that were measured en bloc. The first task was a visually guided finger-thumb opposition movement under normal (NOR) or mirrored (MIR) visual feedback. The video-setup that allowed controlled manipulation of the visual feedback (NOR / MIR) is illustrated in Figure 7. Additionally, they had to keep the hand static under NOR as well as under MIR conditions. The four conditions were conducted in a pseudo-randomized sequence following an acoustic cue ("Start" / "Stop"). However, in this publication only the moving trials were analyzed. The static trials were included in the secondary analysis of Publication 2.

In the second task, the subjects had to perform a pure observation task: The same finger-thumb opposition movement of task 1 was executed by another person and presented on a video, without moving the own hands and fingers (OBS).

The calculated T-contrasts for the first task were: *mirrored* visual feedback condition versus *normal* visual feedback condition, for the left hand (LH) and right hand (RH), respectively (for

the moved trials only). Furthermore, for the mere observation tasks the contrasts LH observation versus RH observation and vice versa were calculated.

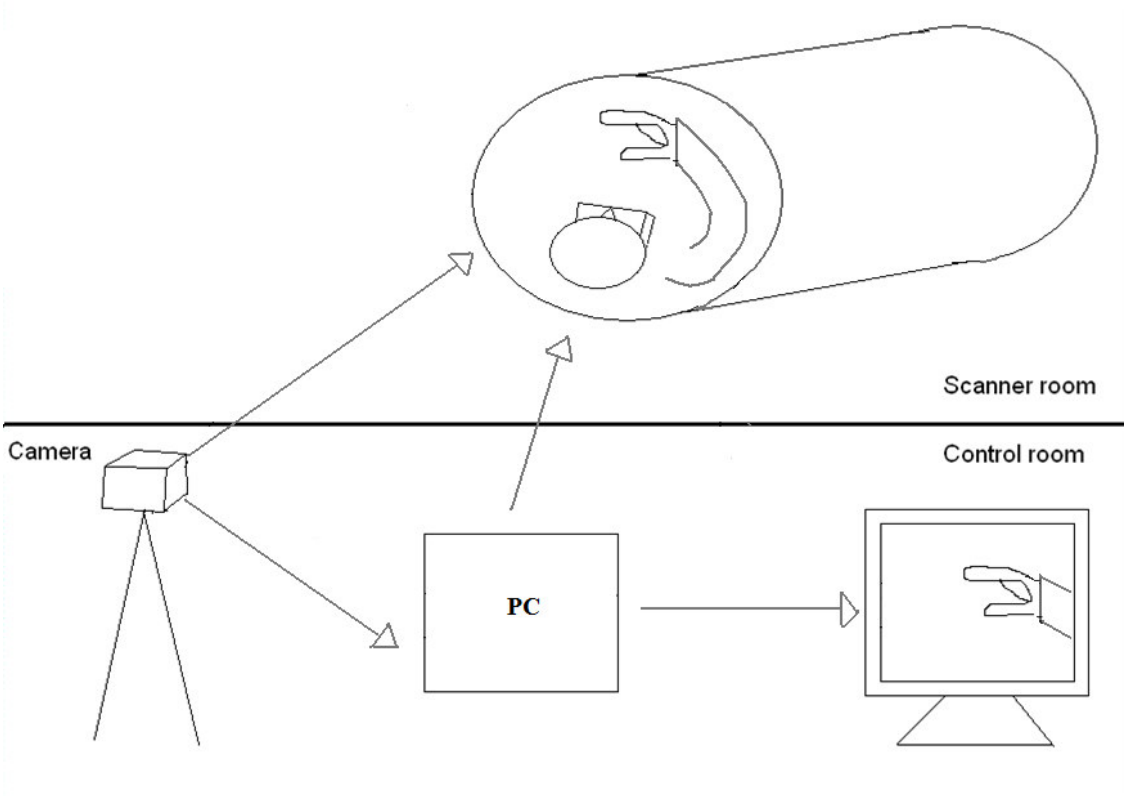


Figure 7: Video-setup: The subject's hand is videoed from outside the fMRI scanner. The image is processed by software on a computer and projected online on fMRI-suitable special goggles worn by the subject.

Although, the right arm was always placed more on the right visual hemifield and the left arm more on the left visual hemifield, hand and fingers - and thus the points of fixation - were always presented centrally. This was constant in all conditions.

3.3.1.2 Results

Different neuronal activity patterns for VT compared with MT were found: Lateralized precuneal activation always contralateral to the perceived limb, even under MIR condition,

was observed. However, lateralized precuneal activation was only found during own active movement performance under visual control, and not during the mere observation tasks. The mere observation of a LH or RH of another person did not elicit the lateralized activation of PC. The contrasts LH observation versus RH observation and vice versa did not reveal any significant activation. That means, while the mirror stimulation elicited additional lateralized activation of PC contralateral to the perceived hand, i.e. ipsilateral to the moving hand, no such lateralization was found in the mere observation of a third person's hand (Figure 8). However, it cannot be excluded that mere observation elicited activation always lateralized to the same hemisphere. Furthermore, the contrasts calculation revealed no differences in M1 activation between movements with normal and mirrored visual feedback.

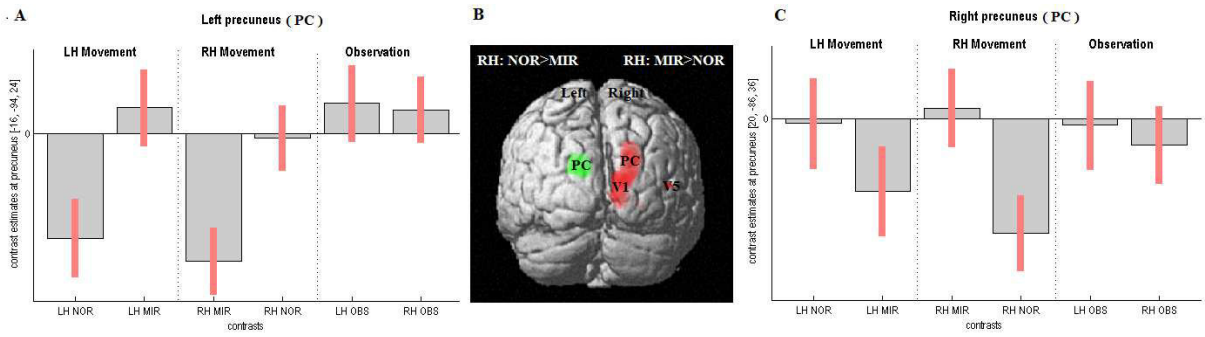


Figure 8: Activation pattern and strength. (B) Activation differences during movements of the right hand (RH) plotted on a standard 3-D image of the brain, viewed from behind. Red: MIR > NOR, green: NOR > MIR. (A, C) Mean standardized effect sizes and 90% confidence intervals at both precunei in all 6 conditions. NOR: normal moving hand; MIR: mirrored moving hand; PC: precuneus; LH: left hand; OBS: pure observation.

3.3.1.3 Conclusions

Firstly, these results confirm the first hypothesis that mirror stimulation (as basis for MT) and VT function via different underlying neuronal mechanisms: While mirror stimulation elicits lateralized activation, there is no difference between observation of a left hand and

observation of a right hand. This could also lead to different implications concerning their therapy applications.

Secondly, visually guided movement execution elicits lateralized precuneal activation that is observed always contralateral to the perceived hand. This is similarly observed under mirrored visual feedback. As no such lateralization was found in mere observation of a third person's hand, this lateralized precuneal activation is not due to mere hemifield stimulation. This is also supported by the asymmetry of the reverse comparison (NOR greater MIR) that generated somehow different activation foci in the hemisphere contralateral to the perceived limb (Figure 8 B).

And finally, a direct modulation of M1 by mirror stimulation can be excluded, as the contrast analysis did not reveal any M1 activation.

3.3.2 Publication 2 - *"Different effects of the mirror illusion on motor and somatosensory processing"*

3.3.2.1 Methods

After comparing MT and VT, a comparison of MT effect on M1 and S1 was aimed and a more detailed examination of the lateralization shift during visually guided hand movements under normal visual feedback (NOR) to mirrored visual feedback (MIR). Therefore, a secondary data analysis of the fMRI data of Publication 1 was conducted. Following concrete hypotheses were tested:

1. The mirror illusion has different effects on the activation of M1 and S1.
2. There is an observable lateralization shift due to the mirror illusion on S1.
3. There is no lateralization shift due to the mirror illusion on M1.

In the first step, for each condition a contrast between moved and static trials was calculated, to find the brain areas where moved trials elicit more activation than the static ones. Afterwards, a lateralization index (LI) was calculated for activations surpassing a certain threshold. The LI was calculated so that an LI of +1 indicates activation that is fully lateralized to the left hemisphere, an LI of -1 indicates activation that is fully lateralized to the right hemisphere, and an LI of 0 indicates full symmetry between both hemispheres. This secondary data analysis was only conducted for functional brain areas that are defined by the SPM toolbox and labeled with the corresponding Brodmann areas (BA). PC is not defined by the SPM toolbox and was thus not included in this analysis.

3.3.2.2 Results

As expected, comparing moved with static trials reveals the motor network to be activated, consisting of M1 (BA 4), premotor cortex and SMA, as well as other areas such as: S1 areas

(BA 2, 3a and 3b) under NOR and MIR conditions. Activation sizes under MIR condition were twice as high as under NOR condition for M1, and five times higher for S1.

No lateralization shift occurred from NOR to MIR on the motor cortex network: M1 remained strictly lateralized to the hemisphere contralateral to the moved hand, independent from the visual feedback and from the hand moving. This was similarly observed for premotor cortex and SMA, where activation remained lateralized to the (dominant) left hemisphere, irrespective of the visual feedback.

Contrary, a lateralization shift on S1 due to the mirror illusion was observed: for BA 2 and BA 3a lateralization changed from ipsilateral to nearly symmetrical and for BA 3b from ipsilateral to contralateral, for left hand movements only. For right hand movements activation remained predominantly contralateral.

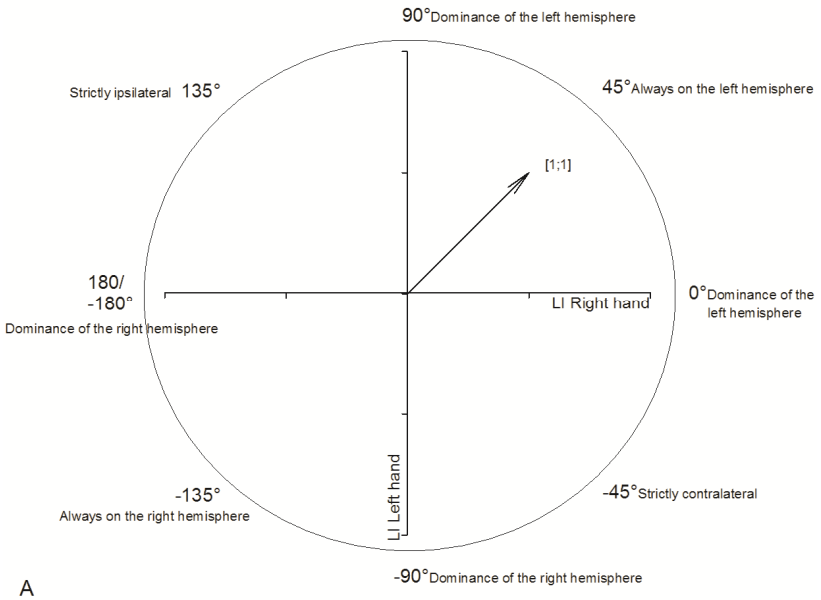
The shifts in lateralization for S1 and M1 are illustrated in Figure 9.

3.3.2.3 Conclusions

It is concluded that M1 is not directly modulated by the mirror illusion, while S1 was found to be modulated directly by the mirror illusion. This confirms the hypothesis of different effects of MT on M1 compared to S1 activation, at least for the left hand. The observed shifts in lateralization in S1 could not be explained by activation differences of visual areas, as for example V5 was equally lateralized to the right hemisphere for NOR and MIR. Thus, the lateralization effect of the mirror illusion cannot be attributed to mere hemifield stimulation.

Furthermore, the direct stimulation of S1 by the mirror illusion could be yet another link between visual stimulation and enhanced neuroplasticity of the motor cortex after MT. This further supports the hypothesis of indirect neuronal pathways to the motor cortex in explaining the effect on motor improvement due to MT. Finally, it reveals for the first time the

underlying different neuronal mechanisms of the motoric and sensory clinical improvements due to MT that differ considerably (cf. Introduction).



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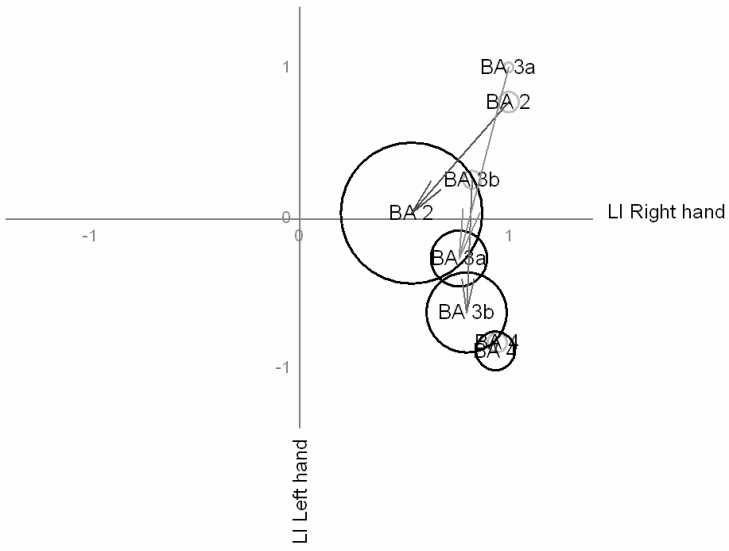

B

Figure 9: Lateralization of left (y-axis) and right (x-axis) hand movements **A: an example** for the correlation of Laterality Vector (LV) to the degree of lateralization. Here LIs for right hand and left hand are 1; thus both literalizing to the left hemisphere. **B:** Lateralization of M1 (BA 4) and S1 areas during NOR and MIR. Activations of M1 and S1 areas during NOR are represented by black-outlined blobs, and during MIR by the grey-outlined blobs. Arrows indicate the laterality shift. The diameter of the blobs represents the size of activations in relation to the respective anatomical areas as given by the Anatomy Toolbox.

3.3.3 Publication 3 - *"Effect of a mirror-like illusion on activation in the precuneus assessed with functional near-infrared spectroscopy"*

3.3.3.1 Methods

As fNIRS measurements for daily clinical routine are much more appropriate than fMRI, Publication 3 aimed at the fNIRS implementation for cerebral activity measurement during mirror stimulation as potential determinant of MT efficacy. Following hypotheses were tested:

1. Lateralized precuneal activity due to mirror illusion in healthy subjects is also measurable by fNIRS.
2. Mirror illusion does immediately modulate PC activity.
3. Mirror illusion does not immediately modulate M1 activity.

Similar to the Publications 1 and 2, the changes in HBO and HBR during the mirror illusion was systematically assessed in predefined ROIs (PC and M1) with fNIRS. Therefore, the video-setup of Publication 1 and 2 was implemented to normal subjects in a sitting position. The visual feedback of the moving hand that performed the finger-thumb opposition task was presented on the monitor in front (Figure 10). Visual feedback (NOR / MIR) and hand laterality (left / right) were manipulated in a randomized way, as well as the movements (movement / static). After standard data pre-processing and filtering the mean beta values over the two ROIs were included in two repeated measure four-way analysis of variance (ANOVA) with the factors hemisphere, hand laterality, mirror and movement.

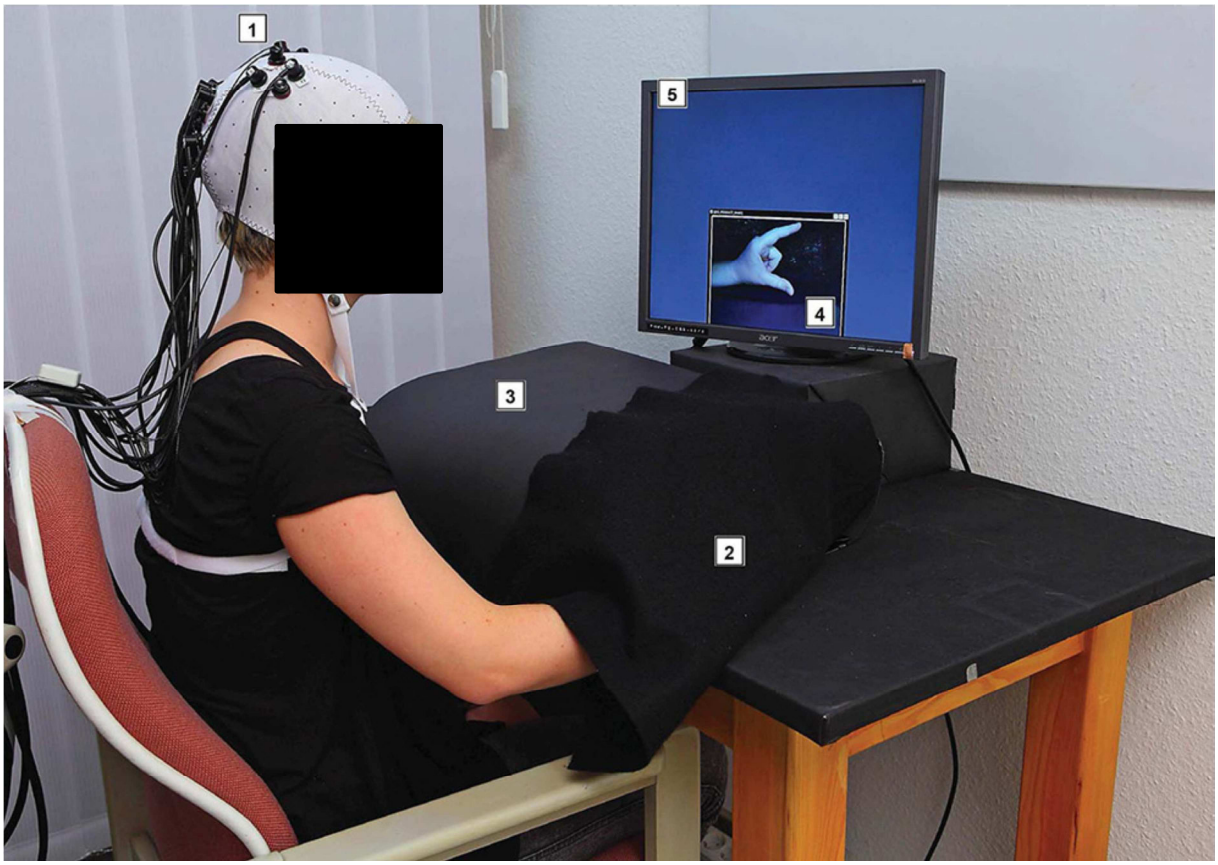

Figure 10: FNIRS setup: 1) Subject wearing an EEG cap with NIRS detectors and sources; 2) subjects hand inside a black paperboard, covered by a black drapery being filmed by 3) a web-cam inside; 4) visual feedback the subject gets of his hand, in this case MIR and 5) the screen on which the visual feedback is displayed. Not in the picture: an additional computer with presentation software running, instructing the investigator to mirror or not to mirror the visual feedback in a randomized way and sending triggers to the NIRS system, the NIRS system recording the blood oxygen concentration changes, as well as the computer with which the visual feedback / the camera (MIR or NOR) was controlled by the principal investigator.

3.3.3.2 Results

Data analysis revealed only in the PC-ROI (not on M1) an increase of activation in the hemisphere contralateral and a parallel decrease of activation of the hemisphere ipsilateral to the perceived hand in the mirror. This interaction of the factors hemisphere and mirror was only significant for HBO, for HBR the effect failed to reach significance, but there was a similar trend. Moreover, the mean interhemispheric differences were positive for NOR and

negative for MIR, indicating an inversion of activity lateralization by the mirror illusion: While there is more activation in the hemisphere contralateral (to the moved hand) under NOR condition, this is inverted by the mirror illusion, so that there is more activation in the hemisphere ipsilateral (to the moved hand, i.e. contralateral to the perceived hand) under MIR condition. In contrast, for the M1-ROI the ANOVA revealed a main effect of the factor movement and a main effect of the factor hemisphere (for the movement trials only), but no interaction effect of the factors mirror and hemisphere. The following Figure 11 illustrates the mean beta values and the mean interhemispheric differences whereat contra- and ipsilateral hemisphere refers to contra- and ipsilateral to the *acting* hand.

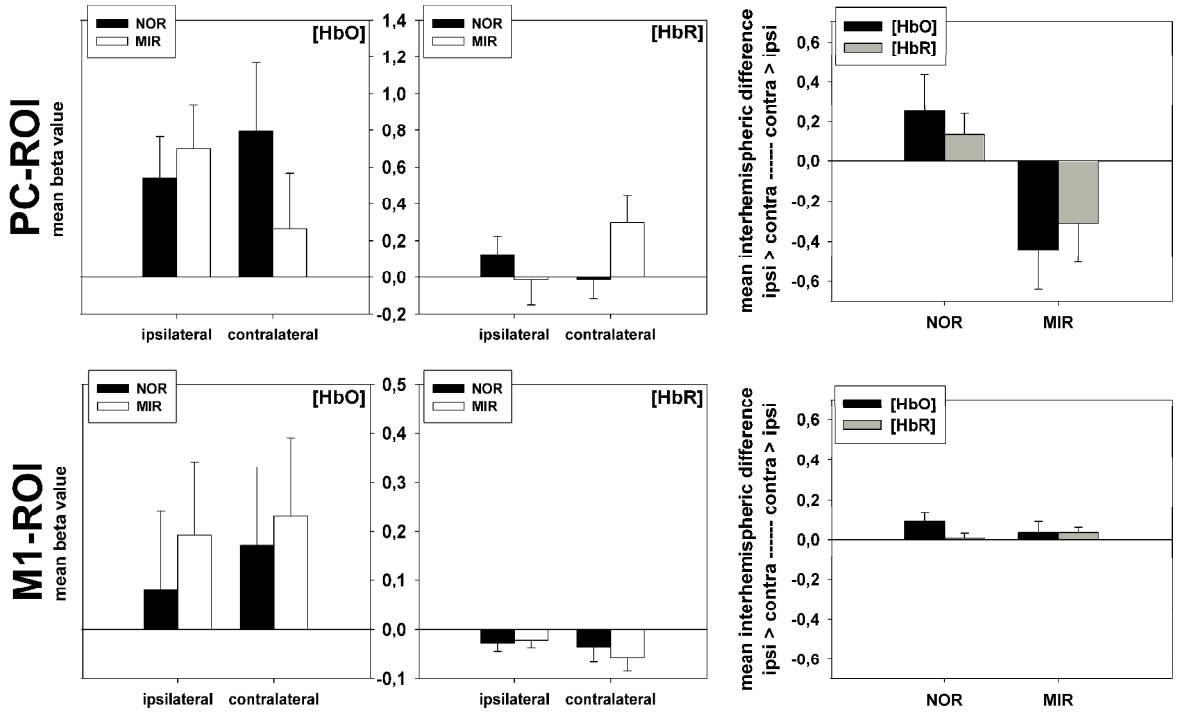

Figure 11: Mean beta values and mean interhemispheric differences, and their corresponding standard errors at the PC-ROI (top row) and M1-ROI (bottom row). Left column: mean beta values for HBO of the ipsi- and contralateral hemisphere for the MIR and NOR conditions; middle column: respectively for HBR; right column: mean interhemispheric differences in the MIR and NOR conditions for HBO and HBR (multiplied by –1). Note: In the right column, values >0 indicate activation in the contralateral > ipsilateral hemisphere, and values <0 indicate activation ipsilateral > contralateral.

3.3.3.3 Conclusions

The results confirm the above listed hypotheses. Firstly, similarly as in Publication 1 and 2, the lateralized precuneal activity due to the mirror stimulation is also measurable with fNIRS. Beyond that, the results of Publication 3 extend those of Publication 1 and 2 by demonstrating an additional decrease of PC activation (ipsilateral to the perceived hand=contralesional) due to the mirror. The mirror stimulation seems to invert the lateralization of activation in PC from contralateral to ipsilateral to the moved hand. Secondly, the results demonstrate that M1 activity is not directly modulated by mirror stimulation. As it was expected M1 is not directly influenced by the change of visual feedback, but rather by the hand movements per se. The inversion of activity lateralization in PC is interpreted as the involvement of the limb specific mental representation according to the limb that is observed: Thus, a *perceived* right limb activates corresponding neuronal correlates in the left hemisphere, which is inversed when the moving limb is *perceived* as left limb by the mirror illusion. The same applies to the left limb.

Translating this phenomenon observed in normal subjects into stroke patients obtaining MT, this would mean that the mirror illusion changes the interhemispheric activation imbalance towards the affected hemisphere: from contralesional to ipsilesional PC. It is well known that the unaffected hemisphere could further inhibit the affected hemisphere via transcallosal inhibition mechanisms, thus deteriorating motor performance (Calautti et al., 2007). Hence, it might be assumed that MT works via this inversion of the interhemispheric activation imbalance in the PC.

3.4 Stroke patients - single-subjects and group analyses

3.4.1 Publication 4 - *"Cerebral activation evoked by the mirror illusion of the hand in stroke patients compared to normal subjects"*

3.4.1.1 Methods

After group-analyses in healthy subjects, it was aimed to examine the lateralized precuneal activation found always contralateral to the perceived hand due to mirror stimulation on a single-subject level. Additionally, it was aimed to compare this activation pattern during mirror stimulation with stroke patients that fulfill the criteria for MT application. Following hypotheses were tested:

1. Lateralized precuneal activity due to mirror illusion is observable in the majority of the single healthy subjects.
2. Lateralized precuneal activity due to mirror illusion is similarly observable on stroke patients, but not in all of them.

Publication 4 is partly a secondary data analysis of Publication 1: The fMRI data during mirror stimulation of the fifteen healthy subjects (mean age: 33.7; range: 22-56) that was already collected in Publication 1 was re-analyzed for Publication 4. Additionally, five stroke patients (mean age: 61; range: 50-72) that fulfilled the criteria for MT application underwent the same measurement protocol with their non-affected hand as the subjects in Publication 1, except for the observation task. In contrast to Publication 1, T-contrasts were not calculated on a group level, but on a single-subject level. T-contrasts were calculated between MIR and NOR for each hand of the healthy subjects and for the non-affected hand of the patients, for each single subject. As a measure of strength of activation the peak T-value in the region of PC was calculated. Furthermore, the 95% confidence interval (CI) of the fifteen healthy subjects was calculated for each hand and for each T-contrast (MIR

greater NOR and vice versa). Finally, the T-value of each patient was compared with this CI-range.

3.4.1.2 Results

Lateralized activation on PC was found in almost every single healthy subject: Ten out of fifteen subjects showed this ipsilateral PC activation during mirror stimulation of their right hand; and thirteen out of fifteen subjects showed ipsilateral PC activation during mirror stimulation of their left hand. There was only one subject that showed no ipsilateral PC activation during mirror stimulation of both hands (Figure 12).

This was similar for the stroke patients: Four out of the five stroke patients had significant lateralized ipsilesional PC activation for the calculated contrast of mirrored visual feedback greater normal visual feedback (Figure 13). For the reverse comparison only two out of the five patients had significant contralesional PC activation. Strength of activation of the patients was always in the range of the corresponding 95%-CI of the fifteen normal subjects.

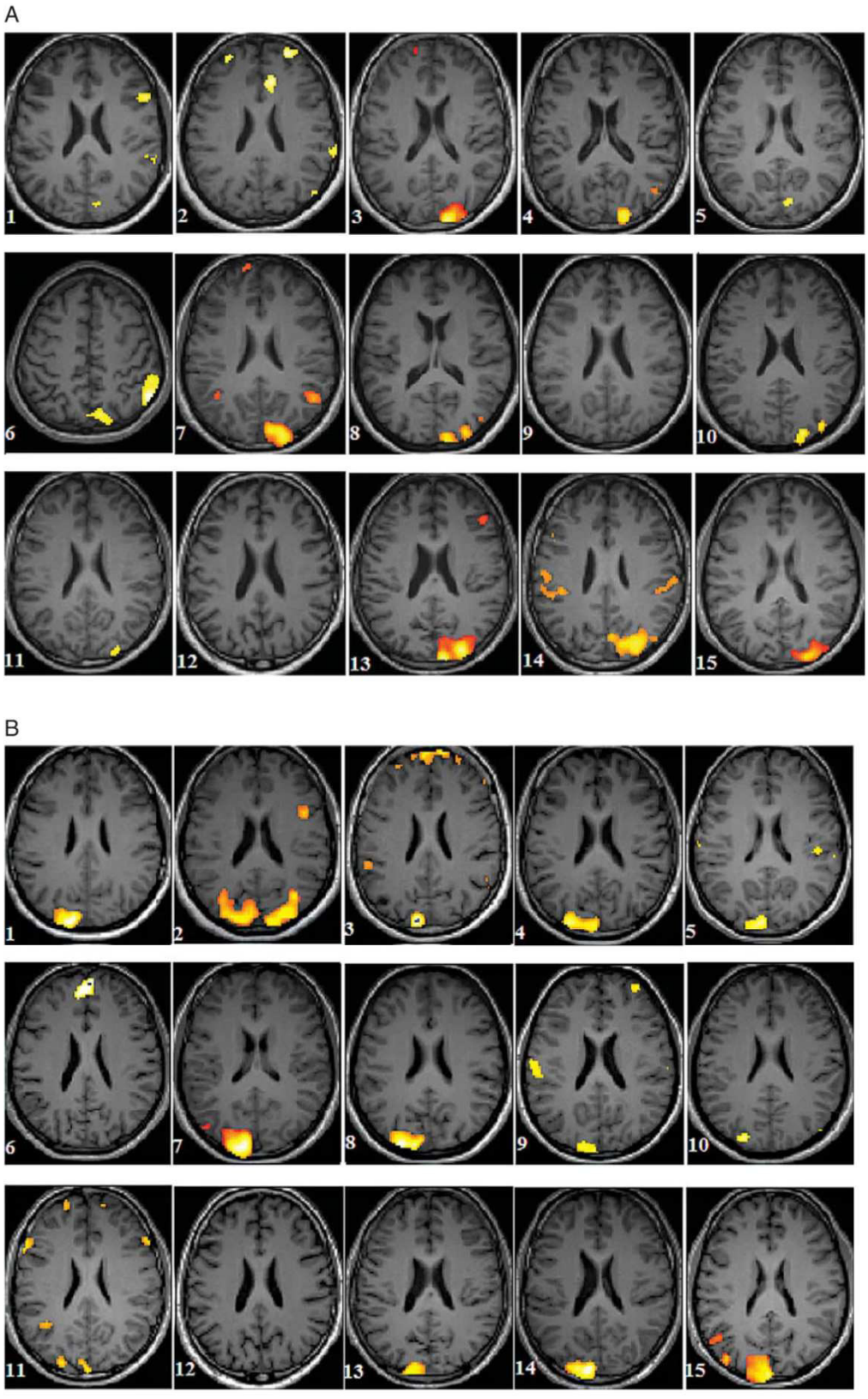

Figure 12: Activations of the fifteen normal subjects due to mirror stimulation (MIR greater NOR). Activation's threshold lies at $p<0.001$ (unc.) with a minimum cluster size of 20 voxels. A: Activations during right hand mirror stimulation. B: Activations during left hand mirror stimulation.

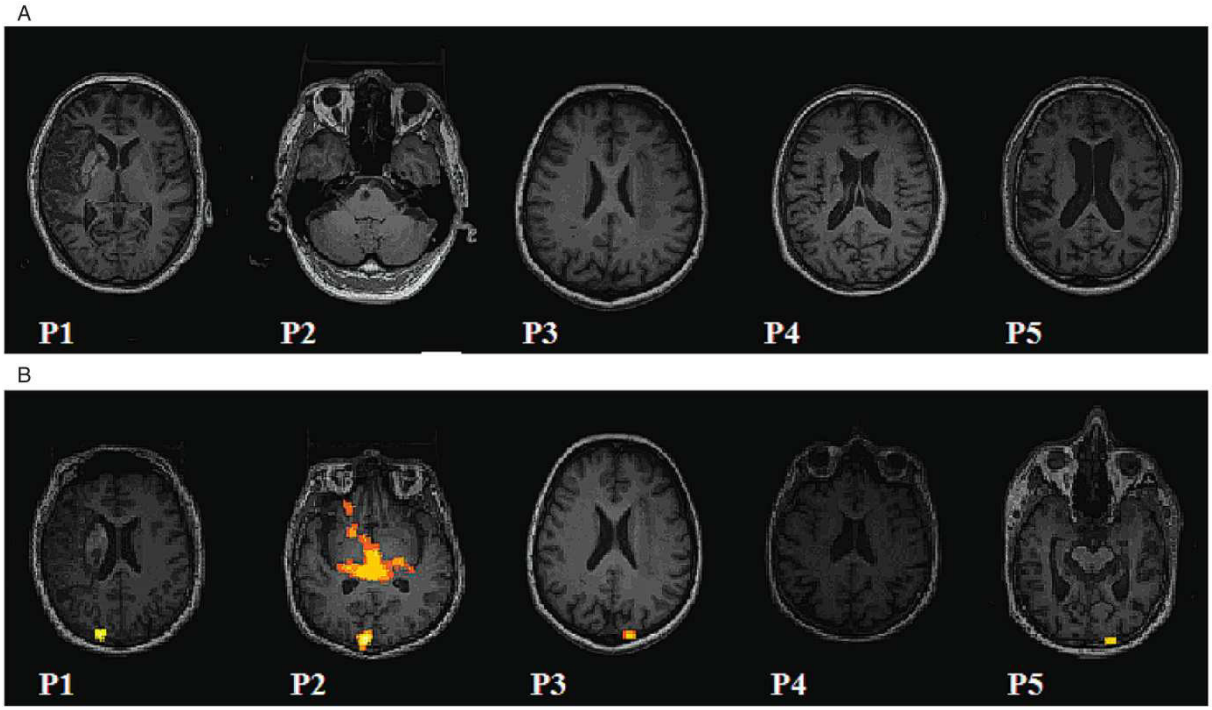

Figure 13: Lesions and cerebral activations of the stroke patients. A: Lesions at the level of maximum infarct volume for each patient as visible in the T1 scans. B: Activation induced by the mirror stimulation of the non-affected hand. Activation's threshold lies at $p<0.001$ (unc.) with a minimum cluster size of 20 voxels.

3.4.1.3 Conclusions

The results of the single-subject analysis show that the lateralized precuneal activity due to mirror stimulation is observable in the majority of healthy subjects. Furthermore, it is also observable in the majority of the stroke patients and does not substantially differ from normal subjects regarding the strength of activation. Thus, despite their severe hemiparesis and despite their advanced age, the lateralized precuneal activity due to mirror stimulation could also be elicited and might therefore be the neurophysiological basis of the clinical improvement of MT.

3.4.2 Manuscript 5 - *"Potential determinants of efficacy of mirror therapy in stroke patients - a pilot study"*

3.4.2.1 Methods

Given that - as hypothesized at the end of Publication 3 - the PC mediates the effect of MT and shows an inter-individual variability, it could be assumed that the responsiveness of PC might serve as a predictor for the success of MT. Therefore, the following study was designed to correlate the inversion of the interhemispheric activation imbalance in PC with therapy success due to MT.

Hypotheses:

1. Patients who show an inversion of the interhemispheric activation imbalance in PC due to mirror stimulation will more likely benefit from MT and will more likely be classified as responders.
2. Contrary, an inversion of the interhemispheric activation imbalance in M1 due to mirror stimulation does not correlate with MT success, as the previous publications of the thesis at hand showed that M1 activity is not directly modulated by mirror stimulation, but rather by the hand movements per se.

Eleven sub-acute stroke patients that fulfilled the criteria for MT application underwent the same fNIRS measurement as the subjects in Publication 3, except that no static trials were given, in order to shorten the measurement procedure for convenience of the patients. Of course, the patients conducted the task solely with their non-affected hand. As an index for the inversion of the interhemispheric activation imbalance, a so called mirror index (MI) on PC-ROI (MI-PC) and on M1-ROI (MI-M1) was calculated. In dependence on the fNIRS results of Publication 3, the MI was calculated as the sum of ipsilesional activation increase and contralesional activation decrease due to mirror stimulation. Hence, the MI represents the immediate lateralization shift due to mirror stimulation on the respective ROI and corresponds to the lateralization shift that was observed on the PC-ROI in healthy subjects in Publication 3, given that it has a positive value. MI-M1 served as a control, as no direct

influence of M1 was observed in healthy subjects. The MI was calculated only for HBO, as in Publication 3 it was only significant for HBO.

Additionally, other factors potentially affecting the motor recovery after stroke, such as initial motor function, lesioned hemisphere, demographic data and neuropsychological status were considered as well.

Responders to MT were defined as patients, whose improvement of active finger motor function in the Fugl-Meyer Assessment (FMA) was > 0. The following factors were included in a discriminant analysis (DA) designed to classify patients as responders or non-responders: Demographic data (days since stroke, age of patient), initial capacity in activities of daily living (Barthel Index), initial motor performance (FMA-finger), initial neuropsychological performance (trail making test), initial fNIRS response (MI-PC, MI-M1) and performance during therapy (percentage of ideal alertness). Note that dichotomous variables (e.g. affected hemisphere or sex) were not included in the DA. In order to reduce the number of potential discriminant factors, the DA was conducted using a backward stepwise procedure designed for the small sample size (Bortz, 2005). Maximum significance (p-value) for the F-ratio of a factor to enter was set at 0.05, and minimum significance (p-value) for the F-ratio of a factor to remove was set at 0.10.

3.4.2.2 Results

Six out of eleven patients were defined as responders, and five out of eleven as non-responders. The stepwise DA revealed that the initial motor function of the fingers measured with FMA was the best factor in classifying responders and non-responders. Additionally, the MI-PC before MT application was the only factor that could explain further variance on motor improvement significantly. Thereby, patients with a positive MI-PC were rather classified as responders (three out of six) compared with patients with a negative MI-PC, who were rather classified as non-responders (five out of five). Three out of the eleven patients of the sample

did not fit in this pattern: They had a negative MI-PC and were nevertheless classified as responders. As expected, MI-M1 had no predictive value on motor recovery. Figure 14 illustrates the range, median and interquartile range (IQR) of all factors included in the DA for responders and non-responders, respectively. The less the boxes overlap, the more the corresponding factor differs between responders and non-responders.

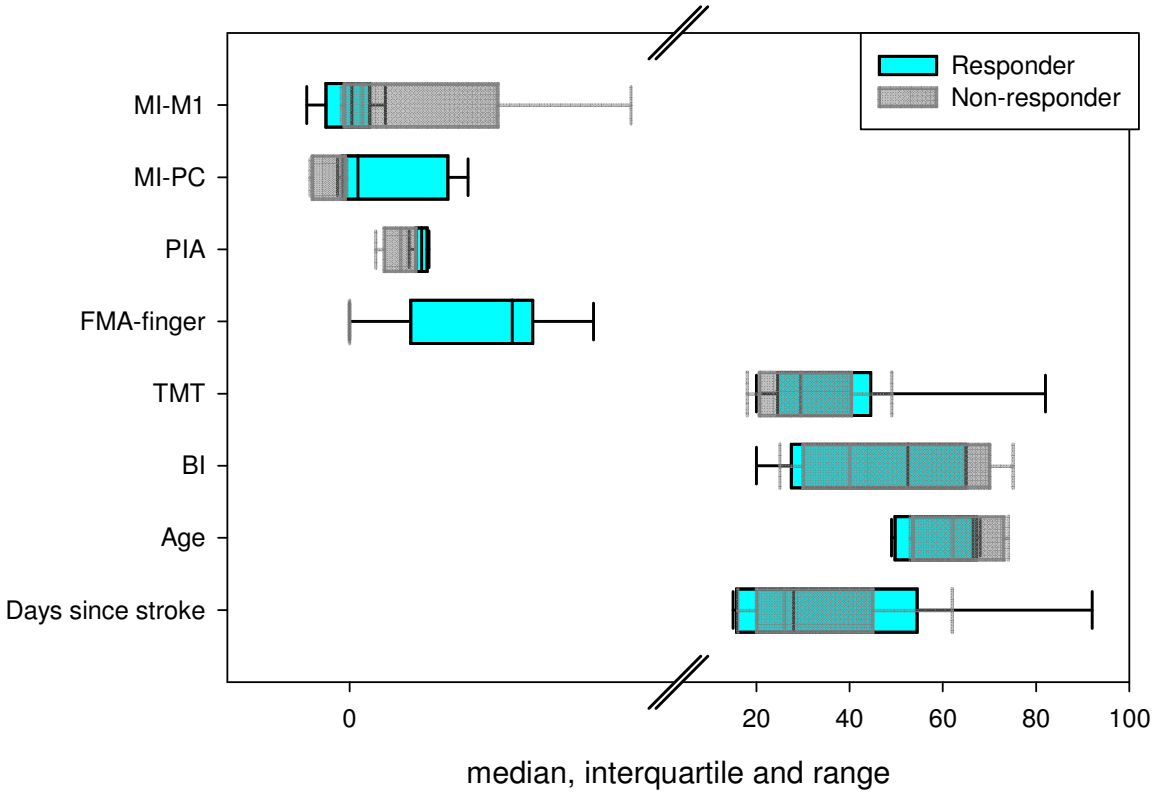

Figure 14: Box-plots with median values, IQR and range of following factors: days since stroke, age, BI, TMT, FMA-finger, PIA, MI-PC, MI-M1; for responders (cyan) and non-responders (grey). Note: For FMA-finger for non-responders there is no box, as they had all 0 points. **Abbreviations**: BI: Barthel Index; TMT: trail making test; FMA: Fugl-Meyer assessment; PIA: percentage of ideal alertness; MI: mirror index; PC: precuneus; M1: primary motor cortex.

3.4.2.3 Conclusions

Patients with an inversion of the interhemispheric activation imbalance in PC due to mirror stimulation seem to be more likely to benefit from MT and be classified as responders. In

other words, patients with a functional brain activation pattern on PC-ROI similar to that of the healthy subjects' sample of Publication 3 are rather classified as responders in contrast to patients without such an activation pattern. Hence, this activation pattern due to mirror stimulation in PC might be the neurophysiological basis and a candidate for determination of the efficacy of MT. Meanwhile, an inversion of the interhemispheric activation imbalance in M1 due to mirror stimulation seems not to be a determinant for MT success. Therefore, an immediate modulation of M1 might be excluded as the underlying neurophysiological mechanism of MT. It could be hypothesized that M1 is modulated by MT only on the long run via PC.

4 Discussion

The study at hand aimed to examine cerebral activation during the mirror illusion, as basis of MT in healthy subjects as well as in stroke patients. It was hypothesized that the MNS and M1 might not be directly modulated by the mirror illusion, in contrast to PC and S1 and that VT and MT elicit different activation patterns. Beyond that, an exploratory examination was conducted to determine if the lateralized precuneal activation observed in normal subjects might be likewise observable in stroke patients or if it differs on a single-subject level. Finally, on the basis of the fNIRS results, it was hypothesized that the lateralized precuneal activation due to the mirror illusion might be one potential determinant between MT responders and non-responders.

The thesis at hand reveals different activation patterns underlying MT and VT. While MT elicits lateralized precuneal activation always contralateral to the observed hand, this was not found during VT. Furthermore, it is confirmed that the mirror illusion does not modulate the MNS and M1 directly, in contrast to PC and S1: While lateralization of S1 and PC changes due to mirror illusion, M1 remains lateralized contralateral to the moving hand. Finally, lateralized precuneal activation due to mirror illusion is similarly observed in stroke patients and seems to be a potential candidate for prediction of MT efficacy additionally to motor functional base level.

4.1 Effect of mirror illusion - healthy subjects and group analyses

4.1.1 Effect of mirror illusion - the role of PC

In three publications and one manuscript of the thesis at hand PC plays a decisive role during mirror illusion. In healthy subjects PC was shown to be activated always contralateral to the perceived hand, with the mirror illusion inverting the interhemispheric activation imbalance in PC. This was similar for stroke patients, whereat its predictive value for motor recovery additionally to the base level motor function was demonstrated. Thus, the important role of PC in facilitating the functional motor recovery of patients with severe hemiparesis after stroke seems to be confirmed.

PC activation due to mirror illusion was also found in other studies based on fMRI with normal subjects (C. Dohle et al., 2004) and with chronic stroke patients conducting a bimanual task (Michielsen, Smits, et al., 2011). Furthermore, a PET study confirms PC as the neuronal correlate for limb transformation during mirror illusion, in contrast to coordination transformation that activated the insular cortex. It is suggested that PC might be the substrate of representation of a left or right arm, lateralized to the hemisphere contralateral to the perceived limb (C. Dohle et al., 2011).

This is similarly supported by studies examining PC's function independently of mirror illusion and MT. These studies demonstrate that neurons situated in the PC-ROI of the thesis at hand are modulated by the orientation of the hand, more precisely by changes of wrist and reach orientation. They corroborate the crucial role of PC during the on-line control of hand movements on a higher-order representational level and its crucial role in processing of upper limb configurations during movement execution (Fattori et al., 2009; Pitzalis et al., 2013). Furthermore, PC seems to have a small overlapping part with the posterior part of the original PRR of monkeys (Connolly et al., 2003; Pitzalis et al., 2013).

In general, it is well known in neuroscience that one body side is controlled and represented by the contralateral hemisphere (Kolb & Whishaw, 1996b). The inversion of the interhemispheric imbalance in PC by the mirror illusion - as demonstrated by the thesis at hand - is not in conflict with this basic concept, as the lateralized activation in PC was always contralateral to the *perceived* limb. When e.g. the right hand was moved under MIR condition, so that it seemed to be the left hand, then an activation increase in the right PC was observed, i.e. contralateral to the *perceived hand*. Hence, the visual perception of the own hand seems to be so strong, to activate correspondent limb specific representational areas in the cortex (Filimon et al., 2007; Hamzei et al., 2002; Pitzalis et al., 2013), even when perceiving it through the mirror (Michielsen, Smits, et al., 2011).

This interpretation is corroborated by several studies examining the area of PC. Yittri and colleagues (2013) demonstrated that neurons of the PRR are limb specific and respond to both ipsi- and contralateral visual fields. Reversibly inactivation of PRR in monkeys slowed reaction time of contralateral reaches only, but not specifically for reaches into either visual field, thus demonstrating that PRR is specific to contralateral limb movements and not to contralateral visual field stimulation (Yttri, Wang, Liu, & Snyder, 2014). Furthermore, Galletti and colleagues (1999) found that neurons of V6A in the macaque monkey represent both contra- and ipsilateral visual fields. V6A seems not to be retinotopically organized (Galletti, Fattori, Kutz, & Gamberini, 1999). These studies demonstrate that PC is limb specific and not modulated by mere visual field stimulation. Thus, the inversion of lateralization of PC activation due to mirror illusion similarly found in fMRI *and* fNIRS studies of the thesis at hand seems not to be caused by hemifield stimulation.

Altogether, it can be concluded that PC is the limb specific cortical representational area of visual and proprioceptive features of the contralateral upper limb, which is activated also during the mirror illusion.

4.1.2 Effect of mirror illusion - the role of M1

In contrast to PC, the inversion of lateralization due to the mirror illusion was not found for M1 where activation was always contralateral to the moved limb, independent from the perceived limb. Nearly all fMRI studies on the mirror illusion show no instant changes of lateralization in M1, including the studies of the thesis at hand (C. Dohle et al., 2004; Matthys et al., 2009; Michielsen, Smits, et al., 2011). Slightly larger activation for the mirror illusion was found only in Publication 2. However, this did not influence the degree of lateralization. Furthermore, this effect was not significant. In contrast, one fMRI study (Diers et al., 2010) and one Magnetoencephalography (MEG) study (Tominaga et al., 2009) found additional ipsilateral M1 activation due to the mirror illusion. Also some TMS studies claim that M1 excitability contralateral to the observed limb changes during mirror illusion (Fukumura et al., 2007; Funase et al., 2007; Garry et al., 2005). However, the heterogeneous results about M1 modulation due to mirror illusion might be attributed to different study designs: Additional activation on M1 was only found when the mirror condition provided the visual image of two synchronously moving hands, e.g. when using a real mirror to create the mirror illusion (Kang et al., 2011; Kang et al., 2012; Saleh, Adamovich, & Tunik, 2013), or when the mirror condition was compared to a neutral stimulus (Fukumura et al., 2007; Funase et al., 2007; Garry et al., 2005), but not when the mirror condition was contrasted with the normal condition (C. Dohle et al., 2004; C. Dohle et al., 2011; Matthys et al., 2009; Michielsen, Smits, et al., 2011). This is supported from an EEG study that refutes a reversal of activation lateralization on M1 due to the mirror illusion (Praamstra et al., 2011).

Altogether, the well-established paradigm in neuroscience that M1 activation is mainly lateralized contralateral to the moving limb during active movements (Pinel, 2001a) is not challenged by the thesis at hand that examined M1 activation due to mirror illusion with diverse methodical approaches, including whole-brain examinations (Publication 1 and 2) as well as analyses on a predefined M1-ROI (Publication 3), one-step (Publication 1) as well as two-step analyses (Publication 2).

On the other hand, clinical studies found changes in M1 activation, following a period of extensive MT training in stroke patients (Michielsen, Selles, et al., 2011) as well as in patients with phantom limb pain syndrome (Giraux & Sirigu, 2003). This was similarly found for healthy subjects: Training of the motor performance via mirrored visual feedback enhanced motor ability of the hand behind the mirror which was correlated to enhanced neuroplasticity in the correspondent motor cortex of the “ipsilesional” hemisphere (Hamzei et al., 2012; Nojima et al., 2012). MT modulates neuroplasticity in the ipsilateral, i.e. ipsilesional M1, but only due to extensive training and not as an immediate, direct response to the mirror illusion (Hamzei et al., 2012; Lappchen et al., 2012). This is supported by the clinical findings about MT that show its beneficial effect for motor recovery only when applied over a rather long time of several weeks (Thieme et al., 2012). This seems to be different for the effect of MT for rehabilitation of sensory deficits, where effects are observed on a much shorter time range (G. Moseley & Wiech, 2009; Ramachandran, Rogers-Ramachandran, & Cobb, 1995).

4.1.3 Effect of mirror illusion - the role of S1

Improvement of sensory function following MT has been reported in clinical studies (Acerra, 2007; C. Dohle, Püllen, et al., 2009; Doyle, Bennett, Fasoli, & McKenna, 2010; G. Moseley & Wiech, 2009), and even in chronic stroke patients (Bowering et al., 2013; Wu, Huang, Chen, Lin, & Yang, 2013). In contrast to motor improvements, significant and lasting improvements of tactile discrimination were already reported after a single session of MT (Acerra, 2007; G. Moseley & Wiech, 2009; G. L. Moseley, 2004). This clinical data support the idea that motor and sensory recoveries following MT are based on different cortical mechanisms. This hypothesis is supported by the thesis at hand, as different activation patterns for M1 and S1 were found due to the mirror illusion, at least for the left hand.

The change of activation lateralization on S1 in the thesis at hand demonstrates that also visuo-motor performance, such as during mirror illusion is able to modulate S1, similar to the

well-known modulation effects elicited by attentional mechanisms. Johansen-Berg and colleagues (2000) demonstrated that directing attention to a touch stimulus increases activation in somatosensory cortex areas (Johansen-Berg, Christensen, Woolrich, & Matthews, 2000). Former studies had shown this similarly (Burton et al., 1999; Mima, Nagamine, Nakamura, & Shibasaki, 1998). Other studies showed even a cross-modal modulation of S1 as e.g. by the visual image of a touched body part of a third person (Schaefer, Heinze, & Rotte, 2005a), or by demonstrating an enhanced sensory threshold due to observation of a hand being touched, but not after observing a video of a hand without being touched. The enhancement was specific to the finger being touched, even several minutes after the visual stimulation (Schaefer, Heinze, & Rotte, 2005b). Beyond that, there are several other studies that demonstrate that vision of the body or body parts, such as the hand, modulates S1 activation (Sambo, Gillmeister, & Forster, 2009), even if this input is not related to the tactile stimulation (Longo, Pernigo, & Haggard, 2011). The fact that S1 and S2 activations were found to be modulated by observation of movements lead some researchers to the conclusion that mirror neurons might be situated also in the somatosensory cortex (Avikainen, Forss, & Hari, 2002; Rossi et al., 2002). It could be speculated, if these effects of visual stimulation modulating S1 activation could be due to reciprocal axonal connections of S1 regions with higher-order visual areas such as BA 5 and 7 as depicted in Figure 3 (Kolb & Whishaw, 1996c).

In spite of these findings, there is little knowledge about the concrete effects of the mirror illusion on somatosensory areas. To the best knowledge of the author, Diers and Wasaka were the only ones that directly analyzed S1 activity during visually guided hand movements under mirrored conditions. Diers and colleagues claim to have found additional activation in M1 and S1, contralateral to the limb seen in the mirror, when comparing the mirror condition with the normal visual feedback condition (Diers et al., 2010). However, as already illustrated in the Introduction of the thesis at hand, this study bears some methodical problems: the conditions mirror and normal visual feedback were not directly contrasted with each other. Furthermore, by application of a real mirror in the scanner to create the mirror illusion the

visual input of two hands is provided and compared with the visual input of one hand in the normal condition, which degrades comparability of the two conditions. In the second study, Wasaka and colleagues found higher activation in the contralateral S2 due to the mirror, but not in S1 (Wasaka & Kakigi, 2012). However, this additional activation in S2 was only found during asymmetric movements, which does not correspond to MT, where symmetric movements are required (Bieniok et al., 2009). S2 is known to be activated by unexpected visual input and to be stronger modulated by attentional effort compared to S1 (Kida et al., 2007). Thus, the S2 modulation observed during the asymmetric task by Wasaka and colleagues might be due to increased attentional demands for the somatosensory information caused by the unexpected visual input (Wasaka & Kakigi, 2012) and less by the mirror illusion per se. However, final conclusions about the different effects on S1 and S2 due to the mirror illusion require further research.

Altogether, these publications are in line with the findings of the thesis at hand, demonstrating that somatosensory cortex is highly dynamical and easily modulated by attentional and visual processing. Furthermore, somatosensory cortex is involved in motor learning and motor control. These characteristics can be utilized for neurological rehabilitation and are probably part of the underlying neuronal mechanisms of MT. The results on PC and S1 of the thesis at hand together with the here mentioned studies suggest a somehow cross-modal interactional link between PC and S1 during MT.

4.1.4 Effect of mirror illusion - the role of the MNS and other structures

As an involvement of the MNS during MT and VT was suggested a number of times (Rizzolatti et al., 2009; Yavuzer et al., 2008), it seemed necessary to address this question in the thesis at hand and to compare MT and VT directly. The results of the thesis at hand exclude the involvement of the MNS in mirror illusion processing, as the original structures of the MNS (VPC, IPL and pars opercularis) (Rizzolatti et al., 2009) were not found to be

modulated by the mirror illusion. This conclusion cannot be drawn for VT. No differences between the observation of a third person's left and right hand were found in Publication 1. Probably, the mere observation of a third person's left or a right hand might both elicited bilateral activations of the MNS. This is in line with former examinations of movement observation that found bilateral activations of the MNS (G. Buccino et al., 2001; G. Buccino et al., 2004). This might generate the idea, that the MNS could similarly be bilaterally active during visually guided own hand movements under normal as well as under mirrored condition. In fact, this cannot entirely be excluded by the thesis at hand. However, the interesting point is to find out what is additionally or differently activated by the mirror illusion compared with normal visual feedback, and not, what these conditions have in common. Furthermore, there is an amount of evidence that visual control of own movements is directed by posterior parietal areas, such as SPL or PC, in contrast to internally generated movements (Battaglia-Mayer et al., 2000; Cavina-Pratesi, Connolly, & Milner, 2013; Hamzei et al., 2002).

The thesis at hand demonstrates that VT and MT are based on different cortical activation patterns. It is well established by now, that VT is based on activation of the MNS (G Buccino, Solodkin, & Small, 2006; Ertelt et al., 2007; Nedelko et al., 2010). In contrast, there is barely any evidence for an involvement of the MNS in MT (Deconinck et al., 2014; Matthys et al., 2009; Michielsen, Smits, et al., 2011).

Beyond that, the social component of the MNS for facilitation of social interaction (Iacoboni & Mazziotta, 2007), is in line with the here suggested exclusion of the MNS in MT. MT does not demand any kind of social interaction or perception, as only own movements are visually perceived, never movements of another person. Furthermore, Rizzolatti emphasized the importance to differentiate between *movements* without any purpose and an *action* that is a movement to achieve something. The MNS is involved only in the observation of action, representing also the goal of an action (Rizzolatti, 2014, March). In contrast, during MT and in all studies of the thesis at hand examining the mirror illusion, abstract movements without any purpose are conducted (Deconinck et al., 2014; C. Dohle et al., 2011).

However, Filimon and colleagues (2007) claim PC to be part of the MNS for reaching, as they found an overlapping activated brain region during execution, observation and imagery of right arm reaching movements that corresponds to the PC-ROI of the thesis at hand. Activation was reported to be stronger in the contralateral (left) hemisphere, however, other than in the thesis at hand, no comparison between left and right hand was done and lateralization was not examined in detail (Filimon et al., 2007). On the other hand, in a review on 200 fMRI studies, PC could not be related neither to the MNS, nor to the metalizing system[3] (Van Overwalle & Baetens, 2009).

Beyond that, Stevens and Stoykov (2003, 2004) subsume MT to rehabilitation strategies using motor imagery claiming that MT might be the same as "visually guided motor imagery" (Stevens & Stoykov, 2003, 2004). Several studies have shown that motor imagery and motor execution, in contrast to the mirror illusion, activate similar neuronal networks, such as SMA, dorsal premotor cortex, supramarginal gyrus and dorsolateral frontal gyrus (Grèzes & Decety, 2001; Porro et al., 1996), or bilateral M1 (Porro, Cettolo, Francescato, & Baraldi, 2000). Additionally, real visual input is given only in MT, while input during imagery is only generated "internally". Thus, on this basis, MT cannot be simply subsumed as a kind of motor imagery.

To conclude, the results of the thesis at hand strongly support the hypothesis that the original MNS, consisting of VPC, IPL and pars opercularis, is not saliently involved in the underlying mechanism of MT. Meanwhile activation of the MNS seems to be a crucial neuronal mechanism for the effect of VT, where actions of a third person are observed (Ertelt et al., 2007). Beyond this, the present thesis demonstrates that different neuronal mechanisms are involved in MT and VT. It is crucial to acknowledge that MT is a fundamentally different therapy procedure compared to VT, learning through imitation or mental imagery, not only in terms of conceptualization, but also with regard to the underlying neuronal mechanisms. With

[3] "[…] when people try to infer intentionality of others in the absence of detailed information on biological motions of social actors, that is, when it receives input on motions of geometric shapes or on more abstract behaviors, beliefs or morality of others (most often via verbal stories)." (Van Overwalle & Baetens, 2009)

regard to the current state of research, MT should not be subsumed to therapies involving the MNS or motor imagery anymore.

4.1.5 Conclusions about healthy subjects (group-analysis level)

In conclusion, the inversion of visual feedback by a mirror during visually guided hand movements activates PC contralateral to the perceived limb just as under normal visual feedback, in spite of the mismatch under mirrored condition between perceived and actually moved limb. This strongly indicates that the mirror illusion is not an isolated phenomenon. However, inversion of the visual feedback seems to be integrated into visuo-motor behavior in the same way as processing of regular non mirrored movements, probably activating higher-order body representations of the visually perceived hand (Hamzei et al., 2002; Pitzalis et al., 2013). Beyond that, also lateralization and activation strength of S1 is significantly modulated by the mirror illusion, although not in the same way as PC. Lateralized precuneal and somatosensory activation is elicited solely by the visual presentation of a right or left hand, independent of the hand that is moved actually. Probably, visually perceiving the own limb automatically triggers corresponding limb specific mental representations (C. Dohle et al., 2011), due to lifelong experience of a left or right limb perception, regardless of the emerged mismatch during the mirror illusion.

Meanwhile, a dissociated activity pattern was found for M1, where activation was only modulated by the laterality of the hand that was actually moved, independent from the visually perceived hand.

In summary, these results show not only dissociated lateralization patterns on different ROIs: Activity in M1 is lateralized opposite to the *moving* hand and in PC opposite to the *visually perceived* hand, independent of each other. These results also demonstrate that M1 is not easily modulated by visual stimulation, while other areas such as PC and S1 are more easily modulated by visual stimulation, adhering more dynamical capabilities that can be utilized for

neurological rehabilitation. Thus, the underlying neuronal mechanism for motor recovery after MT seems not to be a direct one, as the motor cortex was not directly modulated by the mirror illusion. The underlying neuronal mechanism for MT might rather be indirect, activating neuronal pathways and leading to enhanced neuroplasticity in the motor cortex network through PC and S1.

4.2 Effect of mirror therapy - single patients and determinants

The results of both patients studies (Publication 4 and Manuscript 5) support the results found on healthy controls: the lateralized precuneal activity due to the mirror illusion was also observable in the majority of the stroke patients (four out of five) and did not substantially differ from normal subjects regarding the strength of activation, although the controls were not age-matched. Thus, despite their severe hemiparesis and despite their advanced age, the lateralized precuneal activity due to the mirror illusion could also be elicited. Beyond that, in a prospective clinical pilot study (Manuscript 5) the contribution of the initial lateralized PC activation due to the mirror illusion for the beneficial effect of MT was assessed among other factors known to affect the motor recovery after stroke, such as initial motor function, lesioned hemisphere, demographic data and neuropsychological status. As hypothesized, patients who show an inversed interhemispheric activation imbalance in PC due to the mirror illusion - comparable to the healthy controls - will more likely benefit from MT than patients who do not show such an inversion. This was not observed for inversed interhemispheric activation imbalance in M1 due to the mirror illusion. Hence, the patients' results confirm the healthy controls' results and support the hypothesis that inversed interhemispheric activation imbalance on PC, and not on M1, due to the mirror illusion might be the neurophysiological basis of the motor recovery after MT.

4.2.1 The role of motor function base level for benefit from MT

Already in the first ever application of the mirror illusion for the therapeutic purpose of phantom limb pain relief not all patients took benefit. One out of the nine examined patients did not "feel" the illusion, experiencing no pain relief (Ramachandran et al., 1995). Respectively, Dohle and colleagues found high inter-variability between patients who profit

from MT and those who did not (C. Dohle, Püllen, et al., 2009). Thus, the thesis at hand aimed to find determinants for the efficacy of MT. It was found that inversion of the interhemispheric imbalance on PC makes a difference between MT responders and non-responders. The strongest determinant to classify responders and non-responders however, was the finger motor base level function. Patients that were classified as responders to MT were the patients with a better initial FMA score. Actually, there was only one patient with an initial FMA score with 0 that was classified as a responder. All five non-responders had an initial FMA score of 0.

This is in line with the literature about motor recovery after stroke. A review on 56 studies demonstrates that initial voluntary motor behavior (apart from integrity of corticospinal tract) is the best predictor for motor function improvement in hemiparetic stroke patients (Chen & Winstein, 2009). This is confirmed by a study on 102 first ever unilateral stroke in-patients that additionally comes to the conclusion that prediction about motor function outcome of the upper limb can best be made within four weeks after stroke (Kwakkel et al., 2003). This might partly be caused by larger lesions in the motoric network that cause vaster functional losses and might further accelerate neuronal degeneration processes through transneuronal degeneration (Kolb & Whishaw, 1996a).

On the other hand, these findings seem to be somewhat contradictory to the findings of Dohle and colleagues (2009) who found a significant advantage between MT and control therapy only for a subgroup of plegic patients, i.e. MT had a beneficial effect only for patients with an initial FMA score of 0 points.

However, Manuscript 5 is not really comparable with the study of Dohle and colleagues (2009), as it was a controlled randomized trial, whereas Manuscript 5 was a prospective clinical trial without control group, designed to find determinants of MT responders. Furthermore, Dohle and colleagues sample size of 25 patients with an initial FMA score of 0 points was much larger; meanwhile in Manuscript 5 only 6 patients had an initial FMA score

of 0 points. Beyond that, an FMA score of 1 to 3 points in the subscore finger still represents a very low motor functional level (Arya, Verma, & Garg, 2011).

In addition, the therapy protocol used in Manuscript 5 was a “Berlin variant” of “Bonn therapy protocol” that was used in Dohle and colleagues’ study, requiring different abstract limb positions in response to verbal commands (Bieniok et al., 2009). While the Bonn therapy protocol was mainly based on proximal movements combined with distal variations, the “Berlin variant” was defined as working from distal to proximal (Morkisch, Lommack, Kadow, Rietz, & Dohle, 2012). Moreover, in Manuscript 5, MT was conducted for 4 weeks, 5 days per week, for 30 minutes daily, while in Dohle’s study MT was conducted for 6 weeks in the same frequency.

Finally, the two studies agree regarding the inclusion criteria (severe unilateral arm paresis with wrist extension ability < 20° and MCP joint extension ability < 10°). Thus, patients with an initial FMA finger subscore of 0 to 3 points still fitted in this criterion. The important point is, that this inclusion criterion is at the same time the exclusion criteria for CIMT that other than MT requires this minimum of motor function in the affected limb (Uswatte & Taub, 2013) and separates patients with indication for CIMT from patients with indication for MT.

Nevertheless, despite these illustrated differences and commonalities there is no satisfying explanation for this contradictory conclusions between Manuscript 5 and the clinical study of Dohle and colleagues (2009).

Concerning FMA in general, it is the gold standard in assessing motor function and thus motor functional rehabilitation after stroke. Gross hand functions, such as grasping tasks are involved, but FMA might not be sensitive enough in detecting improvements of individual fingers. Anyway, in the domain of research FMA is a well validated and reliable tool for assessment of motor functions (Gladstone, Danells, & Black, 2002; Hsieh et al., 2009).

Overall, the finding that patients with better base level motor function will probably benefit more from a therapy intervention such as MT is not surprising as it is in line with recent studies about motor recovery after stroke in general.

4.2.2 The role of inversion of interhemispheric imbalance for benefit from MT

It is well known by now that the unaffected hemisphere could further inhibit the affected hemisphere via transcallosal inhibition mechanisms, thus deteriorating motor performance after stroke (Calautti et al., 2007) and that inversion of this interhemispheric activation imbalance in favor of the affected hemisphere could facilitate motor recovery (Grefkes et al., 2010). The inversion of the interhemispheric imbalance in favor of the affected hemisphere could be mediated by PC, as this was observed in healthy controls during the mirror illusion by the thesis at hand. Additionally, this inversion was shown to be one potential candidate for prediction of the efficacy of MT in stroke patients (Manuscript 5) additionally to the initial motor function. In other words, if the mirror illusion leads to an inversion of the interhemispheric activation imbalance towards the affected hemisphere in PC, then patients do benefit from MT. That means that an activation pattern in response to the mirror illusion measured before MT application more comparable to the one of healthy subjects determinates MT efficacy. An inversion of the interhemispheric activation imbalance on M1 however, did not predict motor recovery. Hence, the hypothesis of the thesis at hand about an indirect underlying neuronal mechanism of MT leading to a long-term enhancement of neuroplasticity in the motor cortex network through PC, seems to be further confirmed.

4.2.3 Precuneus and S1 and their role for motor recovery in mirror therapy

For all that, PC' function is not primary motoric, so the question remains why PC activation imbalance shift should play a crucial role for motor recovery of hemiparesis after stroke? This could possibly be explained by intra-hemispheric reciprocal connections of PC with the SMA and with the dorsal premotor area (Cavanna & Trimble, 2006). Furthermore, fMRI studies found also V6A, a subarea of PC, to be directly connected with the dorsal premotor cortex (Galletti et al., 2001; Marconi et al., 2001). Thus, activation increase of ipsilesional PC and activation decrease of contralesional PC during MT could stimulate the lesioned parts of the

motor cortex via an parieto-frontal network, respectively (Hamzei et al., 2002), consequently boosting neuroplasticity for motor functional recovery. Indeed, this was similarly shown recently by an fMRI study investigating brain connectivity with dynamic causal modelling (DCM) on stroke patients during the mirror illusion. Increased functional, facilitatory interactions between ipsi- and contralesional posterior parietal cortex with the ipsilesional M1 were found due to mirror illusion (Saleh et al., 2013). Beyond that, increased interconnections between both premotor regions and the ipsilateral SMA were found by functional connectivity analysis. In turn, SMA was found to develop increased interconnectivity with the ipsilateral sensorimotor cortex due to MT training of healthy subjects (Hamzei et al., 2012).

Hence, PC seems to be an intermediary cortical structure that receives and processes the visual feedback of the hand in the mirror and transfers it to the ipsilesional premotor cortex and SMA as an underlying mechanism for motor recovery due to MT. This is in line with the function of the posterior parietal and posterior medial cortex that is known to interconnect (Figure 3) the visual and the motor system (Cauda et al., 2010; Pinel, 2001a). Indeed, PC is part of a crucial node of the fronto-parietal network for planning and execution of visuo-motor tasks (Archambault, Ferrari-Toniolo, Caminiti, & Battaglia-Mayer, 2014).

Although S1 activation was not explicitly examined in the patients' studies of the thesis at hand, S1 was shown to be modulated during left hand movements by the mirror illusion in the healthy controls. Hence, S1 could also play a crucial role in MT. In all probability, S1 has an important role in motor recovery in general and is involved during motoric learning in healthy subjects. An fMRI study on the functional connectivity during motor-skill learning demonstrated the involvement of S1 in learning of novel motor tasks. During the acquisition of novel motor tasks, higher intra- and interhemispheric coupling was found between S1 and M1, premotor cortex and SMA in contrast to already learned motor tasks (Sun, Miller, Rao, & D'Esposito, 2007).

Furthermore, examinations demonstrated that S1 has descending pyramidal tract neurons for motor control additionally to the ascending ones (Fromm & Evarts, 1982; Kolb &

Whishaw, 1996c). Beyond that, several studies demonstrate strong interconnections between M1 and S1 (Jones, Coulter, & Hendry, 1978) that might also be effective during motor recovery.

The crucial role of S1 for motor control and recovery after stroke is also emphasized by the fact that additional severe sensory loss predicts poor motor recovery after stroke. Additionally, sensory loss can also even result in motor impairments similar to hemiparesis (Nelles et al., 1999).

However, the fact that the lateralization shift was observed only for the left hand in S1 leaves room for speculations. One possibility might be that the neuronal correlate for movements of the dominant hand is more stable irrespective of additional stimulation. However, there is barely any literature about this subject as only very few studies have analyzed the somatosensory cortex and its lateralization (Aziz-Zadeh, Maeda, Zaidel, Mazziotta, & Iacoboni, 2002; Eickhoff, Grefkes, Fink, & Zilles, 2008; Ferretti et al., 2004; Jung, Baumgartner, Magerl, & Treede, 2008).

Altogether, this emphasizes the crucial role of PC activation during MT for MT efficacy. MT seems to provide an interhemispheric balance in favor for the affected hemisphere via lateralization inversion in PC, thereby fostering motor recovery. Meanwhile, S1 modulation during MT could stimulate M1 activation. Finally, the activation pattern on PC seems to be stable over time, MI-PC did not change by MT. Thus, the cortical response to the mirror illusion seems to be a personal invariant characteristic, which is not affected by application of MT. It remains speculative whether this personal characteristic was already present before stroke or if it was caused by the lesion. The fact that only the responder group's MI was comparable to that of normal subjects, however, might suggest that the stroke lesion caused a change in the responsiveness to the mirror illusion. Further studies are necessary to verify this hypothesis.

4.2.4 Comparison of the results of Publication 3 and Manuscript 5

Concerning the fNIRS measurements, in Publication 3 (healthy subjects) and Manuscript 5 (stroke patients) the same setup was applied on the same ROIs, except some minor changes in order to make the procedure more convenient for patients (overall fewer trials and optodes, no static trials and a break separating the measurement in two blocks), which was not expected to influence the results considerably.

The group analysis on the M1-ROI in both studies revealed a modulation by the laterality of the hand that is actually moved, with higher activations in the contralateral hemisphere, as it was to be expected. Furthermore, neither a direct influence of the mirrored visual feedback, nor a shift in activation lateralization due to the mirror was observed in both studies on M1. Hence, concerning the role of M1 the two studies come to the same conclusion: MT does not influence M1 directly.

In contrast to the results on the M1-ROI, the group analysis on the PC-ROI in both studies revealed different group effects: while in Publication 3 an interaction between hemisphere and mirror was found, no such interaction was found on a group level in Manuscript 5. On the one hand this could be explained by the smaller patients' sample size (Manuscript 5: n=11; Publication 3: n=20). On the other hand, this could also be explained by inter individual differences. Comparing the values of MI-PC, it becomes clear that the patients' distribution offers a higher variability than the normal subjects' distribution. This is in line with recent studies that found higher inter-variability among stroke patients concerning their BOLD signal which might be caused by pathologic alterations of the BOLD signal (Krainik, Hund-Georgiadis, Zysset, & von Cramon, 2005). Furthermore, patients' MI-PC distribution differs clearly between responders and non-responders. All non-responders had a negative MI-PC, while 50% of responders had a positive MI-PC, which is more comparable to the normal subjects' sample. Also among the normal subjects 50 % had a positive MI-PC.

4.2.5 Specific fMRI-fNIRS comparison and results of Publication 4 and Manuscript 5

Cerebral activation in the thesis at hand was measured with fMRI as well as with fNIRS. Methods and results of these two measurement techniques are compared in this chapter, especially with regard to the results of Publication 4 and Manuscript 5. The five patients of the fMRI study of Publication 4 also participated in the study of Manuscript 5, so that their results can be directly compared. As already described above in the methods' section, for the fMRI data the T-contrasts MIR greater NOR for the ipsilesional and NOR greater MIR for the contralesional hemisphere were calculated. Meanwhile, MI-PC unifies an ipsilateral activation increase with a contralateral activation decrease, for the HBO beta-values of fNIRS. Positive values correspond to an activation pattern of the healthy controls, respectively. The clinical outcome is also considered. In the following table, a detailed overview of fMRI, fNIRS and FMA results of these five patients is given:

Patient	MIR>NOR (fMRI)	NOR>MIR (fMRI)	MI-PC (fNIRS)	Responder? ∆ FMA
1	5.01	5.31	-0.43	Non-Res (0)
2	7.59	n.s.	0.22	Res (+4)
3	8.74	8.42	1.13	Res (+6)
4	n.s.	n.s.	1.46	Res (+1)
5	4.28	n.s.	-0.03	Non-Res (0)

Table 1: FMRI and fNIRS results of the five patients that participated in both studies (Publication 4 and Manuscript 5) and improvement of the FMA subtest finger (n.s.=not significant; Res=responder; Non-Res=non-responder).

Not for all five patients the results of fMRI correspond to fNIRS and to the clinical outcome. For the Patients 2 and 3, the significant MIR greater NOR contrast in the ipsilesional hemisphere is in line with a positive MI-PC and simultaneously with the motor functional improvement measured with FMA, classifying these patients as responders. The other three patients must be considered more in detail. For the Patients 1, 4 and 5 fNIRS and fMRI

results do not correspond. Comparing the Patients 1 and 4, fNIRS and fMRI results do not correspond at all. While the fMRI results in both hemispheres correspond to the activation pattern of healthy controls, the MI-PC (fNIRS) has a negative value for Patient 1. The opposite is true for Patient 4 where fMRI could not detect an activation difference, while the MI-PC (fNIRS) has a strong positive value. Evaluation for Patient 5 is less clear. On the one hand, the MI-PC is slightly negative, which corresponds to the contrast NOR greater MIR in the contralateral hemisphere that became not significant. On the other hand, the contrast MIR greater NOR was significant. This indicates that the activation decrease in the contralesional hemisphere was probably not strong enough, so that the MI-PC became negative.

Probably, differences in specific analysis approaches could explain for the different results in two out of the five patients. While the fNIRS analysis was done for a pre-defined PC-ROI, the MNI coordinates were variable in Publication 4, where a peak analysis was conducted, leading to individually different locations in the broader area of PC. Furthermore, fNIRS is known to measure only cortical structures on superficial layers of the brain as its depth penetration is strongly limited (Obrig, 2014), so that the PC-ROI referred to in the fNIRS measurement rather corresponds to an overlapping region between the superior parts of PC, the medial part of SPL and parts of the anterior medial parieto-occipital cortex (Figure 5). Meanwhile, fMRI allows to measure activity also in the parts of PC lying deeper in the medial longitudinal fissure (Figure 2). Due to this higher “flexibility” one could assume the fMRI to be more sensitive in detecting the activation differences due to the mirror illusion as compared with fNIRS. This could be an explanation for Patient 1, but not for Patient 4, for whom fNIRS measurement revealed an inversion of the inter-hemispheric balance while no activation differences were detected by fMRI.

Beyond that, MI-PC is calculated for HBO, while fMRI corresponds rather to HBR of fNIRS (Steinbrink et al., 2006). The inversion of lateralization in PC activity in the healthy controls (Publication 3) became significant only for HBO; although the results for HBR were similar, they failed to reach significance. This could be explained by the higher amplitudes that are

usually observed in HBO compared with HBR leading rather to significant results, but worsening at the same time the signal-to-noise ratio (Obrig & Villringer, 2003). This in turn, could be an explanation for the different results for Patient 4, who showed a significant inversion of the inter-hemispheric balance for the fNIRS measurement only, but no significant activation differences in the fMRI.

In comparing the imaging data with the clinical outcome, MI-PC measured with fNIRS seems to be more appropriate to predict clinical outcome of MT than fMRI measurement, at least for these five patients. For all of the five patients a positive value of MI-PC is accompanied with motor functional improvements, meanwhile a negative value of MI-PC is accompanied with no motor functional improvements (Table 1). However, Patients 1 and 5 could be patients on the edge. Perhaps, these patients would have been responders after two more weeks of MT. Whereas, it cannot be excluded that one point of improvement in the FMA of Patient 4 might be due to any measurement error (Table 1), which argues for the predictive power of the fMRI results.

Altogether, based on these five patients, it is difficult to draw final conclusions, as e.g. measurement errors, deviations or coincidence can distort the results considerably as compared with a larger sample size. Comparison of Publication 4 and Manuscript 5 leaves room for further speculations.

4.2.6 Conclusions about stroke patients and single-subject level

In conclusion, the lateralized precuneal activation measured with fMRI is also observable on a single-subject level in healthy subjects and stroke patients. Stroke patients' activation strength in lateralized precuneal activation does not differ from normal subjects substantially, at least in fMRI. The MI-PC measured with fNIRS differs between stroke patients and healthy subjects and correlates with MT outcome in stroke patients. The MI-PC of responders does not substantially differ from healthy subjects, but the MI-PC of non-responders seems to be

different. Hence, responsiveness to the mirror illusion in the rather superficial area of PC that is comparable to normal subjects might be a crucial underlying mechanism for the efficacy of mirror therapy. This should be verified in further randomized controlled studies.

4.3 Limitations

In the studies of the thesis at hand the mirror illusion was implemented by means of a video-setup providing on-line visual feedback of the moving hand that was presented either on the monitor in front (fNIRS) or on goggles (fMRI). This kind of operationalization of the mirror illusion comprises some limitations. First, the direction of gaze in this setup is not the same as in MT with a real mirror. During MT gaze is directed towards the affected limb, meanwhile, in the video-setup it is directed towards the screen in front. This causes also a lack of congruency between visual feedback and proprioception. Thus, one could expect the mirror illusion in the video-setup to be not as strong as in the MT setup.

However, in spite of this incongruity, the subjects in the studies of the present thesis reported to have experienced a mirror illusion. The mirrored visual feedback of a left hand induced the impression of a moving right hand and vice versa. Furthermore, studies about virtual reality examined this incongruity problem by means of questionnaires. They found out, that a video-setup with a frontally direction of gaze elicits a mirror illusion comparable with the mirror illusion elicited by a real mirror. It is concluded that a computer-mediated generation of visual illusions, comparable with the video-setup of the thesis at hand, holds all capabilities of the traditional MT (Regenbrecht et al., 2012). Furthermore, Hoermann and colleagues demonstrated that virtual reality with a frontally displayed limb on the screen allows the users to develop a strong sense of ownership of the displayed limb and referred sensations in the displayed limb similar to those in the optical mirror box (Hoermann, Franz, & Regenbrecht, 2012).

Beyond that, there are good experimental reasons to implement a video-setup instead of a real mirror, because when we see our limb through a sagittal placed mirror as in the setup of the MT, not only the limb per se is mirrored, so that a left arm resembles a right arm and vice versa, but also the space-coordinates are mirrored (moving to the left looks like moving to the right and vice versa) (C. Dohle et al., 2011). The implementation of the video-setup

allows isolated and randomized variation of mirrored and non-mirrored visual feedback of the moving hand. Furthermore, when compared to the utilization of a real mirror in the fMRI scanner, which provides the visual feedback of two hands in the mirror compared with one hand in the normal condition, the video-setup provides real advantages. Despite these experimental advantages of the video-setup, it is suggested to compare activations due to mirror illusion in the video-setup with mirror illusion in the original MT setup in further studies.

Furthermore, it cannot be fully excluded that the lateralized activation found in PC was due to visual hemifield stimulation, as a part of the lower arm was always placed in the peripheral hemifield. Although, hand and fingers, and thus the points of fixation, were always presented centrally, eye movements were not controlled. However, the results argue against this demur. Firstly, V5 was equally lateralized to the right hemisphere for normal and mirrored feedback. If the lateralized precuneal activation would be due to mere hemifield stimulation, then activation in V5 should have been lateralized, too. Secondly, the reverse comparison of normal visual feedback greater mirrored visual feedback generated somehow different activation foci in the hemisphere contralateral to the perceived limb (Figure 8 B) as the comparison mirrored greater normal. And finally, no such lateralization was found in mere observation of a third person's hand, even though also during observation the lower arm was always placed in the peripheral hemifield. Thus, the lateralized precuneal activation is not due to mere hemifield stimulation, but due to the mirror illusion. Beyond that, researchers demonstrated that PC activation is limb specific and represents both contra- and ipsilateral visual fields (Galletti et al., 1999; Yttri et al., 2014).

Another limitation concerning the fNIRS measurements is that the inversion of lateralization in PC activation in the healthy controls became significant only for HBO; although the results for HBR were similar, they failed to reach significance. Higher amplitudes are usually observed in HBO compared with HBR leading rather to significant results, but worsening at the same time the signal-to-noise ratio (Obrig & Villringer, 2003).

Beyond that, MI-PC is calculated for HBO, while fMRI corresponds rather to the HBR value of fNIRS (Steinbrink et al., 2006). Furthermore, fNIRS is known to measure only cortical structures on superficial layers of the brain as its depth penetration is strongly limited (Obrig, 2014), so that the PC-ROI referred to in the fNIRS measurement rather corresponds to a superficial part of PC, meanwhile fMRI allows to measure activity also in the parts of PC lying deeper in the medial longitudinal fissure. Altogether, this makes the fNIRS and fMRI results of the thesis at hand less comparable. Nevertheless, conclusions drawn out of the fMRI and fNIRS studies are similar and not contradictory.

The major limitation of the patients' studies are the small sample sizes (Publication 4: n=5; Manuscript 5: n=11). On this basis they could only be rated as pilot studies. Although statistical analysis was accordingly adapted, using median and range to compare the groups on a descriptive way, or backward stepwise discriminant analysis (Bortz, 2005), the generalizability of the results remains limited. Further studies with higher sample size, preferable in a multi-center study design should be conducted in order to verify the results of the present study and answer further questions.

Another crucial limitation is the difficulty or even impossibility to separate the effect of the conventional rehabilitation procedure from that of MT, as the patients received MT as an adjacent treatment additionally to conventional treatment procedures, according to the German Practice Guideline (Platz & Roschka, 2009). Further studies including a control group are necessary to separate these two effects and their corresponding determinants more precisely. However, responders and non-responders did not differ concerning the number of conventional therapy units they received during MT treatment. Hence, it could be concluded that at least the classification as a responder or a non-responder was not simply due to the conventional therapy. Further studies with a control group are still recommended to separate the effect of conventional and MT more precisely. Then, it could be e.g. answered, if the discrimination of responders and non-responders by means of the initial motor functional level might be attributed to the general rehabilitation process effect. Meanwhile, it is tempting to attribute the discrimination of responders and non-responders by

means of the MI-PC to the MT effect. A randomized controlled trial could answer the question if a MI-PC that corresponds to an activation pattern of healthy controls might be a predictor for motor functional rehabilitation in general, or if it might be a predictor specific for MT efficacy.

4.4 Theory about mirror therapy

In the following, a new theory on the basis of the presented data in the thesis at hand is developed to explain the underlying neuronal mechanism of the beneficial effect of MT.

MT generates the impression of the paretic hand suddenly moving. This activates the limb-specific mental representation through lateralized ipsilateral PC activation (C. Dohle et al., 2011), which in turn stimulates ipsilesional premotor cortex and SMA via reciprocal intra-hemispheric connections (Cavanna & Trimble, 2006; Marconi et al., 2001).

Figure 15 illustrates a model of cortical structures and neuronal pathways activated during MT on the basis of the results of the thesis at hand. The "+" next to PC indicates an increase in the ipsilesional hemisphere, meanwhile the "-" indicates an activation decrease in the contralesional hemisphere. The red arrows illustrate neuronal projections to SMA and premotor cortex deriving from PC activated during MT, which in turn have connections with M1. This pathway would correspond to recent functional connectivity studies that found increasing interconnections between premotor cortex, SMA and ipsilateral sensorimotor cortex due to MT (Hamzei et al., 2012).

The inversion of the interhemispheric imbalance of PC activation is in favor of the affected hemisphere, i.e. increasing activation in the ipsilesional and decreasing activation in the contralesional hemisphere, which might be also projected to M1. Thus, lateralized precuneal activation due to MT might not only change intra-hemispheric activation of the motor network, but could even invert an interhemispheric imbalance in favor of the ipsilesional hemisphere through parieto-fronto projections (Hamzei et al., 2002). This is in line with the notion that an interhemispheric activity imbalance or rather an excessive inhibition of the affected hemisphere by the contralesional hemisphere across transcallosal inhibition is related to poor motor performance after stroke (Calautti et al., 2007; Murase et al., 2004). This is also supported by a combined rTMS / DCM study, where the contralesional (non-affected) M1 was inhibited via rTMS. This did not only lead to less inhibition of ipsilesional (affected) M1

by the non-affected M1, but caused also enhanced coupling of SMA and ipsilesional M1 and improved motor function (Grefkes et al., 2010). The thesis at hand provides evidence of a similar functioning of MT through an inversion of the impedimental interhemispheric activation imbalance in stroke patients via PC.

Another working mechanism could be the somatosensory path: As demonstrated in Publication 2, S1 is directly modulated by the mirror illusion. This is in line with the notion of Altschuler and colleagues that argued that the lacking proprioceptive input in stroke patients with hemiplegia might be compensated with the visual input that provides MT (Altschuler et al., 1999). Thus, the direct connections from S1 to M1 could also be stimulated via S1 modulation during MT (Figure 15, green arrows). However, S1 modulation due to mirror illusion was not the same as PC modulation. As the lateralization in S1 changed from ipsilateral to nearly symmetrical, this was not in favor of the affected hemisphere and is thus not in line with the above mentioned well-established theory about excessive transcallosal inhibition of the affected hemisphere (Murase et al., 2004). That means that if MT would work via S1 modulation, then the underlying mechanism would not be an inversion of the impedimental interhemispheric activation imbalance as suggested above. Instead, the underlying mechanism would correspond rather to a take-over of the motor control functions in corresponding areas of the non-affected hemisphere. This might be provided by an intensifying of the uncrossed corticospinal tract and functional reorganizing of the non-affected hemisphere (Ago et al., 2003; Song et al., 2005; Yamamoto et al., 2007). It could be suggested that the descending pyramidal tract neurons of S1 for motor control additionally to the ascending ones (Fromm & Evarts, 1982; Kolb & Whishaw, 1996c) could be intensified, if MT works via S1. Beyond that, as sensory loss can also even result in motor impairments similar to hemiparesis and severe sensory loss predicts poor motor recovery after stroke (Nelles et al., 1999), it could be suggested that the fast recovery of sensory deficits after MT promotes motor recovery across the strong interconnections between M1 and S1 (Jones et al., 1978).

However, it cannot be excluded, that both mechanisms occur during MT. A recent study clearly illustrated that motor functional recovery after stroke can be based on different kinds of cortical reorganization (Hamzei et al., 2006). Another possibility is a combination of a simultaneous modulation of PC and S1 by MT and their corresponding connections and interconnections (Figure 15, pink arrows). Future studies are necessary to test for these different possible underlying neuronal pathways.

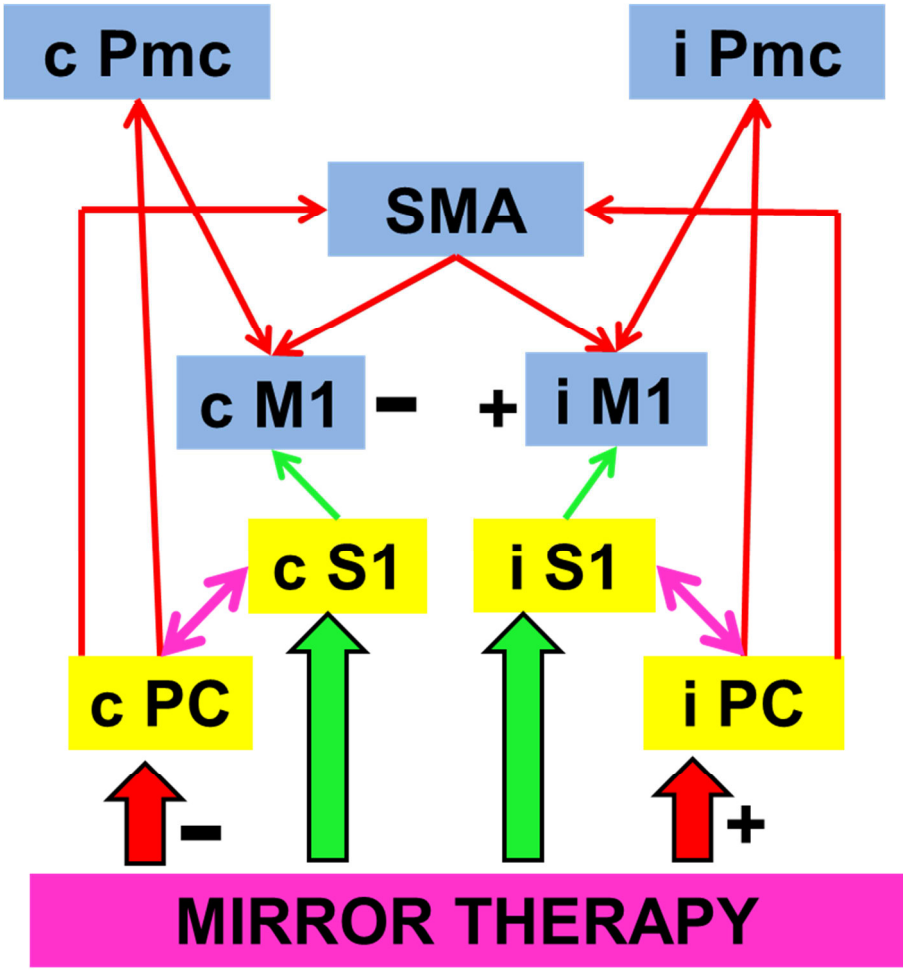

Figure 15: Model of anatomical structures and neuronal pathways activated during MT. **Red arrows:** neuronal pathways stimulated by PC activation; **green arrows:** neuronal pathways stimulated by S1 activation; **pink arrows:** neuronal interconnection between S1 and PC. **Abbreviations:** c: contralesional, i: ipsilesional, Pmc: premotor cortex, SMA: supplementary motor area, M1: primary motor cortex, S1: primary somatosensory cortex, PC: precuneus.

4.4.1 Forecast

Despite the above described experimental advantages of the video-setup, it is suggested to compare activations during mirror illusion in the video-setup with mirror illusion in the original MT setup. The findings of the thesis at hand render further fNIRS studies possible examining the brain activation during MT in setups with more congruent anatomical positions to MT or even application of a real mirror and compare them with the results of the thesis at hand. Due to technical and financial resource limitations this could not be implemented in the thesis at hand. However, the experimental difficulty of measurements in the real MT setup should be taken into account, such as implementation of a control condition. The control condition of the thesis at hand was normal visual feedback of the hand. In the real MT setup this could be provided by a change in direction of gaze, which would entail head movements that could lead to artefacts in the signal. Ultimately, this type of experiments might help to validate findings of rather restricted fMRI studies in comparison to real-world settings.

Furthermore, with the implemented fNIRS paradigm of the thesis at hand, now broader clinical studies are afforded investigating the interhemispheric balance, how it changes in the longitudinal time course after stroke and how this correlates to motor rehabilitation.

Analysis of effective and functional connectivity could be conducted to enhance comprehension about MT induced neuroplasticity, to test the models about functional connectivity presented in chapter 4.4 and to examine whether MT operates via S1, PC or both (Figure 15). Especially, PC could be added in the analyzed network models, which was not included in former connectivity studies analyzing only premotor cortex, SMA and ipsilateral sensorimotor (Hamzei et al., 2012). Therefore, results of DCM analysis before and after MT treatment should be compared with each other. FNIRS recently was proven to sufficiently analyze functional connectivity networks during resting periods (Lu et al., 2010; White et al., 2009) and a first experiment shows that also DCM analysis using NIRS data is reliable (Mehnert, not published).

Beyond that, to test the generalizability of the clinical findings of the thesis at hand a randomized controlled clinical trial with higher sample size would be necessary. Thus the precuneal lateralized activation could be further tested as predictor for MT outcome. In addition, with a higher sample size further questions could be addressed such as about a potential hemispherical advantage for MT effect. The fMRI Publication 1 demonstrated the lateralization shift on PC to be more pronounced for right hand movements than for LH. In contrast, in Publication 2 the lateralization shift on S1 activity was more pronounced for left hand movements. Thus, no consistent hand laterality advantage concerning MT can be concluded. However, it could be speculated an advantage for left hemisphere lesions, if MT functions predominantly via S1, meanwhile an advantage for right hemisphere lesions is suggested, if MT functions predominantly via PC. No concrete hand laterality, or hemisphere lesion advantage concerning MT efficacy is assumed if MT should function via both structures in parallel - S1 and PC (Figure 15).

It would also further be interesting to compare the neuronal correlate of MT variations, such as including bimanual movements, additional sensory stimulation of the affected limb and object manipulation via fNIRS (C. Dohle et al., 2011).

5 Literature

Acerra, N. E. (2007). *Sensorimotor dysfunction in CRPS1 and stroke: characterisation, prediction and intervention.*

Ago, T., Kitazono, T., Ooboshi, H., Takada, J., Yoshiura, T., Mihara, F., . . . Iida, M. (2003). Deterioration of pre-existing hemiparesis brought about by subsequent ipsilateral lacunar infarction. *J Neurol Neurosurg Psychiatry, 74*(8), 1152-1153.

Altschuler, E. L., Wisdom, S. B., Stone, L., Foster, C., Galasko, D., Llewellyn, D. M., & Ramachandran, V. S. (1999). Rehabilitation of hemiparesis after stroke with a mirror. *Lancet, 353*(9169), 2035-2036.

Archambault, P. S., Ferrari-Toniolo, S., Caminiti, R., & Battaglia-Mayer, A. (2014). Visually-guided correction of hand reaching movements: The neurophysiological bases in the cerebral cortex. *Vision Res*. doi: 10.1016/j.visres.2014.09.009

Arya, K. N., Verma, R., & Garg, R. K. (2011). Estimating the minimal clinically important difference of an upper extremity recovery measure in subacute stroke patients. *Top Stroke Rehabil, 18 Suppl 1*, 599-610. doi: 10.1310/tsr18s01-599

Arzy, S., Overney, L. S., Landis, T., & Blanke, O. (2006). Neural mechanisms of embodiment: asomatognosia due to premotor cortex damage. *Arch Neurol, 63*(7), 1022-1025. doi: 10.1001/archneur.63.7.1022

Avikainen, S., Forss, N., & Hari, R. (2002). Modulated activation of the human SI and SII cortices during observation of hand actions. *Neuroimage, 15*(3), 640-646. doi: 10.1006/nimg.2001.1029

Aziz-Zadeh, L., Maeda, F., Zaidel, E., Mazziotta, J. C., & Iacoboni, M. (2002). Lateralization in motor facilitation during action observation: a TMS study. *Exp Brain Res, 144*(1), 127-131.

Baier, B., & Karnath, H. O. (2008). Tight link between our sense of limb ownership and self-awareness of actions. *Stroke, 39*(2), 486-488. doi: 10.1161/STROKEAHA.107.495606

Battaglia-Mayer, A., Ferraina, S., Mitsuda, T., Marconi, B., Genovesio, A., Onorati, P., . . . Caminiti, R. (2000). Early coding of reaching in the parietooccipital cortex. *J Neurophysiol, 83*(4), 2374-2391.

Bieniok, A., Govers, J., & Dohle, C. (2009). *Spiegeltherapie in der Neurorehabilitation* (Vol. 1). Germany: Schulz-Kirchner Verlag.

Boggio, P. S., Nunes, A., Rigonatti, S. P., Nitsche, M. A., Pascual-Leone, A., & Fregni, F. (2007). Repeated sessions of noninvasive brain DC stimulation is associated with motor function improvement in stroke patients. *Restor Neurol Neurosci, 25*(2), 123-129.

Bortz, J. (2005). *Statistik für Sozialwissenschaftler* (6. ed.): Springer Medizin Verlag Heidelberg.

Bowering, K. J., O'Connell, N. E., Tabor, A., Catley, M. J., Leake, H. B., Moseley, G. L., & Stanton, T. R. (2013). The Effects of Graded Motor Imagery and Its Components on Chronic Pain: A Systematic Review and Meta-Analysis. *The Journal of Pain, 14*(1), 3-13.

Brodmann, K. (1909). *Vergleichende Lokalisationslehre der Großhirnrinde*. Leipzig: Barth.

Broeks, J. G., Lankhorst, G. J., Rumping, K., & Prevo, A. J. (1999). The long-term outcome of arm function after stroke: results of a follow-up study. *Disabil Rehabil, 21*(8), 357-364.

Buccino, G., Binkofski, F., Fink, G. R., Fadiga, L., Fogassi, L., Gallese, V., . . . Freund, H. J. (2001). Action observation activates premotor and parietal areas in a somatotopic manner: an fMRI study. *Eur J Neurosci, 13*(2), 400-404.

Buccino, G., Gatti, R., Giusti, M. C., Negrotti, A., Rossi, A., Calzetti, S., & Cappa, S. F. (2011). Action observation treatment improves autonomy in daily activities in Parkinson's disease patients: results from a pilot study. *Mov Disord, 26*(10), 1963-1964. doi: 10.1002/mds.23745

Buccino, G., Solodkin, A., & Small, S. (2006). Functions of the mirror neuron system: implications for neurorehabilitation. *Cogn Behav Neurol., 19*(1), 55-63.

Buccino, G., Vogt, S., Ritzl, A., Fink, G. R., Zilles, K., Freund, H. J., & Rizzolatti, G. (2004). Neural circuits underlying imitation learning of hand actions: an event-related fMRI study. *Neuron, 42*(2), 323-334.

Burton, H., Abend, N. S., MacLeod, A. M., Sinclair, R. J., Snyder, A. Z., & Raichle, M. E. (1999). Tactile attention tasks enhance activation in somatosensory regions of parietal cortex: a positron emission tomography study. *Cereb Cortex, 9*(7), 662-674.

Butefisch, C., Hummelsheim, H., Denzler, P., & Mauritz, K. H. (1995). Repetitive training of isolated movements improves the outcome of motor rehabilitation of the centrally paretic hand. *Journal of the Neurological Sciences, 130*(1), 59-68.

Calautti, C., Naccarato, M., Jones, P. S., Sharma, N., Day, D. D., Carpenter, A. T., . . . Baron, J. C. (2007). The relationship between motor deficit and hemisphere activation balance after stroke: A 3T fMRI study. *Neuroimage, 34*(1), 322-331. doi: 10.1016/j.neuroimage.2006.08.026

Camacho, A., & Massieu, L. (2006). Role of glutamate transporters in the clearance and release of glutamate during ischemia and its relation to neuronal death. *Arch Med Res, 37*(1), 11-18. doi: 10.1016/j.arcmed.2005.05.014

Cauda, F., Geminiani, G., D'Agata, F., Sacco, K., Duca, S., Bagshaw, A. P., & Cavanna, A. E. (2010). Functional connectivity of the posteromedial cortex. *PLoS One, 5*(9). doi: 10.1371/journal.pone.0013107

Cavanna, A. E., & Trimble, M. R. (2006). The precuneus: a review of its functional anatomy and behavioural correlates. *Brain, 129*, 564-583.

Cavina-Pratesi, C., Connolly, J. D., & Milner, A. D. (2013). Optic ataxia as a model to investigate the role of the posterior parietal cortex in visually guided action: evidence from studies of patient M.H. *Front Hum Neurosci, 7*, 336. doi: 10.3389/fnhum.2013.00336

Chen, S., & Winstein, C. (2009). A systematic review of voluntary arm recovery in hemiparetic stroke: critical predictors for meaningful outcomes using the international classification of functioning, disability, and health. *J Neurol Phys Ther, 33*(1), 2-13.

Connolly, J. D., Andersen, R. A., & Goodale, M. A. (2003). FMRI evidence for a 'parietal reach region' in the human brain. *Exp Brain Res, 153*(2), 140-145. doi: 10.1007/s00221-003-1587-1

Corkin, S., Milner, B., & Rasmussen, T. (1970). Somatosensory thresholds--contrasting effects of postcentral-gyrus and posterior parietal-lobe excisions. *Arch Neurol, 23*(1), 41-58.

Deconinck, F. J., Smorenburg, A. R., Benham, A., Ledebt, A., Feltham, M. G., & Savelsbergh, G. J. (2014). Reflections on Mirror Therapy: A Systematic Review of the Effect of Mirror Visual Feedback on the Brain. *Neurorehabil Neural Repair*. doi: 10.1177/1545968314546134

Diers, M., Christmann, C., Koeppe, C., Ruf, M., & Flor, H. (2010). Mirrored, imagined and executed movements differentially activate sensorimotor cortex in amputees with and without phantom limb pain. *Pain, 149*(2), 296-304. doi: 10.1016/j.pain.2010.02.020

Dohle, C., Kleiser, R., Seitz, R. J., & Freund, H.-J. (2004). Body scheme gates visual processing. *J Neurophysiol, 91*(5), 2376-2379.

Dohle, C., Morkisch, N., Lommack, R., & Kadow, L. (2011). Spiegeltherapie. *neuroreha, 3*(4), 184-190. doi: 10.1055/s-0031-1295557

Dohle, C., Püllen, J., Nakaten, A., Kuest, J., Rietz, C., & Karbe, H. (2009). Mirror therapy promotes recovery from severe hemiparesis: A randomized, controlled trial. *Neurorehabil Neural Repair, 23*(3), 209-217.

Dohle, C., Pullen, J., Nakaten, A., Kust, J., Rietz, C., & Karbe, H. (2009). Mirror therapy promotes recovery from severe hemiparesis: a randomized controlled trial. *Neurorehabil Neural Repair, 23*(3), 209-217. doi: 10.1177/1545968308324786

Dohle, C., Stephan, K. M., Valvoda, J. T., Hosseiny, O., Tellmann, L., Kuhlen, T., . . . Freund, H. J. (2011). Representation of virtual arm movements in precuneus. *Exp Brain Res, 208*(4), 543-555. doi: 10.1007/s00221-010-2503-0

Doyle, S., Bennett, S., Fasoli, S. E., & McKenna, K. T. (2010). Interventions for sensory impairment in the upper limb after stroke. *Cochrane Database Syst Rev*(6), CD006331. doi: 10.1002/14651858.CD006331.pub2

Eickhoff, S. B., Grefkes, C., Fink, G. R., & Zilles, K. (2008). Functional lateralization of face, hand, and trunk representation in anatomically defined human somatosensory areas. *Cereb Cortex, 18*(12), 2820-2830. doi: 10.1093/cercor/bhn039

Elbert, T., Pantev, C., Wienbruch, C., Rockstroh, B., & Taub, E. (1995). Increased cortical representation of the fingers of the left hand in string players. *Science, 270*(5234), 305-307.

Ertelt, D., Small, S., Solodkin, A., Dettmers, C., McNamara, A., Binkofski, F., & Buccino, G. (2007). Action observation has a positive impact on rehabilitation of motor deficits after stroke. *Neuroimage, 36 Suppl 2*, T164-173.

Fattori, P., Breveglieri, R., Marzocchi, F., Filippini, D., Bosco, A., & Galletti, C. (2009). Hand Orientation during Reach-to-Grasp Movements Modulates Neuronal Activity in the Medial Posterior Parietal Area V6A. *The Journal of Neuroscience, 29*(6), 1928 –1936.

Feigin, V. L., Forouzanfar, M. H., Krishnamurthi, R., Mensah, G. A., Connor, M., Bennett, D. A., . . . the, G. B. D. S. E. G. (2014). Global and regional burden of stroke during 1990-2010: findings from the Global Burden of Disease Study 2010. *Lancet, 383*(9913), 245-254.

Ferretti, A., Del Gratta, C., Babiloni, C., Caulo, M., Arienzo, D., Tartaro, A., . . . Romani, G. L. (2004). Functional topography of the secondary somatosensory cortex for nonpainful and painful stimulation of median and tibial nerve: an fMRI study. *Neuroimage, 23*(3), 1217-1225. doi: 10.1016/j.neuroimage.2004.08.003

Filimon, F., Nelson, J. D., Hagler, D. J., & Sereno, M. I. (2007). Human cortical representations for reaching: mirror neurons for execution, observation, and imagery. *Neuroimage, 37*(4), 1315-1328. doi: 10.1016/j.neuroimage.2007.06.008

Fletcher, P. C., Frith, C. D., Grasby, P. M., Shallice, T., Frackowiak, R. S., & Dolan, R. J. (1995). Brain systems for encoding and retrieval of auditory-verbal memory. An in vivo study in humans. *Brain, 118 (Pt 2)*, 401-416.

Fromm, C., & Evarts, E. V. (1982). Pyramidal tract neurons in somatosensory cortex: central and peripheral inputs during voluntary movement. *Brain Res, 238*(1), 186-191.

Fukumura, K., Sugawara, K., Tanabe, S., Ushiba, J., & Tomita, Y. (2007). Influence of mirror therapy on human motor cortex. *Int J Neurosci, 1117*(7), 1039-1048.

Funase, K., Tabira, T., Higashi, T., Liang, N., & Kasai, T. (2007). Increased corticospinal excitability during direct observation of self-movement and indirect observation with a mirror box. *Neurosci Lett, 419*(2), 108-112.

Galletti, C., Fattori, P., Kutz, D. F., & Gamberini, M. (1999). Brain location and visual topography of cortical area V6A in the macaque monkey. *European Journal of Neuroscience, 11*(2), 575-582.

Galletti, C., Gamberini, M., Kutz, D. F., Fattori, P., Luppino, G., & Matelli, M. (2001). The cortical connections of area V6: an occipito-parietal network processing visual information. *Eur J Neurosci, 13*(8), 1572-1588.

Garry, M. I., Loftus, A., & Summers, J. J. (2005). Mirror, mirror on the wall: viewing a mirror reflection of unilateral hand movements facilitates ipsilateral M1 excitability. *Exp Brain Res, 163*(1), 118-122.

Giraux, P., & Sirigu, A. (2003). Illusory movements of the paralyzed limb restore motor cortex activity. *Neuroimage, 20*, S107-S111.

Gladstone, D., Danells, C., & Black, S. (2002). The Fugl-Meyer Assessment of Motor Recovery after stroke: A Critical Review of its measurement properties. *Neurorehabil Neural Repair, 16*, 232-240.

Grefkes, C., & Fink, G. R. (2011). Reorganization of cerebral networks after stroke: new insights from neuroimaging with connectivity approaches. *Brain, 134*(5), 1264-1276. doi: 10.1093/brain/awr033

Grefkes, C., Nowak, D. A., Wang, L. E., Dafotakis, M., Eickhoff, S. B., & Fink, G. R. (2010). Modulating cortical connectivity in stroke patients by rTMS assessed with fMRI and dynamic causal modeling. *Neuroimage, 50*(1), 233-242. doi: 10.1016/j.neuroimage.2009.12.029

Grèzes, J., & Decety, J. (2001). Functional Anatomy of Execution, Mental Simulation, Observation and Verb Generation of Actions: A Meta-Analysis. *Hum Brain Mapp, 12*, 1-19.

Habermehl, C., Holtze, S., Steinbrink, J., Koch, S. P., Obrig, H., Mehnert, J., & Schmitz, C. H. (2012). Somatosensory activation of two fingers can be discriminated with ultrahigh-density diffuse optical tomography. *Neuroimage, 59*(4), 3201-3211. doi: 10.1016/j.neuroimage.2011.11.062

Hamzei, F., Dettmers, C., Rijntjes, M., Glauche, V., Kiebel, S., Weber, B., & Weiller, C. (2002). Visuomotor control within a distributed parieto-frontal network. *Exp Brain Res, 146*(3), 273-281. doi: 10.1007/s00221-002-1139-0

Hamzei, F., Lappchen, C. H., Glauche, V., Mader, I., Rijntjes, M., & Weiller, C. (2012). Functional plasticity induced by mirror training: the mirror as the element connecting both hands to one hemisphere. *Neurorehabil Neural Repair, 26*(5), 484-496. doi: 10.1177/1545968311427917

Hamzei, F., Liepert, J., Dettmers, C., Weiller, C., & Rijntjes, M. (2006). Two different reorganization patterns after rehabilitative therapy: an exploratory study with fMRI and TMS. *Neuroimage, 31*(2), 710-720. doi: 10.1016/j.neuroimage.2005.12.035

Hesse, S., Waldner, A., Mehrholz, J., Tomelleri, C., Pohl, M., & Werner, C. (2011). Combined transcranial direct current stimulation and robot-assisted arm training in subacute stroke patients: an exploratory, randomized multicenter trial. *Neurorehabil Neural Repair, 25*(9), 838-846. doi: 10.1177/1545968311413906

Hesse, S., Werner, C., Schonhardt, E. M., Bardeleben, A., Jenrich, W., & Kirker, S. G. (2007). Combined transcranial direct current stimulation and robot-assisted arm training in subacute stroke patients: a pilot study. *Restor Neurol Neurosci, 25*(1), 9-15.

Hodics, T., Cohen, L. G., & Cramer, S. C. (2006). Functional imaging of intervention effects in stroke motor rehabilitation. *Arch Phys Med Rehabil, 87*(12 Suppl 2), S36-42. doi: 10.1016/j.apmr.2006.09.005

Hoermann, S., Franz, E. A., & Regenbrecht, H. (2012). Referred sensations elicited by video-mediated mirroring of hands. *PLoS One, 7*(12), e50942. doi: 10.1371/journal.pone.0050942

Hsieh, Y. W., Wu, C. Y., Lin, K. C., Chang, Y. F., Chen, C. L., & Liu, J. S. (2009). Responsiveness and validity of three outcome measures of motor function after stroke rehabilitation. *Stroke, 40*(4), 1386-1391. doi: 10.1161/STROKEAHA.108.530584

Hummel, F., Celnik, P., Giraux, P., Floel, A., Wu, W. H., Gerloff, C., & Cohen, L. G. (2005). Effects of non-invasive cortical stimulation on skilled motor function in chronic stroke. *Brain, 128*(Pt 3), 490-499. doi: 10.1093/brain/awh369

Iacoboni, M., & Mazziotta, J. C. (2007). Mirror neuron system: basic findings and clinical applications. *Ann Neurol, 62*(3), 213-218. doi: 10.1002/ana.21198

Ietswaart, M., Johnston, M., Dijkerman, H. C., Joice, S., Scott, C. L., MacWalter, R. S., & Hamilton, S. J. (2011). Mental practice with motor imagery in stroke recovery: randomized controlled trial of efficacy. *Brain, 134*(Pt 5), 1373-1386. doi: 10.1093/brain/awr077

Jeannerod, M., Decety, J., & Michel, F. (1994). Impairment of grasping movements following a bilateral posterior parietal lesion. *Neuropsychologia, 32*(4), 369-380.

Jenkins, W. M., Merzenich, M. M., Ochs, M. T., Allard, T., & Guic-Robles, E. (1990). Functional reorganization of primary somatosensory cortex in adult owl monkeys after behaviorally controlled tactile stimulation. *J Neurophysiol, 63*(1), 82-104.

Johansen-Berg, H., Christensen, V., Woolrich, M., & Matthews, P. M. (2000). Attention to touch modulates activity in both primary and secondary somatosensory areas. *Neuroreport, 11*(6), 1237-1241.

Johnston, S., Mendis, S., & Mathers, C. (2009). Global variation in stroke burden and mortality: estimates from monitoring, surveillance, and modeling. *Lancet Neurol*(8), 345–354.

Jones, E. G., Coulter, J. D., & Hendry, S. H. (1978). Intracortical connectivity of architectonic fields in the somatic sensory, motor and parietal cortex of monkeys. *J Comp Neurol, 181*(2), 291-347. doi: 10.1002/cne.901810206

Jorgensen, H. S., Nakayama, H., Raaschou, H. O., Vive-Larsen, J., Stoier, M., & Olsen, T. S. (1995). Outcome and time course of recovery in stroke. Part I: Outcome. The Copenhagen Stroke Study. *Arch Phys Med Rehabil, 76*(5), 399-405.

Jung, P., Baumgartner, U., Magerl, W., & Treede, R. D. (2008). Hemispheric asymmetry of hand representation in human primary somatosensory cortex and handedness. *Clin Neurophysiol, 119*(11), 2579-2586. doi: 10.1016/j.clinph.2008.04.300

Kaas, J. H., Merzenich, M. M., & Killackey, H. P. (1983). The reorganization of somatosensory cortex following peripheral nerve damage in adult and developing mammals. *Annu Rev Neurosci, 6*, 325-356. doi: 10.1146/annurev.ne.06.030183.001545

Kang, Y. J., Ku, J., Kim, H. J., & Park, H. K. (2011). Facilitation of corticospinal excitability according to motor imagery and mirror therapy in healthy subjects and stroke patients. *Ann Rehabil Med, 35*(6), 747-758. doi: 10.5535/arm.2011.35.6.747

Kang, Y. J., Park, H. K., Kim, H. J., Lim, T., Ku, J., Cho, S., . . . Park, E. S. (2012). Upper extremity rehabilitation of stroke: facilitation of corticospinal excitability using virtual mirror paradigm. *J Neuroeng Rehabil, 9*, 71. doi: 10.1186/1743-0003-9-71

Karnath, H. O., & Perenin, M. T. (2005). Cortical control of visually guided reaching: evidence from patients with optic ataxia. *Cereb Cortex, 15*(10), 1561-1569. doi: 10.1093/cercor/bhi034

Kato, H., Izumiyama, M., Koizumi, H., Takahashi, A., & Itoyama, Y. (2002). Near-infrared spectroscopic topography as a tool to monitor motor reorganization after hemiparetic stroke: a comparison with functional MRI. *Stroke, 33*(8), 2032-2036.

Kida, T., Inui, K., Wasaka, T., Akatsuka, K., Tanaka, E., & Kakigi, R. (2007). Time-varying cortical activations related to visual-tactile cross-modal links in spatial selective attention. *J Neurophysiol, 97*(5), 3585-3596. doi: 10.1152/jn.00007.2007

Kircher, T. T., Brammer, M., Bullmore, E., Simmons, A., Bartels, M., & David, A. S. (2002). The neural correlates of intentional and incidental self processing. *Neuropsychologia, 40*(6), 683-692.

Kirilina, E., Jelzow, A., Heine, A., Niessing, M., Wabnitz, H., Bruhl, R., . . . Tachtsidis, I. (2012). The physiological origin of task-evoked systemic artefacts in functional near infrared spectroscopy. *Neuroimage, 61*(1), 70-81. doi: 10.1016/j.neuroimage.2012.02.074

Klawans, H. L. (1990). *Newton's madness: Furhter tales of clinical neurology*. New York: Harper & Row.

Koch, S. P., Habermehl, C., Mehnert, J., Schmitz, C. H., Holtze, S., Villringer, A., . . . Obrig, H. (2010). High-resolution optical functional mapping of the human somatosensory cortex. *Front Neuroenergetics, 2*, 12. doi: 10.3389/fnene.2010.00012

Koessler, L., Maillard, L., Benhadid, A., Vignal, J. P., Felblinger, J., Vespignani, H., & Braun, M. (2009). Automated cortical projection of EEG sensors: anatomical correlation via the international 10-10 system. *Neuroimage, 46*(1), 64-72.

Kolb, B., & Whishaw, I. Q. (1996a). Die Erholung gestörter Hirnfunktionen. In M. Pritzel (Ed.), *Neuropsychologie* (2 ed., pp. 454-478). Heidelberg Berlin Oxford: Spektrum Akademischer Verlag.

Kolb, B., & Whishaw, I. Q. (1996b). Die Organisation des Nervensystems. In M. Pritzel (Ed.), *Neuropsychologie* (2 ed., pp. 34-54). Heidelberg Berlin Oxford: Spektrum Akademischer Verlag.

Kolb, B., & Whishaw, I. Q. (1996c). Die Organisation sensorischer Systeme. In M. Pritzel (Ed.), *Neuropsychologie* (2 ed., pp. 85-102). Heidelberg Berlin Oxford: Spektrum Akademischer Verlag.

Krainik, A., Hund-Georgiadis, M., Zysset, S., & von Cramon, D. Y. (2005). Regional impairment of cerebrovascular reactivity and BOLD signal in adults after stroke. *Stroke, 36*(6), 1146-1152. doi: 10.1161/01.STR.0000166178.40973.a7

Krause, B. J., Schmidt, D., Mottaghy, F. M., Taylor, J., Halsband, U., Herzog, H., . . . Muller-Gartner, H. W. (1999). Episodic retrieval activates the precuneus irrespective of the imagery content of word pair associates. A PET study. *Brain, 122 (Pt 2)*, 255-263.

Kwakkel, G., Kollen, B. J., van der Grond, J., & Prevo, A. J. (2003). Probality of Regaining Dexterity in the Flaccid Upper Limb. Impact of Severity of Paresis and Time Since Onset in Acute Stroke. *Stroke, 34*, 2181-2186.

Lappchen, C. H., Ringer, T., Blessin, J., Seidel, G., Grieshammer, S., Lange, R., & Hamzei, F. (2012). Optical illusion alters M1 excitability after mirror therapy: a TMS study. *J Neurophysiol, 108*(10), 2857-2861. doi: 10.1152/jn.00321.2012

Liepert, J., Bauder, H., Wolfgang, H. R., Miltner, W. H., Taub, E., & Weiller, C. (2000). Treatment-induced cortical reorganization after stroke in humans. *Stroke, 31*(6), 1210-1216.

Liepert, J., Graef, S., Uhde, I., Leidner, O., & Weiller, C. (2000). Training-induced changes of motor cortex representations in stroke patients. *Acta Neurol Scand, 101*(5), 321-326.

Liepert, J., Miltner, W. H., Bauder, H., Sommer, M., Dettmers, C., Taub, E., & Weiller, C. (1998). Motor cortex plasticity during constraint-induced movement therapy in stroke patients. *Neurosci Lett, 250*(1), 5-8.

Liepert, J., Storch, P., Fritsch, A., & Weiller, C. (2000). Motor cortex disinhibition in acute stroke. *Clin Neurophysiol, 111*(4), 671-676.

Longo, M. R., Pernigo, S., & Haggard, P. (2011). Vision of the body modulates processing in primary somatosensory cortex. *Neurosci Lett, 489*(3), 159-163. doi: 10.1016/j.neulet.2010.12.007

Lu, C. M., Zhang, Y. J., Biswal, B. B., Zang, Y. F., Peng, D. L., & Zhu, C. Z. (2010). Use of fNIRS to assess resting state functional connectivity. *J Neurosci Methods, 186*(2), 242-249.

Lynch, J. (1980). The functional organization of posterior parietal association cortex. *Behavioral and Brain Sciences, 3*, 485-499. doi: 10.1017/S0140525X00006324

Mansur, C. G., Fregni, F., Boggio, P. S., Riberto, M., Gallucci-Neto, J., Santos, C. M., . . . Pascual-Leone, A. (2005). A sham stimulation-controlled trial of rTMS of the unaffected hemisphere in stroke patients. *Neurology, 64*(10), 1802-1804. doi: 10.1212/01.WNL.0000161839.38079.92

Marconi, B., Genovesio, A., Battaglia-Mayer, A., Ferraina, S., Squatrito, S., Molinari, M., . . . Caminiti, R. (2001). Eye-hand coordination during reaching. I. Anatomical relationships between parietal and frontal cortex. *Cerebral Cortex, 11*, 513-527.

Matthys, K., Smits, M., Van der Geest, J. N., Van der Lugt, A., Seurinck, R., Stam, H. J., & Selles, R. W. (2009). Mirror-induced visual illusion of hand movements: a functional magnetic resonance imaging study. *Arch Phys Med Rehabil., 90*(4), c675-681.

Mehrholz, J., Platz, T., Kugler, J., & Pohl, M. (2008). Electromechanical and robot-assisted arm training for improving arm function and activities of daily living after stroke. *Cochrane Database Syst Rev.*(4), CD006876.

Merzenich, M. M., Nelson, R. J., Stryker, M. P., Cynader, M. S., Schoppmann, A., & Zook, J. M. (1984). Somatosensory cortical map changes following digit amputation in adult monkeys. *J Comp Neurol, 224*(4), 591-605. doi: 10.1002/cne.902240408

Michielsen, M. E., Selles, R. W., van der Geest, J. N., Eckhardt, M., Yavuzer, G., Stam, H. J., . . . Bussmann, J. B. (2011). Motor recovery and cortical reorganization after mirror therapy in chronic stroke patients: a phase II randomized controlled trial. *Neurorehabil Neural Repair, 25*(3), 223-233. doi: 10.1177/1545968310385127

Michielsen, M. E., Smits, M., Ribbers, G. M., Stam, H. J., van der Geest, J. N., Bussmann, J. B., & Selles, R. W. (2011). The neuronal correlates of mirror therapy: an fMRI study

on mirror induced visual illusions in patients with stroke. *J Neurol Neurosurg Psychiatry, 82*(4), 393-398. doi: 10.1136/jnnp.2009.194134
Mima, T., Nagamine, T., Nakamura, K., & Shibasaki, H. (1998). Attention modulates both primary and second somatosensory cortical activities in humans: a magnetoencephalographic study. *J Neurophysiol, 80*(4), 2215-2221.
Moore, C. I., Stern, C. E., Corkin, S., Fischl, B., Gray, A. C., Rosen, B. R., & Dale, A. M. (2000). Segregation of somatosensory activation in the human rolandic cortex using fMRI. *J Neurophysiol, 84*(1), 558-569.
Morkisch, N., Lommack, R., Kadow, L., Rietz, C., & Dohle, C. (2012). *Evaluation eines standardisierten Therapieprotokolls zur Spiegeltherapie*. Paper presented at the 22. Jahrestagung der Deutschen Gesellschaft für Neurorehabilitation e.V. (DGNR), Fürth, Germany.
Moseley, G., & Wiech, K. (2009). The effect of tactile discrimination training is enhanced when patients watch the reflected image of their unaffected limb during training. *Pain, 144*(3), 314-319.
Moseley, G. L. (2004). Graded motor imagery is effective for long-standing complex regional pain syndrome: a randomised controlled trial. *Pain, 108*, 192-198.
Mountcastle, V., Lynch, J., Georgopoulos, A., Sakata, H., & Acuna, C. (1975). Posterior parietal association cortex of the monkey: command functions for operation within extrapersonal space. *J Neurophysiol, 38*, 871-908.
Muehlschlegel, S., Selb, J., Patel, M., Diamond, S. G., Franceschini, M. A., Sorensen, A. G., . . . Schwamm, L. H. (2009). Feasibility of NIRS in the neurointensive care unit: a pilot study in stroke using physiological oscillations. *Neurocrit Care, 11*(2), 288-295.
Mulder, T. (2007). Motor imagery and action observation: cognitive tools for rehabilitation. *J Neural Transm, 114*, 1265-1278. doi: 10.1007/s00702-007-0763-z
Murase, N., Duque, J., Mazzocchio, R., & Cohen, L. G. (2004). Influence of interhemispheric interactions on motor function in chronic stroke. *Ann Neurol, 55*(3), 400-409. doi: 10.1002/ana.10848
Nedelko, V., Hassa, T., Hamzei, F., Weiller, C., Binkofski, F., Schoenfeld, M. A., . . . Dettmers, C. (2010). Age-independent activation in areas of the mirror neuron system during action observation and action imagery. A fMRI study. *Restor Neurol Neurosci, 28*(6), 737-747. doi: 10.3233/RNN-2010-0542
Nelles, G., Spiekermann, G., Jueptner, M., Leonhardt, G., Muller, S., Gerhard, H., & Diener, H. C. (1999). Reorganization of sensory and motor systems in hemiplegic stroke patients. A positron emission tomography study. *Stroke, 30*(8), 1510-1516.
Nilsen, D. M., Gillen, G., DiRusso, T., & Gordon, A. M. (2012). Effect of imagery perspective on occupational performance after stroke: a randomized controlled trial. *Am J Occup Ther, 66*(3), 320-329. doi: 10.5014/ajot.2012.003475
Nojima, I., Mima, T., Koganemaru, S., Thabit, M. N., Fukuyama, H., & Kawamata, T. (2012). Human motor plasticity induced by mirror visual feedback. *J Neurosci, 32*(4), 1293-1300. doi: 10.1523/JNEUROSCI.5364-11.2012
Nowak, D. A., Grefkes, C., Ameli, M., & Fink, G. R. (2009). Interhemispheric competition after stroke: brain stimulation to enhance recovery of function of the affected hand. *Neurorehabil Neural Repair, 23*(7), 641-656. doi: 10.1177/1545968309336661
Nudo, R. J. (1997). Remodeling of cortical motor representations after stroke: implications for recovery from brain damage. *Mol Psychiatry, 2*(3), 188-191.
Nudo, R. J., Jenkins, W. M., Merzenich, M. M., Prejean, T., & Grenda, R. (1992). Neurophysiological correlates of hand preference in primary motor cortex of adult squirrel monkeys. *J Neurosci, 12*(8), 2918-2947.
Nudo, R. J., Milliken, G. W., Jenkins, W. M., & Merzenich, M. M. (1996). Use-dependent alterations of movement representations in primary motor cortex of adult squirrel monkeys. *J Neurosci, 16*(2), 785-807.
Obrig, H. (2014). NIRS in clinical neurology - a 'promising' tool? *Neuroimage, 85 Pt 1*, 535-546. doi: 10.1016/j.neuroimage.2013.03.045
Obrig, H., & Villringer, A. (2003). Beyond the visible--imaging the human brain with light. *J Cereb Blood Flow Metab, 23*(1), 1-18.

Orjuela-Rojas, J. M., Ramirez-Bermudez, J., Martinez-Juarez, I. E., Kerik, N. E., Diaz Meneses, I., & Perez-Gay, F. J. (2014). Visual hallucinations of autobiographic memory and asomatognosia: a case of epilepsy due to brain cysticercosis. *Neurocase*, 1-7. doi: 10.1080/13554794.2014.962548

Page, S. J., Levine, P., & Leonard, A. C. (2005). Effects of mental practice on affected limb use and function in chronic stroke. *Arch Phys Med Rehabil, 86*(3), 399-402.

Page, S. J., Levine, P., Sisto, S. A., & Johnston, M. V. (2001). Mental practice combined with physical practice for upper-limb motor deficit in subacute stroke. *Physical Therapy, 81*(8), 1455-1462.

Pascual-Leone, A., Nguyet, D., Cohen, L. G., Brasil-Neto, J. P., Cammarota, A., & Hallett, M. (1995). Modulation of muscle responses evoked by transcranial magnetic stimulation during the acquisition of new fine motor skills. *J Neurophysiol, 74*(3), 1037-1045.

Penfield, W., & Boldrey, E. (1937). Somatic motor and sensory representation in the cerebral cortex of man as studied by electrical stimulation. *Brain, 60*(4), 389-443. doi: http://dx.doi.org/10.1093/brain/60.4.389

Perenin, M.-T. (2006). Optische Ataxie. In H.-O. Karnath & P. Thier (Eds.), *Neuropsychologie* (pp. 308-319). Heidelberg: Springer Medizin Verlag.

Perenin, M.-T., & Vighetto, A. (1988). Optic ataxia: A specific disruption in visuomotor mechanisms. I. Different aspects for the deficit in reaching for objects. *Brain, 111*, 643-674.

Pinel, J. P. (2001a). Das sensomotorische System. In B. W. (Ed.), *Biopsychologie* (2 ed., pp. 234-260). Heidelberg Berlin: Spektrum Akademischer Verlag GmbH.

Pinel, J. P. (2001b). Mechanismen der Wahrnehmung, des Bewusstseins und der Aufmerksamkeit. In B. W. (Ed.), *Biopsychologie* (2 ed., pp. 198-233). Heidelberg Berlin: Spektrum Akademischer Verlag GmbH.

Pinel, J. P. (2001c). Neuronale Plastizität: Entwicklung, Lernen und Erholung von Hirnschädigungen. In B. W. (Ed.), *Biopsychologie* (2 ed., pp. 423-457). Heidelberg Berlin: Spektrum Akademischer Verlag GmbH.

Pitzalis, S., Sereno, M. I., Committeri, G., Fattori, P., Galati, G., Tosoni, A., & Galletti, C. (2013). The human homologue of macaque area V6A. *Neuroimage, 82*, 517-530. doi: 10.1016/j.neuroimage.2013.06.026

Platz, T., & Roschka, S. (2009). Rehabilitative Therapie bei Armparese nach Schlaganfall. *Neurol Rehabil, 15*(2), 17-42.

Porro, C. A., Cettolo, V., Francescato, M. P., & Baraldi, P. (2000). Ipsilateral involvement of primary motor cortex during motor imagery. *Eur J Neurosci, 12*(8), 3059-3063.

Porro, C. A., Francescato, M. P., Cettolo, V., Diamond, M. E., Baraldi, P., Zuiani, C., . . . di Prampero, P. E. (1996). Primary motor and sensory cortex activation during motor performance and motor imagery: a functional magnetic resonance imaging study. *J Neurosci, 16*(23), 7688-7698.

Praamstra, P., Torney, L., Rawle, C. J., & Miall, R. C. (2011). Misconceptions about mirror-induced motor cortex activation. *Cereb Cortex, 21*(8), 1935-1940. doi: 10.1093/cercor/bhq270

Ramachandran, V. S., & Rogers-Ramachandran, D. (1996). Synaesthesia in phantom limbs induced with mirrors. *Proc Royal Soc London - Biol Sci, 263*, 377-386.

Ramachandran, V. S., Rogers-Ramachandran, D., & Cobb, S. (1995). Touching the phantom limb. *Nature, 377*, 489-490.

Regenbrecht, H., Hoermann, S., McGregor, G., Dixon, B., Franz, E., Ott, C., . . . Hoermann, J. (2012). Visual manipulations for motor rehabilitation. *Computers & Graphics, 36*(7), 819–834.

Rizzolatti, G. (2014, March). *The mirror mechanism – a neural mechanism to understand others.* Paper presented at the 30. International Congress of Clinical Neurophysiology (ICCN), Berlin.

Rizzolatti, G., Fabbri-Destro, M., & Cattaneo, L. (2009). Mirror neurons and their clinical relevance. *Nat Clin Pract Neurol, 5*(1), 24-34. doi: 10.1038/ncpneuro0990

Rondot, P., de Recondo, J., & Dumas, J. L. (1977). Visuomotor ataxia. *Brain, 100*(2), 355-376.

Rossi, S., Tecchio, F., Pasqualetti, P., Ulivelli, M., Pizzella, V., Romani, G. L., . . . Rossini, P. M. (2002). Somatosensory processing during movement observation in humans. *Clin Neurophysiol, 113*(1), 16-24.

Saleh, S., Adamovich, S. V., & Tunik, E. (2013). Visual Feedback Discordance Mediates Changes in Brain Activity and Effective Connectivity: A Stroke fMRI Dynamic Causal Modeling Study. *2nd International Conference on Advances in Biomedical Engineering*.

Sambo, C. F., Gillmeister, H., & Forster, B. (2009). Viewing the body modulates neural mechanisms underlying sustained spatial attention in touch. *Eur J Neurosci, 30*(1), 143-150. doi: 10.1111/j.1460-9568.2009.06791.x

Sanes, J. N., Donoghue, J. P., Thangaraj, V., Edelman, R. R., & Warach, S. (1995). Shared neural substrates controlling hand movements in human motor cortex. *Science, 268*(5218), 1775-1777.

Schaefer, M., Heinze, H. J., & Rotte, M. (2005a). Seeing the hand being touched modulates the primary somatosensory cortex. *Neuroreport, 16*(10), 1101-1105.

Schaefer, M., Heinze, H. J., & Rotte, M. (2005b). Viewing touch improves tactile sensory treshold. *Neuroreport, 16*(4), 367-370.

Schieber, M. H. (1990). How might the motor cortex individuate movements? *Trends Neurosci, 13*(11), 440-445.

Schwartz, A. B. (1994). Distributed motor processing in cerebral cortex. *Curr Op Neurobiology, 4*, 840-846.

Song, Y. M., Lee, J. Y., Park, J. M., Yoon, B. W., & Roh, J. K. (2005). Ipsilateral hemiparesis caused by a corona radiata infarct after a previous stroke on the opposite side. *Arch Neurol, 62*(5), 809-811. doi: 10.1001/archneur.62.5.809

Steinbrink, J., Villringer, A., Kempf, F., Haux, D., Boden, S., & Obrig, H. (2006). Illuminating the BOLD signal: combined fMRI-fNIRS studies. *Magn Reson Imaging, 24*(4), 495-505.

Stevens, J. A., & Stoykov, M. E. (2003). Using motor imagery in the rehabilitation of hemiparesis. *Arch Phys Med Rehabil, 84*(7), 1090-1092.

Stevens, J. A., & Stoykov, M. E. (2004). Simulation of bilateral movement training through mirror reflection: A case report demonstrating an occupational therapy technique for hemiparesis. *Top Stroke Rehabil, 11*(1), 59-66.

Strangman, G., Goldstein, R., Rauch, S., & Stein, J. (2006). Near-infrared spectroscopy and imaging for investigating stroke rehabilitation: test-retest reliability and review of the literature. *Arch Phys Med Rehabil, 87*(12 Suppl 2), S12-19.

Sun, F., Miller, L., Rao, A., & D'Esposito, M. (2007). Functional Connectivity of Cortical Networks Involved in Bimanual Motor Sequence Learning. *Cerebral Cortex, 17*, 1227-1234. doi: 10.1093/cercor/bhl033

Takahashi, T., Takikawa, Y., Kawagoe, R., Shibuya, S., Iwano, T., & Kitazawa, S. (2011). Influence of skin blood flow on near-infrared spectroscopy signals measured on the forehead during a verbal fluency task. *Neuroimage, 57*(3), 991-1002. doi: 10.1016/j.neuroimage.2011.05.012

Taub, E., Crago, J. E., & Uswatte, G. (1998). Constraint-induced movement therapy: a new approach to treatment in physical medicine. *Rehabil Psychol, 43*, 152-170.

Taub, E., Miller, N. E., Novack, T. A., Cook, E. W., 3rd, Fleming, W. C., Nepomuceno, C. S., . . . Crago, J. E. (1993). Technique to improve chronic motor deficit after stroke. *Arch Phys Med Rehabil, 74*(4), 347-354.

Taub, E., Uswatte, G., & Elbert, T. (2002). New treatments in neurorehabilitation founded on basic research. *Nat Rev Neurosci, 3*(3), 228-236. doi: 10.1038/nrn754

Thieme, H., Bayn, M., Wurg, M., Zange, C., Pohl, M., & Behrens, J. (2013). Mirror therapy for patients with severe arm paresis after stroke--a randomized controlled trial. *Clin Rehabil, 27*(4), 314-324. doi: 10.1177/0269215512455651

Thieme, H., Mehrholz, J., Pohl, M., Behrens, J., & Dohle, C. (2012). Mirror therapy for improving motor function after stroke (Review). *The Cochrane Library*(3).

Tominaga, W., Matsubayashi, J., Deguchi, Y., Minami, C., Kinai, T., Nakamura, M., . . . Mitani, A. (2009). A mirror reflection of a hand modulates stimulus-induced 20-Hz activity. *Neuroimage, 46*(2), 500-504.

Touzalin-Chretien, P., & Dufour, A. (2008). Motor cortex activation induced by a mirror: evidence from lateralized readiness potentials. *J Neurophysiol, 100*(1), 19-23.

Twitchell, T. E. (1951). The restoration of motor function following hemiplegia in man. *Brain, 74*(4), 443-480.

Uswatte, G., & Taub, E. (2013). Constraint-induced movement therapy: a method for harnessing neuroplasticity to treat motor disorders. *Prog Brain Res, 207*, 379-401. doi: 10.1016/B978-0-444-63327-9.00015-1

Vaalto, S., Julkunen, P., Saisanen, L., Kononen, M., Maatta, S., & Karhu, J. (2013). Long-term plasticity may be manifested as reduction or expansion of cortical representations of actively used muscles in motor skill specialists. *Neuroreport, 24*(11), 596-600. doi: 10.1097/WNR.0b013e3283628636

Van Overwalle, F., & Baetens, K. (2009). Understanding others' actions and goals by mirror and mentalizing systems: a meta-analysis. *Neuroimage, 48*(3), 564-584. doi: 10.1016/j.neuroimage.2009.06.009

Wall, P. D., & Egger, M. D. (1971). Formation of new connexions in adult rat brains after partial deafferentation. *Nature, 232*(5312), 542-545.

Wang, J., Fritzsch, C., Bernarding, J., Krause, T., Mauritz, K. H., Brunetti, M., & Dohle, C. (2013). Cerebral activation evoked by the mirror illusion of the hand in stroke patients compared to normal subjects. *NeuroRehabilitation*. doi: 10.3233/NRE-130999

Wasaka, T., & Kakigi, R. (2012). Conflict caused by visual feedback modulates activation in somatosensory areas during movement execution. *Neuroimage, 59*(2), 1501-1507. doi: 10.1016/j.neuroimage.2011.08.024

White, B. R., Snyder, A. Z., Cohen, A. L., Petersen, S. E., Raichle, M. E., Schlaggar, B. L., & Culver, J. P. (2009). Resting-state functional connectivity in the human brain revealed with diffuse optical tomography. *Neuroimage, 47*(1), 148-156.

Wolpert, D. M. (1997). Computational approaches to motor control. *Trends Cogn Sci, 1*(6), 209-216. doi: 10.1016/S1364-6613(97)01070-X

Wu, C. Y., Huang, P. C., Chen, Y. T., Lin, K. C., & Yang, H. W. (2013). Effects of mirror therapy on motor and sensory recovery in chronic stroke: a randomized controlled trial. *Arch Phys Med Rehabil, 94*(6), 1023-1030. doi: 10.1016/j.apmr.2013.02.007

Yamada, T., Umeyama, S., & Matsuda, K. (2009). Multidistance probe arrangement to eliminate artifacts in functional near-infrared spectroscopy. *J Biomed Opt, 14*(6), 064034. doi: 10.1117/1.3275469

Yamamoto, S., Takasawa, M., Kajiyama, K., Baron, J. C., & Yamaguchi, T. (2007). Deterioration of hemiparesis after recurrent stroke in the unaffected hemisphere: Three further cases with possible interpretation. *Cerebrovasc Dis, 23*(1), 35-39. doi: 10.1159/000095756

Yavuzer, G., Selles, R., Sezer, N., Sutbeyaz, S., Bussmann, J. B., Koseoglu, F., . . . Stam, H. J. (2008). Mirror therapy improves hand function in subacute stroke: a randomized controlled trial. *Arch Phys Med Rehabil, 89*(3), 393-398.

Yttri, E. A., Wang, C., Liu, Y., & Snyder, L. H. (2014). The parietal reach region is limb specific and not involved in eye-hand coordination. *J Neurophysiol, 111*(3), 520-532. doi: 10.1152/jn.00058.2013

Zeff, B. W., White, B. R., Dehghani, H., Schlaggar, B. L., & Culver, J. P. (2007). Retinotopic mapping of adult human visual cortex with high-density diffuse optical tomography. *Proc Natl Acad Sci U S A, 104*(29), 12169-12174. doi: 10.1073/pnas.0611266104

Internet sources of the figures

Figure 1:

http://classconnection.s3.amazonaws.com/917/flashcards/770260/png/homunculus.png

Retrieved the 20th November, 2014 at 2:35 pm

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http://en.wikipedia.org/wiki/Brodmann_area_7

Retrieved the 26th February, 2014 at 11:43 am

Abstract

Stroke is the second leading cause of death worldwide and one of the leading causes for disability at advanced age. In about one third of the patients, stroke causes hemiplegia, complete paralysis of the upper limb. Low rates of complete recovery even after intensive rehabilitation procedures are probably due to the lack of appropriate therapy strategies for hemiplegic patients. The aim of the present thesis was to examine the underlying neuronal mechanisms of mirror therapy (MT) that creates a mirror illusion by placing a mirror in the mid-sagittal plane in front of the sitting patient, so that the patient sees the reflection of the movements of the non-affected limb as if they were movements from the affected one. Another therapy procedure implementing visual input in motor recovery is Video Therapy (VT) that is based on mere observation of a third person's movements.

It was hypothesized that the mirror neuron system (MNS) and the primary motor cortex (M1) might not be directly modulated by the mirror illusion, in contrast to precuneus (PC) and the primary somatosensory cortex (S1). PC plays a crucial role during the on-line control of hand movements and is part of a crucial node of the fronto-parietal network for planning and execution of visuo-motor tasks. S1 is known to be involved in motor learning and recovery. Moreover, it was suggested that VT and MT elicit different activation patterns. Beyond that, an exploratory examination was conducted to determine if the lateralized precuneal activation observed in normal subjects might be likewise observable in stroke patients or if it differs on a single-subject level or due to pathological factors. Finally, on the basis of the results of healthy controls, it was hypothesized that the lateralized precuneal activation due to the mirror illusion might be a determining factor to classify MT responders and non-responders. The methods of choice were functional near-infrared-spectroscopy (fNIRS) as well as functional magnetic resonance imaging (fMRI).

Different activation patterns underlying MT and VT were found. While MT elicits lateralized precuneal activation always contralateral to the observed hand, this was not found during VT. Furthermore, the results demonstrate dissociated lateralization patterns on different ROIs: Activity in M1 is lateralized opposite to the *moving* hand and in PC opposite to the *visually perceived* hand, independent of each other. For S1 lateralization changed from ipsilateral to symmetrical, for the left hand only. Moreover, it is confirmed that the mirror illusion does not modulate the MNS. Beyond that, lateralized precuneal activation due to mirror illusion is similarly observed in stroke patients and seems to be a potential candidate for determination of MT efficacy additionally to motor functional base level of the affected hand.

It is concluded that MT operates by inverting the interhemispheric imbalance in favor of the affected hemisphere across PC. Thereby, PC seems to be an intermediary structure that receives and processes the visual feedback of the hand in the mirror and transfers it across its intracortical connections to the ipsilesional premotor cortex and SMA. An additional beneficial effect due to immediate S1 modulation during MT could be exerted across the strong interconnections between M1 and S1.

The findings of the thesis at hand contribute to a better understanding of the underlying neuronal mechanisms of MT and provide a potential neurophysiological predictor for MT efficacy. A basis is created for further fNIRS and fMRI connectivity analysis of underlying neuronal networks across which MT might operate.

Zusammenfassung

Schlafanfall belegt weltweit Rang Zwei der Todesursachen und ist eine der Hauptursachen für eine erworbene Behinderung im Alter. Bei circa einem Drittel der Menschen, verursacht Schlaganfall Hemiplegie, d.h. eine komplette Halbseitenlähmung der oberen Extremität. Die niedrigen Raten für eine vollständige Erholung, selbst nach intensiver Rehabilitation, sind vermutlich auf ungeeignete Therapiestrategien für Patienten mit Hemiplegie zurückzuführen. Ziel der vorliegenden Arbeit war es, die zugrundeliegenden, neuronalen Mechanismen der Spiegeltherapie (ST) zu untersuchen. ST erzeugt eine Spiegelillusion, indem ein Spiegel sagittal vor dem sitzenden Patienten aufgestellt wird, sodass er das Spiegelbild der Handbewegungen seiner nicht betroffenen Seite als Handbewegungen seiner gelähmten Seite wahrnimmt. Eine weitere Therapie, die auf visuellen Input zur motorischen Erholung nach Schlaganfall setzt, ist die Videotherapie (VT). Sie basiert auf reiner Beobachtung von Handbewegungen einer dritten Person.

Es wurde angenommen, dass das Spiegelneuronen System (SNS) und der primär motorische Kortex (M1) nicht direkt durch die Spiegelillusion moduliert werden, im Gegensatz zum Precuneus (PC) und primär somatosensorischen Kortex (S1). PC spielt eine wichtige Rolle bei der on-line Kontrolle von Handbewegungen und ist Teil eines relevanten Knotenpunktes des fronto-parietalen Netzwerks zur Planung und Durchführung visuell-motorischer Aufgaben. S1 ist bekanntlich maßgebend an motorischem Lernen und motorischer Erholung beteiligt. Darüber hinaus wurde angenommen, dass ST und VT unterschiedliche Aktivierungsmuster hervorrufen. Zusätzlich wurde exploratorisch untersucht, ob die lateralisierte Aktivierung des PC, die bereits bei Gesunden beobachtet wurde, auch bei Schlaganfallpatienten zu beobachten sei, oder ob sich diese auf Einzelsubjektebene, bzw. aufgrund pathologischer Faktoren unterscheide. Schließlich wurde analysiert, ob die lateralisierte Aktivierung des PC durch die Spiegelillusion ein entscheidender Faktor zur Klassifizierung von ST Respondern und Non-Respondern sei. Die Methoden der Wahl waren

funktionelle Nah-Infrarot-Spektroskopie (fNIRS), sowie funktionelle Magnetresonanz-tomographie (fMRT).

ST und VT führten zu unterschiedlichen Aktivierungsmustern. Während ST eine lateralisierte Aktivierung des PC kontralateral zur wahrgenommenen Hand hervorrief, war dies bei VT nicht der Fall. Außerdem fanden sich unterschiedliche, voneinander unabhängige Lateralisierungsmuster: Während M1 Aktivierung immer kontralateral zur sich *bewegenden* Hand lateralisiert war, war PC kontralateral zur *visuell wahrgenommenen* Hand lateralisiert. S1 Lateralisierung veränderte sich durch die Spiegelillusion von ipsilateral zu symmetrisch, allerdings nur für die linke Hand. Darüber hinaus bestätigten die Ergebnisse, dass das SNS nicht direkt moduliert wird. Schließlich wurde die lateralisierte PC Aktivierung ähnlich auch bei Schlaganfallpatienten nachgewiesen und scheint ein potentieller Faktor zur Vorhersage der ST Wirksamkeit zu sein, zusätzlich zum funktionell motorischen Ausgangsniveau der betroffenen Hand.

Es lässt sich also schlussfolgern, dass ST eine Umkehrung des interhemisphärischen Ungleichgewichts zugunsten der betroffenen Hemisphäre mittels PC bewirkt. Dabei scheint PC eine zwischengeschaltete Struktur zu sein, die Informationen zum visuellen Feedback der gespiegelten Hand erhält und verarbeitet und es dann weiter über intrakortikale Verbindungen zum ipsiläsionalen prämotorischen Kortex und supplementär motorischen Kortex übermittelt. Ein zusätzlicher heilsamer Effekt wird über die Verbindungen zwischen M1 und S1 ausgeübt, indem S1 während der ST direkt moduliert wird.

Die Erkenntnisse der vorliegenden Arbeit tragen zu einem besseren Verständnis der zugrundeliegenden neuronalen Wirkmechanismen der ST bei und liefern zudem einen potentiellen neurophysiologischen Prädiktor für die Wirksamkeit der ST. Es wird die Grundlage für zukünftige fNIRS und fMRT Konnektivitätsanalysen geschaffen, welche die zugrundeliegenden neuronalen Netzwerke weiter durchleuchten können, über welche die ST wirkt.

List of publications

Brunetti, M., Morkisch, N., Fritzsch, C., Mehnert, J., Steinbrink, J., Niedeggen, M., & Dohle, C. Potential determinants of efficacy of mirror therapy in stroke patients - a pilot study. (under revision in Restor Neurol Neurosci)

Fritzsch, C., Wang, J., Dos Santos, L. F., Mauritz, K. H., Brunetti, M., & Dohle, C. (2014). Different effects of the mirror illusion on motor and somatosensory processing. *Restor Neurol Neurosci, 32*(2), 269-280. doi: 10.3233/RNN-130343

Mehnert, J.*, Brunetti, M.*, Steinbrink, J., Niedeggen, M., & Dohle, C. (2013). Effect of a mirror-like illusion on activation in the precuneus assessed with functional near-infrared spectroscopy. *J Biomed Opt, 18*(6), 066001. doi: 10.1117/1.JBO.18.6.066001

Wang, J., Fritzsch, C., Bernarding, J., Holtze, S., Mauritz, K. H., Brunetti, M., & Dohle, C. (2013). A comparison of neural mechanisms in mirror therapy and movement observation therapy. *J Rehabil Med, 45*(4), 410-413. doi: 10.2340/16501977-1127

Wang, J., Fritzsch, C., Bernarding, J., Krause, T., Mauritz, K. H., Brunetti, M., & Dohle, C. (2013). Cerebral activation evoked by the mirror illusion of the hand in stroke patients compared to normal subjects. *NeuroRehabilitation*. doi: 10.3233/NRE-130999

* Both authors contributed equally to this work.

Conference proceedings

Brunetti, M., Mehnert, J., Steinbrink, J., Niedeggen, M., & Dohle, C. (2011). *Effekt der Spiegelillusion auf die Hirnaktivität im Praecuneus gemessen mit der Nahinfrarotspektroskopie.* Poster presented at the 84. Kongress der Deutschen Gesellschaft für Neurologie mit Fortbildungsakademie, Wiesbaden, Germany.

Brunetti, M., Wang, J., Fritzsch, C., Bernarding, J., Holtze, S., Mauritz, K.-H., & Dohle, C. (2012). *Experience of agency during movement observation causes lateralized cerebral activations.* Poster presented at the 56. Jahrestagung der Deutschen Gesellschaft für Klinische Neurophysiologie und funktionelle Bildgebung, Köln, Germany.

Fritzsch, C., Wang, J., Mauritz, K.-H., Dos Santos, L., Brunetti, M., & Dohle, C. (2013). *Different effects of mirror therapy on motor and sensory processing.* Poster presented at the 57. Jahrestagung der Deutschen Gesellschaft für Klinische Neurophysiologie und funktionelle Bildgebung, Leipzig, Germany.

Curriculum Vitae

Der Lebenslauf ist in der Online-Version

aus Gründen des Datenschutzes nicht enthalten.

Appendix

Publication 1

"A comparison of neural mechanisms in mirror therapy and movement observation therapy"

Wang, J., Fritzsch, C., Bernarding, J., Holtze, S., Mauritz, K. H., Brunetti, M., & Dohle, C.

http://dx.doi.org/10.2340/16501977-1127

Publication 2

"Different effects of the mirror illusion on motor and somatosensory processing"

Fritzsch, C., Wang, J., Mauritz, K.-H., Dos Santos, L., Brunetti, M., & Dohle, C.

http://dx.doi.org/10.3233/RNN-130343

Publication 3

"Effect of a mirror-like illusion on activation in the precuneus assessed with functional near-infrared spectroscopy"

Mehnert, J., Brunetti, M., Steinbrink, J., Niedeggen, M., & Dohle, C.

http://dx.doi.org/10.1117/1.JBO.18.6.066001

Publication 4

"Cerebral activation evoked by the mirror illusion of the hand in stroke patients compared to normal subjects"

Wang, J., Fritzsch, C., Bernarding, J., Krause, T., Mauritz, K. H., Brunetti, M., & Dohle, C.

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Manuscript 5 (under revision)

"Potential determinants of efficacy of mirror therapy in stroke patients - a pilot study"

Brunetti, M., Morkisch, N., Fritzsch, C., Mehnert, J., Steinbrink, J., Niedeggen, M., & Dohle, C.

Potential determinants of efficacy of mirror therapy in stroke patients - a pilot study

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Abstract

Background: Mirror therapy (MT) was found to improve motor function after stroke. However, there is high variability between patients regarding motor recovery.

Objectives: The following pilot study was designed to identify potential factors determining this variability between patients with severe upper limb paresis, receiving MT.

Methods: Eleven sub-acute stroke patients with severe upper limb paresis were recruited, receiving in-patient rehabilitation. After a set of pre-assessments (including measurement of brain activity at the primary motor cortex and precuneus during the mirror illusion, using near-infrared spectroscopy as described previously (Mehnert, Brunetti, Steinbrink, Niedeggen, & Dohle, 2013)), four weeks of MT were applied including recording of performance indicators during therapy, followed by a set of post-assessments. Discriminant group analysis for MT responders and non-responders was performed.

Results: Six out of eleven patients were defined as responders and five as non-responders on the basis of their functional motor improvement. The initial motor function and the activity shift in both precunei (mirror index) were found to discriminate significantly between responders and non-responders.

Conclusions: In line with earlier results, initial motor function was confirmed as crucial determinant of motor recovery. Additionally, activity response to the mirror illusion in both precunei was found to be a candidate for determination of the efficacy of MT.

Keywords: mirror therapy, stroke, motor recovery, fNIRS, precuneus

1. Introduction

Today, in the aging society of industrial countries, stroke is the leading cause for disability (Johnston, Mendis, & Mathers, 2009; Murray, 1996). About one third of all stroke patients suffer from a severe arm paresis (Heller et al., 1987; Jorgensen et al., 1995). Therapy for patients with severe arm paresis is largely focused on assisted movements of the affected limb (Broeks, Lankhorst, Rumping, & Prevo, 1999). Many therapies requiring active movements, like constraint-induced therapy (Taub, Crago, & Uswatte, 1998) or repetitive active arm training, are not realizable for patients with severe arm paresis. Given that the severely affected paretic arm is mostly accompanied by additional somatosensory deficits (Broeks et al., 1999), this patient group is furthermore disadvantaged.

An alternative is the application of visual input in the therapy, e.g. by mirror therapy (MT). In MT, a mirror is placed mid-sagittally on a table in front of the sitting patient, with the affected limb placed behind and the non-affected in front of the mirror. When the patient looks into the mirror, the movements of his non-affected arm mask the view of the affected arm, eliciting the so called mirror-illusion (Ramachandran, 1994). Especially over the last years, several studies demonstrated the beneficial effects of MT on upper limb motor function, activities of daily living (Thieme, Mehrholz, Pohl, Behrens, & Dohle, 2012) and pain for patients after stroke (Bowering et al., 2013). However, inter-individual variability in the outcome can be high: In randomized clinical trials, some patients took benefit from the application of MT while others did not, so that patients might be classified as MT "responders" and "non-responders" (Dohle et al., 2009). Up to now, it is unknown which factors determine these differences on efficacy of the MT.

The outcome is probably determined by the functional state of the neural mechanisms mediating the effect of MT. However, the underlying neuronal mechanisms of MT are not

fully understood. Some researchers assumed the mirror neuron system (MNS) to play a crucial role in the effect of MT (Hamzei et al., 2012). The MNS is activated during observation of one's own or another person's limb movements (Rizzolatti, Fabbri-Destro, & Cattaneo, 2009). Another possible mediating structure identified in fMRI studies is the precuneus (PC). In normal subjects, Wang and colleagues observed differences in the cerebral activation patterns during movement observation and movement mirroring: While the mirrored visual feedback of one's own hand elicited additional activation in the PC strictly lateralized contralateral to the perceived hand, the observation of a third person's hand showed no such lateralization (Wang, Fritzsch, Bernarding, Holtze, et al., 2013). These results are in line with previous findings of Dohle and colleagues, who found additional activation of PC due to the mirror strictly contralateral to the perceived hand (Dohle, Kleiser, Seitz, & Freund, 2004). Results from a study on a single-subject analysis level indicate that this effect seems to be similar when comparing normal subjects and stroke patients with severe arm paresis (Wang, Fritzsch, Bernarding, Krause, et al., 2013).

This effect of the mirror illusion on activity in PC was confirmed using functional near-infrared-spectroscopy (fNIRS). Just as fMRI, fNIRS measures the blood oxygen level dependent (BOLD) signal, recording oxygenated (HBO) and deoxygenated (HBR) blood in the cerebral cortex (Obrig & Villringer, 2003), but is easier to apply, especially in a clinical setting (Muehlschlegel et al., 2009; Strangman, Goldstein, Rauch, & Stein, 2006). In their fNIRS study on the mirror illusion in normal subjects, Mehnert and colleagues (2013) not only confirmed the above mentioned fMRI results that ipsilateral activations in the PC increase due to the mirror illusion. Additionally, they demonstrated that this was accompanied by decreased activation of the contralateral PC, while no such interaction was found for activation of the primary motor cortex (M1). In other words, the limb specific representation in PC is activated according to the observed limb (Mehnert et al., 2013).

That means that at least for PC, the mirror illusion changes the inter-hemispheric activation balance towards the ipsilateral hemisphere. Translating this phenomenon observed in normal subjects into patients obtaining MT, this would mean that the mirror illusion changes the inter-hemispheric activation balance towards the affected hemisphere. It is well known by now that the unaffected hemisphere could further inhibit the affected hemisphere via transcallosal inhibition mechanisms, thus deteriorating motor performance (Calautti et al., 2007) and that inversion of the inter-hemispheric activation balance in favor of the affected hemisphere could facilitate motor recovery (Grefkes et al., 2010). Thus, it might be assumed that MT works via this inversion of the inter-hemispheric activation balance across PC.

Given that the PC mediates the effect of MT and shows a remarkable inter-individual variability, we assumed that the responsiveness of PC might serve as a predictor for the success of MT. Therefore, the following study was designed to correlate the inversion of the inter-hemispheric activation balance in PC with therapy success due to MT. In order to estimate the contribution of the initial PC activation following stroke, other factors affecting the motor recovery after stroke, such as initial motor function, lesioned hemisphere, demographic data and neuropsychological status were considered as well. We hypothesized that patients who show an inversed inter-hemispheric activation balance in PC by the mirror illusion will more likely benefit from MT and be classified as responders than patients who do not show such an inversion.

Besides, we aimed to explore whether this inversion of inter-hemispheric activation balance in PC by the mirror illusion changes after the application of MT.

2. Materials and methods

2.1. Participants - inclusion and exclusion criteria

Stroke patients with severe arm paresis were recruited in the department of inpatient rehabilitation at the MEDIAN Klinik Berlin-Kladow from August 2010 to August 2011. Patients had to suffer from a first-ever unilateral ischemic stroke confirmed by computer tomography (CT) and/or MRI resulting in severe unilateral arm paresis with a wrist extension ability of less than 20 degrees and a metacarpophalangeal joint extension ability of less than 10 degrees. Patients had to be able to understand the instructions and to sustain the pre- and post-assessments and the daily 30 minutes of MT as described below. Exclusion criteria were previous experienced strokes or orthopedic, rheumatic or other diseases curtailing their ability to move the non-affected upper limb. The study was approved by the Ethics Committee of the Charité - Universitätsmedizin Berlin. 13 patients (six female, seven male) fulfilled these criteria during the recruitment period and were included in the study. All subjects gave informed consent prior to participation.

2.2. Study design

The study was applied in addition to the regular inpatient rehabilitation program. Participants underwent a set of physiological and behavioral pre-assessments, consisting of Fugl-Meyer assessment (FMA), motor and somatosensory evoked potentials (MEPs and SSEPs), Barthel-index (BI), neuropsychological testing and functional near infrared spectroscopy of the mirror illusion (fNIRS), all applied within one week. Afterwards, the participants received four weeks of MT with recording of the percentage of ideal alertness (PIA) during therapy. Post-assessments were the same as the pre-assessments except for the recording of MEPs and SSEPs.

2.3. Assessments

2.3.1. Fugl-Meyer assessment

Upper extremity motor subscales of the Fugl-Meyer assessment (FMA) was conducted and video-taped (and rated) by trained occupational therapists in order to measure upper limb motor function, consisting of finger, hand, and arm function measurements (Fugl-Meyer, Jaasko, Leyman, Olsson, & Steglind, 1975). Reflex and coordination items were left out due to its poor psychometric properties (Woodbury et al., 2007). After data acquisition, FMA was rated by another blinded trained occupational therapist, who was neither involved in measurements nor in therapy procedure. As a previous study on severe arm paresis after stroke found an effect of MT especially on distal upper limb function (Dohle et al., 2009), only the subscore for finger movements was taken into account for further analysis. In this subscore, a total sum of 14 points could be achieved.

2.3.2. MEPs and SSEPs

MEPs were elicited using a flat coil and a stimulator (MAGSTIM 200, Novametrix Medical Systems, USA) and recorded at the fist dorsal interosseus muscle via surface electromyography (EMG) recording machine (Medtronic Keypoint V5.06, Medtronic A/S, Skovlunde, Denmark).

SSEPs were elicited using a stimulator (Medtronic, USA) to induce electrical stimulation of the ulnar nerve and recorded at the somatosensory cortex of the patient's scalp by an EEG electrode (Medtronic, USA).

MEPs and SSEPs recorded on both sides - ipsi- and contralesional side - were compared and results from the affected side were rated by a trained neurologist. As previous studies indicated that the presence, rather than the magnitude, of the evoked potentials was crucial for recovery (Hendricks, Hageman, & van Limbeek, 1997; Wohrle, Behrens, Mielke,

& Hennerici, 2004), they were rated with 0, if not derivable and with 1, if they were recorded at all.

2.3.3. Barthel index

Ability in activities of daily living (ADL) was assessed by the 100 points Barthel index (BI) (Mahoney & Barthel, 1965) recorded by an experienced nurse who knew the patient from daily ward routine.

2.3.4. Neuropsychological measurements

For assessment of attention, the trail making test (TMT, German: Zahlenverbindungstest) a subscale of the "Nürnberger Altersinventar", which is a speed test, was conducted by the patients with their non-affected hand. Mean speed time was recorded and used as index for overall cognitive fluency and neuropsychological status. The "Nürnberger Altersinventar" is an intelligence test also validated for an older inpatients sample and therefore applicable for the patients' population of the present study (Oswald, 1986). To control for potential advantages for patients that performed the TMT with their dominant hand (right hemisphere lesions), an univariate ANOVA for the mean reaction time with the factor hand (dominant / non-dominant) was conducted, with a significance level of $p = 0.05$.

2.3.5. FNIRS measurement

The paradigm for recording the mirror index (MI) due to the mirror induced illusion with fNIRS is based on an earlier fNRIS study of Mehnert and colleagues in normal subjects (Mehnert et al., 2013).

2.3.5.1. Experimental task

Patients had to perform a finger-thumb opposition movement task with their non-affected hand under online visual control. Neither hand could be seen directly, but the non-affected, moving hand could be seen via a video chain on a screen placed in front of the patient. By means of a software package (Logitech Webcam Software v1.1, frame rate: 50 Hertz) the image was displayed in real-time with 0° eccentricity on the screen (Acer, 1280 x 1024 Pixel, frame rate: 60 Hertz) and could be inverted horizontally in such a way that the subjects' right hand appeared as if it was their left hand and vice versa ("mirror"). This setup provided two possible conditions: normal visual feedback (NOR) and mirrored visual feedback (MIR). Each trial was initiated by an acoustic cue ("start") and lasted 10 seconds, followed by a rest period with an average duration of 15 seconds (jittered from 10-20 seconds) during which a plain grey screen was shown, serving as baseline for the correction in the analysis. Each condition was presented randomly 20 times, resulting in a total measurement time of 20 minutes. The measurement was interrupted after 10 minutes by the investigator, in order to give the patients a small break and ensure their alertness on the task. In contrast to the previous study on normal subjects (Mehnert et al., 2013), no static task with no hand movement was required, in order to reduce the measurement time for the patients and thus make it more convenient. The patients' adherence to the task was ensured by the investigator who was present during the entire experiment.

2.3.5.2. Data acquisition

During the experiment, the blood oxygenation at the surface of the subjects' brain was measured with an fNIRS system which offers up to 16 detectors and 8 emitters (NIRScout 8-16, NIRx Medizintechnik GmbH, Berlin, Germany) at two wavelengths (850 and 760 nm). FNIRS data were continuously sampled at 6.26 Hz, instead of 3.13 Hz as applied by Mehnert

and colleagues, due to the lower number of emitters. Based on previous findings on the role of the PC during movement mirroring (Dohle et al., 2004; Dohle et al., 2011; Mehnert et al., 2013), we chose optode positions to cover the occipito-parietal and precentral areas of the subject's head bilaterally, providing a total of 24 useful channels where source and detector were placed at a distance between 2.5 and 3 cm from each other (Figure 1). Emitters and detectors were integrated into a commercially available EEG cap (www.easycap.de) with 128 possible positions.

2.3.5.3. Pre-processing of fNIRS data and regions of interest (ROI)

Data analysis was performed using software routines employing the software packet Matlab (Mathworks Inc., USA, student version R2012a, 7.14.0.739). FNIRS data were corrected for movement artifacts by a semi-automated approach, which replaces contaminated data segments by linear interpolation (Koch, Werner, Steinbrink, Fries, & Obrig, 2009). Subsequently, attenuation changes of both wavelengths were transformed to concentration changes of oxy- and deoxygenated hemoglobin (HBO and HBR) using a modified Beer-Lambert law (Differential Path length Factors: 5.98 (higher wavelength: 850 nm), 7.15 (lower wavelength: 760 nm), extinction coefficients for HBO 2.53/1.49 (higher/lower wavelength) and HBR 1.80/3.84 (higher/lower wavelength), and an inter-optode-distance of 3 cm)(Cope & Delpy, 1988). Data were then band-pass filtered between 0.2 and 0.016 Hz (using a 3rd order Butterworth filter) to attenuate for heartbeat, breathing-related changes and drifts.

A GLM was performed and the resulting beta-values were averaged across the channels of the regions of interest (ROIs). ROIs as shown in Figure 1 (M1-ROI: red, PC-ROI: green) were defined in line with the identified ROIs of the above-mentioned study (Mehnert et al., 2013). For visual inspection and demonstration, the freeware MATLAB toolbox NFRI (http://www.jichi.ac.jp/brainlab/tools.html) was employed (Singh, Okamoto, Dan, Jurcak, &

Dan, 2005), which takes EEG 10-20 positions as references to estimate brain regions underlying the channel locations.

2.3.5.4. Calculation of the mirror index

As Mehnert and colleagues (2013) found an interaction between the factors hemisphere and mirror due to the mirror illusion, a so-called mirror index MI was calculated for both ROIs as following:

$$MI = (Ipsi\_MIR - Ipsi\_NOR) + (Contra\_NOR - Contra\_MIR)$$

Ipsi_MIR refers to the activation in the ipsilesional hemisphere under mirrored visual feedback, Ipsi_NOR stands for the activation in the ipsilesional hemisphere under normal visual feedback; and Contra_NOR and Contra_MIR stand for the activations in the contralesional hemisphere under the respective condition.

Thus, the MI quantifies the sum of augmentation of activation in the ipsilesional hemisphere and decrease of activation in the contralesional hemisphere due to the mirror illusion. Higher values of the MI indicate a stronger influence of the mirrored visual feedback on inter-hemisphere activation balance. The value MI-PC refers to the index obtained in the precuneus region, the value MI-M1 to the value in the primary motor area (M1). As the interaction between hemisphere and mirror was found to be significant for the oxygenated hemoglobin (HBO) only, the MI was calculated only for HBO (Mehnert et al., 2013).

2.4. Application of MT

During MT, a mirror was placed on the mid sagittal plane on a table in front of the patients. The paretic limb was placed behind the mirror, the non-affected arm was in front of the mirror and the gaze was oriented towards the mirror. The MT protocol was a variant of "Bonn therapy protocol" used in a previous study (Dohle et al., 2009), requiring presentation

of abstract limb positions in response to verbal commands (Bieniok, Govers, & Dohle, 2009). The Bonn therapy protocol is mainly based on proximal movements combined with distal variations, but since the clinical study (Dohle et al., 2009) indicated mainly effects on distal motor function, a "Berlin variant" of this protocol was defined, working from distal to proximal (Morkisch, Lommack, Kadow, Rietz, & Dohle, 2012).

MT was conducted for 4 weeks, 5 days per week, for 30 minutes daily. Applying occupational therapists rotated across patients in order to exclude a therapist effect. Each therapist recorded the exact time (in minutes) the patients gazed into the mirror. For each therapy session the patients' attention was rated from one to four (1 = unchallenged, 2 = concentrated, 3 = temporary focused, 4 = overstrained). Thus, a score of two points represented an ideal amount of attention. For further analysis, the amount of time spent in the ideal attention was set in proportion to the total amount of time spent gazing into the mirror, providing the value of PIA (percentage of ideal attention) in the moment of MT.

2.5. Other rehabilitation services

Following other rehabilitation services were delivered during the intervention time: Physiotherapy (upper and lower extremity, Apotic), activities of daily living (ADL), occupational therapy (upper extremity, ADL) and others, such as neuropsychology or logopedics. The different therapies were rearranged in four types:

1. ADL (occupational therapy)
2. Upper extremity (physiotherapy, occupational therapy and Apotic)
3. Lower extremity (physiotherapy, treadmill)
4. Others (neuropsychology, logopedics, massage)

The therapy units (1 unit = 30 min) that were delivered during the four weeks of MT intervention time of the study, were counted. To control for differences in the number of

delivered therapy units between responders and non-responders, an univariate ANOVA for the number of therapy units with the factor group (responder / non-responder) was conducted for each of the four therapy types, with an uncorrected significance level of $p = 0.05$.

2.6. Statistical analysis

Statistical analysis was performed with the software packet SPSS (IBM SPSS Statistics, USA, version 21.0.0). The fNIRS measurements were analyzed comparing corresponding mean beta values of the ROIs in both hemispheres, resulting in a 2 x 2 study design with the two factors mirror (MIR / NOR) and hemisphere (ipsilesional / contralesional). For statistical group analysis, including evaluation of the treatment effect, a three-way repeated-measure ANOVA with the three factors time (pre-measurement / post-measurement), mirror (MIR / NOR) and hemisphere (ipsilesional / contralesional) was conducted for each ROI.

Beyond this, responders to MT were defined as patients, whose improvement of active finger motor function in the FMA was > 0. The following factors were included in a discriminant analysis (DA) designed to classify patients as responders or non-responders: Demographic data (days since stroke, age of patient), initial capacity in activities of daily living (BI), initial motor performance (FMA-finger), initial neuropsychological performance (TMT), initial fNIRS response (MI-PC, MI-M1) and performance during therapy (PIA). Note that dichotomous variables (e.g. affected hemisphere or sex) were not included in the DA.

In order to reduce the number of potential discriminant factors, the DA was conducted using a backward stepwise procedure designed for the small sample size (Bortz, 2005). Maximum significance (p-value) for the F-ratio of a factor to enter was set at 0.05, and minimum significance (p-value) for the F-ratio of a factor to remove was set at 0.10.

3. Results

3.1. Patients sample

During the recruitment period, thirteen patients fulfilled the criterions and agreed to participate in the study. Two patients did not complete the measurements, as they were discharged from the hospital earlier: one patient for disciplinary reasons, the other patient due to medical complications (urosepsis). The other eleven patients (four female, seven male; mean age: 62; range: 49-74) completed the full procedure of the study. Their characteristics are presented in Table 1, as well as the clinical improvements (CI) for BI, FMA-total, FMA-finger and TMT. All patients were right handed, as assessed with the German version of the Edinburgh Handedness Inventory (Oldfield, 1971) and had normal or corrected-to-normal vision. Unfortunately, recordings of MEPs and SSEPs were incomplete. Both MEPs and SSEPS were only recorded for 8 patients each: MEPs were recorded for 5 responders and 3 non-responders, and SSEPs were recorded for 4 responders and 4 non-responders. Median value of the TMT for patients performing with their dominant hand (n=4) was 25.7 sec and 31.0 sec for patients performing with their non-dominant hand (n=7).The univariate ANOVA revealed no significant difference in TMT performance between hands ($F(1,9)=1.44$; $p=0.26$). The univariate ANOVAs for the number of therapy units revealed no significant difference between responders and non-responders: The median value for upper extremity therapy units for responders was M=24, for non-responders M=21 ($F(1,9)=0.07$; $p=0.79$). The median value for lower extremity therapy units for responders was M=52, for non-responders M=41 ($F(1,9)=1.11$; $p=0.32$). The median value for ADLs therapy units for responders was M=6, for non-responders M=7 ($F(1,9)=0.34$; $p=0.58$). And median value for other therapy units for responders was M=27, for non-responders M=36 ($F(1,9)=1.07$; $p=0.33$).

3.2. Pre- and post-measurement of fNIRS

The estimated marginal means for the mean beta values for PC-ROI and M1-ROI are shown in Table 2. The top row depicts the values for the total sample, in the middle row the values for the responders and in the bottom row the values for the non-responders are shown.

The repeated measures three-way ANOVA over the mean beta values of the PC-ROI revealed no significant effect. Especially, neither the two-way interaction between the factors hemisphere x mirror ($F(1,10)=0.67$; $p=0.43$, $\eta^2=0.06$, for HBO and $F(1,10)=0.65$; $p=0.44$, $\eta^2=0.06$, for HBR) nor the three-way interaction between the factors time x mirror x hemisphere ($F(1,10)=0.34$; $p=0.57$, $\eta^2=0.03$, for HBO and $F(1,10)=0.34$; $p=0.57$, $\eta^2=0.03$, for HBR) reached significance.

The repeated measures three-way ANOVA over the mean beta values of the M1-ROI revealed a significant main effect of the factor hemisphere ($F(1,10)=7.90$; $p<0.05$, $\eta^2=0.44$, for HBO. There was a similar trend for this main effect for HBR, which barely did not reach significance ($F(1,10)=4.63$; $p=0.057$, $\eta^2=0.32$). The estimated marginal means indicate more activation in the contralesional compared to the ipsilesional hemisphere (ipsilesional: 0.25; contralesional: 0.35 for HBO; ipsilesional: -0.06; contralesional: -0.11 for HBR). No other effects reached significance in the M1-ROI.

3.3. Comparison and description of responder versus non-responder group

Applying the definition as stated above (Chapter *2.5. Statistical analysis*), six out of eleven patients were classified as responders, and five were classified as non-responders. An overview of their characteristics is given in Table 1 and Figure 2. In the responder group, there were more left-hemisphere lesions (5 out of 6) than in the non-responder group (2 out of 5). Responders and non-responders were comparable in sex (two females in both groups). Furthermore, as depicted in Figure 2, there is much overlap between responders and non-

responders range for the factors: days since stroke, age, BI and TMT. This seems to be less pronounced for MI-M1. In contrast, only little overlap could be found in MI-PC and PIA: For the MI-PC all non-responders had negative values ranging from -0.49 to -0.03 (median: -0.11), while responders values ranged from -0.15 to 1.46 (median: +0.10). In detail, 3 out of 6 responders had positive values (0.22; 1.13; 1.46) and 3 had slightly negative values (-0.15; -0.07; -0.007). Finally, there is hardly any overlap of the initial FMA-finger and FMA-total: while responders' FMA-finger score reached from 0 to 3, all non-responders had 0 points.

3.4. Stepwise discriminant analysis

Two variables were identified by the stepwise DA: FMA-finger base level and MI-PC. The overall Chi-square test was highly significant (Wilks λ=0.224, χ^2=11.98, df=2, Canonical correlation=0.881, p=0.002); the two variables extracted accounted for 77.6 % of the variance in finger motor improvement. The standardized canonical discriminant function coefficients were 0.88 for MI-PC and 1.06 for FMA-finger base level (Table 3, upper row). Pooled within-groups correlations between discriminating variables and standardized canonical discriminant functions were 0.64 for FMA-finger base level and 0.37 for MI-PC (Table 3, lower row).

4. Discussion

In the present pilot study, we were able to integrate functional imaging measurements into a clinical trial as a first step for identification of potential determinants of therapy success of MT.

Among several possible neurophysiological, behavioral, functional and demographic determinants, the stepwise DA selected two factors to classify patients as responders or non-responders: *FMA-finger base level* and the *MI-PC* as measured with fNIRS. With these two factors, 77.6 % of variance among responders and non-responders could be explained. The prominent role of these factors was also confirmed by the direct comparison of the distribution of the variables in the two groups, revealing only little overlap between responder and non-responder group. Although the factor *days since stroke* had a higher pooled within-groups correlation with the standardized canonical discriminant function than MI-PC, it was not selected by the stepwise DA. This is due to the fact that in stepwise analysis the factors are selected by their unique contribution (the semi-partial correlation) to the explained variation, and not by means of the direct correlation e.g. between dependent and independent variable. Thus, MI-PC is the only factor among all assessed variables that significantly explains variation additionally to the initial FMA-finger.

4.1. Role of initial motor function

As the patients in this study received MT as an adjacent treatment additionally to conventional treatment procedure, which is in line with the German *Practice Guideline* (Platz & Roschka, 2009), it is difficult to separate the effect of general rehabilitation outcome from the MT specific outcome. Therefore, it is not surprising that the motor function base level is a significant discriminant of responders and non-responders. In their review on 56 studies, Chen and Winstein found the initial voluntary motor behavior (apart from integrity of corticospinal

tract) to be the best predictor for motor function improvement in hemiparetic stroke patients (Chen & Winstein, 2009). Thus, the discrimination of responders and non-responders by means of the initial motor function level might be attributed to the general rehabilitation process effect. Meanwhile, it is tempting to attribute the variance explained by the MI-PC to the MT effect. Further studies with a control group are recommended to separate these effects more precisely.

4.2. The role of precuneus

The repeated measures three-way ANOVA over the mean beta values of the fNIRS measurement revealed no significant main or interaction effects on the PC-ROI. Thus, the interaction effect of hemisphere and mirror, as previously found in normal subjects (Mehnert et al., 2013), could not be replicated for the entire group of stroke patients on the basis of the data at hand. Obviously, this might be due to their inter-individual differences. Actually, the individually calculated MI reveals that only three out of eleven (27%) patients in this study in total had a positive MI-PC. Comparison of responders and non-responders clarify that they differ concerning the distribution of the MI-PC: All patients classified as non-responders had a negative MI-PC, while 50 % of responders had a positive MI-PC (3 out of 6 had strong positive, and 3 out of 6 had slightly negative values). The latter distribution of the MI-PC is similar to the normal subjects' sample of Mehnert and colleagues: 50 % of this normal subjects' sample had a positive MI-PC, reaching from -0.36 to 4.53, Median: 0.001; IQR: [-0.09; 0.92], with bigger values for positive MI-PC and lower values for negative MI-PC (Mehnert et al., 2013) (values not published). As described in the results section, this was similar for the distribution of MI-PC for the responders among the patient group (-0.15 to 1.46; IQR: [-0.09; 1.21]); but not for the non-responders (-0.49 to -0.03; IQR: [-0.46; -0.06]) (Table 4). That means that the activation pattern on PC evoked by the mirror illusion

(ipsilesional increase, contralesional decrease) of the responders is comparable to that of the normal subjects (Mehnert et al., 2013), while this is not the case for non-responders. Thus, activation shift in the area of both precunei as observed in normal subjects might indeed play a crucial role for the efficacy of MT. This extends previous findings on the immediate role of PC for cerebral processing of the mirror illusion in normal subjects (Dohle et al., 2011; Wang, Fritzsch, Bernarding, Holtze, et al., 2013) and patients (Wang, Fritzsch, Bernarding, Krause, et al., 2013). The PC plays a decisive role for self-related body representation (Cavanna & Trimble, 2006) and is involved especially for hand orientation during reach-to-grasp movements (Fattori et al., 2009). This prompts the idea that the mirror illusion activates the neural substrate for the visual representation of the paretic hand, as it evokes the impression that the paretic hand is moving. To the best knowledge of the authors, the present study is the first one to correlate the cerebral activation due to the mirror illusion before intervention with the efficacy of MT.

Why should PC, situated in the occipito-parietal cortex, play a crucial role for motor recovery of hemiparesis after stroke, if the function of PC is not primary motoric? PC is connected with the supplementary motor area (SMA) (Cavanna & Trimble, 2006). Furthermore, fMRI studies found V6A, a subarea of PC, to be directly connected with the dorsal premotor cortex (Galletti et al., 2001; Marconi et al., 2001). Thus, activation of PC could stimulate the lesioned parts of the motor cortex, boosting neuroplasticity for a motor functional recovery. This was recently shown by an fMRI study investigating brain connectivity with dynamic causal modelling (DCM) on stroke patients during the mirror illusion. Facilitatory connections between posterior parietal cortex, PC and the ipsilesional M1 were found due to mirror illusion (Saleh, Adamovich, & Tunik, 2013).

In our study, the activation pattern on PC seems to be stable over time, as there was no main effect of the factor time of the ANOVA. Thus, the cortical response to the mirror illusion seems to be a personal invariant characteristic, which is not affected by application of

MT. It remains speculative whether this personal characteristic was already present before stroke or caused by the lesion. The fact that only the responder group's MI was comparable to that of normal subjects, however, might suggest that the stroke lesion caused a change in the responsiveness to the mirror illusion. Further studies are necessary to verify this hypothesis.

4.3. The role of primary motor cortex

There are divergent results concerning the role of M1 during MT: While some researchers hypothesized that the mirror illusion might enhance activity on M1 directly (Fukumura, Sugawara, Tanabe, Ushiba, & Tomita, 2007; Funase, Tabira, Higashi, Liang, & Kasai, 2007; Tominaga et al., 2009), others could not replicate these findings (Praamstra, Torney, Rawle, & Miall, 2011). These heterogeneous results might be due to different study designs: Additional activation on M1 was only found when the mirror condition provided the visual image of two synchronously moving hands (Kang, Ku, Kim, & Park, 2011; Saleh et al., 2013), but not when only one hand was mirrored (Dohle et al., 2004; Fritzsch et al., 2013; Matthys et al., 2009; Michielsen et al., 2011).

The ANOVA in the present study, revealed a significant main effect of the factor hemisphere on the M1-ROI with stronger activation on the hemisphere contralateral to the hand moved, just as it was expected. In line with the previous study on normal subjects, no interaction between hemisphere and mirror was found on M1 (Mehnert et al., 2013). Furthermore, the MI-M1 did not have a discriminative value for the motor recovery after MT in addition to the FMA-finger base level. This finding supports results from former fMRI studies that did not find an immediate effect of the mirror illusion on M1, but on other areas, such as PC (Dohle et al., 2011) or primary somatosensory cortex (Fritzsch et al., 2013).

Like PC, the activation pattern on M1 seems to be stable over time, as there was no main effect of the factor time of the ANOVA.

4.4. The role of attention towards the mirror

Concerning PIA, in Table 1 and Figure 2, there is also hardly any apparent overlap between responders and non-responders. So why did the stepwise DA not select PIA as determinant? The explanation here is similar to the above mentioned unique contribution to the explained variation: the correlation between finger motor base level and PIA was relatively high (Spearman correlation: r=0.67; p=0.025), meanwhile there was no correlation between finger motor base level and MI-PC (Spearman correlation: r=0.39; p=0.24). Thus, the variance of responders and non-responders that could be explained by PIA could also - and even better - be explained by finger motor base level. In other words, the alertness during MT has no additional information value to the motoric function at base level. In contrast, the variance explained by the MI-PC is "different" from the variance that is explained by finger motor base level. It might be speculated if the correlation between finger motor base level and PIA is due to the fact that the slightly less affected patients in motor function, at the same time, are less affected concerning their attention, so that they are more likely to concentrate and to be alert during MT (or rather being perceived as such by the therapist). On this point it should be acknowledged that the alertness in the study at hand was not measured by a validated scale, but rather represents a subjective estimation by the treating therapist. Hence, it can be deliberated if PIA is indeed a reliable approach to assess alertness.

4.5. The role of the lesioned hemisphere

There seemed to be somewhat more left hemisphere lesions in the responder group (5 out of 6) as compared with the non-responder group (2 out of 5). As due to the small sample size, conclusions must be drawn cautiously. However, this could lead to the speculation that patients with left hemispheric lesions might profit more from MT due to possible neglect

pathology stroke patients suffer mainly after right hemisphere lesions. There is indirect evidence against this speculation: For example there was no hemisphere advantage in a randomized controlled trial (Dohle et al., 2009). Another controlled randomized trial showed that MT has two different effects on motoric and neglect improvements, which can be regarded as two different components that are affected by MT (Thieme et al., 2013). Thus, we can indirectly conclude that neglect has no effect on MT. Furthermore, the total study sample was not balanced (7 out of 11 with left hemispheric lesions). However, to answer this question properly, further studies are required to test the hypothesis about a left hemisphere lesion advantage or a neglect pathology disadvantage for MT effect.

4.6. Implications for application of MT

As stated above, the present study shows that the MI-PC correlates with therapy success: The higher this value (i.e. the more it was comparable to a normal subject's sample), the higher was the patient's chance to be classified as responder. Patients with a MI different to the normal subjects sample (i.e. no activation shift due to the mirror illusion) were rather classified as non-responders. Even as this finding is based on a pilot study with certain limitations, as stated in the following chapter, it might open a way for the use of activation patterns for identification of therapy responders (prior to therapy): In the present study, all patients with a positive MI benefitted from MT, while patients with a negative MI have a lower probability to take benefit.

4.7. Limitations of the study

A crucial limitation of the study is the difficulty or even impossibility to separate the effect of the conventional rehabilitation procedure from that of MT. Further studies including a control

group are necessary to separate these two effects and their corresponding determinants more precisely. This should also include a higher percentage of successfully recorded MEPs and SSEPs that might provide additional information about the integrity of the pyramidal and somatosensory pathways. However, as the univariate ANOVAs on the number of conventional therapy units revealed no significant difference between responders and non-responders, we can conclude that at least in the context of this pilot-study, the classification as a responder or a non-responder was not simply due to the conventional therapy.

Another eye-catching limitation is the small sample size. Due to time and limited resources, the recruitment and assessment period had to be stopped after one year. Although statistical analysis was accordingly adapted - using median and range to compare the groups on a descriptive way, or backward stepwise DA (Bortz, 2005) - further studies with higher sample size, preferable in a multi-center study design should be conducted in order to verify the results of the present study and answer further questions. Nevertheless, the results and conclusions of our study can stimulate future research on the use of cerebral activation patterns for therapeutic decision regarding application of MT.

5. Conclusion

In conclusion, the base level of finger motor function was confirmed to be a crucial determinant of upper distal limb motor recovery, being in line with former findings. Additionally, this study demonstrated that activation shift in the area of PC due to the mirror illusion might be considered as a determinant for efficacy of mirror therapy. I.e. a cerebral activation pattern due to the mirror illusion in the area of precuneus that is comparable to normal subjects might be a crucial underlying mechanism for the efficacy of mirror therapy. This should be verified in further controlled studies.

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Declaration of Conflicting Interests

The author(s) declare no potential conflicts of interest with respect to the authorship and / or publication of this article.

References

Bieniok, A., Govers, J., & Dohle, C. (2009). *Spiegeltherapie in der Neurorehabilitation* (Vol. 1). Germany: Schulz-Kirchner Verlag.

Bortz, J. (2005). *Statistik für Sozialwissenschaftler* (6. ed.): Springer Medizin Verlag Heidelberg.

Bowering, K. J., O'Connell, N. E., Tabor, A., Catley, M. J., Leake, H. B., Moseley, G. L., & Stanton, T. R. (2013). The Effects of Graded Motor Imagery and Its Components on Chronic Pain: A Systematic Review and Meta-Analysis. *The Journal of Pain, 14*(1), 3-13.

Broeks, J. G., Lankhorst, G. J., Rumping, K., & Prevo, A. J. (1999). The long-term outcome of arm function after stroke: results of a follow-up study. *Disabil Rehabil, 21*(8), 357-364.

Calautti, C., Naccarato, M., Jones, P. S., Sharma, N., Day, D. D., Carpenter, A. T., . . . Baron, J. C. (2007). The relationship between motor deficit and hemisphere activation balance after stroke: A 3T fMRI study. *Neuroimage, 34*(1), 322-331. doi: 10.1016/j.neuroimage.2006.08.026

Cavanna, A. E., & Trimble, M. R. (2006). The precuneus: a review of its functional anatomy and behavioural correlates. *Brain, 129*, 564-583.

Chen, S., & Winstein, C. (2009). A systematic review of voluntary arm recovery in hemiparetic stroke: critical predictors for meaningful outcomes using the international classification of functioning, disability, and health. *J Neurol Phys Ther, 33*(1), 2-13.

Cope, M., & Delpy, D. T. (1988). System for long-term measurement of cerebral blood and tissue oxygenation on newborn infants by near infra-red transillumination. *Med Biol Eng Comput, 26*(3), 289-294.

Dohle, C., Kleiser, R., Seitz, R. J., & Freund, H.-J. (2004). Body scheme gates visual processing. *J Neurophysiol, 91*(5), 2376-2379.

Dohle, C., Püllen, J., Nakaten, A., Kuest, J., Rietz, C., & Karbe, H. (2009). Mirror therapy promotes recovery from severe hemiparesis: A randomized, controlled trial. *Neurorehabil Neural Repair, 23*(3), 209-217.

Dohle, C., Stephan, K. M., Valvoda, J. T., Hosseiny, O., Tellmann, L., Kuhlen, T., . . . Freund, H. J. (2011). Representation of virtual arm movements in precuneus. *Exp Brain Res, 208*(4), 543-555. doi: 10.1007/s00221-010-2503-0

Fattori, P., Breveglieri, R., Marzocchi, F., Filippini, D., Bosco, A., & Galletti, C. (2009). Hand Orientation during Reach-to-Grasp Movements Modulates Neuronal Activity in the Medial Posterior Parietal Area V6A. *The Journal of Neuroscience, 29*(6), 1928 – 1936.

Fritzsch, C., Wang, J., Mauritz, K.-H., Dos Santos, L., Brunetti, M., & Dohle, C. (2013). Different effects of mirror therapy on motor and sensory processing. *Restor Neurol Neurosci*.

Fugl-Meyer, A. R., Jaasko, L., Leyman, I., Olsson, S., & Steglind, S. (1975). The post-stroke hemiplegic patient. 1. a method for evaluation of physical performance. *Scand J Rehabil Med, 7*(1), 13-31.

Fukumura, K., Sugawara, K., Tanabe, S., Ushiba, J., & Tomita, Y. (2007). Influence of mirror therapy on human motor cortex. *Int J Neurosci, 1117*(7), 1039-1048.

Funase, K., Tabira, T., Higashi, T., Liang, N., & Kasai, T. (2007). Increased corticospinal excitability during direct observation of self-movement and indirect observation with a mirror box. *Neurosci Lett, 419*(2), 108-112.

Galletti, C., Gamberini, M., Kutz, D. F., Fattori, P., Luppino, G., & Matelli, M. (2001). The cortical connections of area V6: an occipito-parietal network processing visual information. *Eur J Neurosci, 13*(8), 1572-1588.

Grefkes, C., Nowak, D. A., Wang, L. E., Dafotakis, M., Eickhoff, S. B., & Fink, G. R. (2010). Modulating cortical connectivity in stroke patients by rTMS assessed with fMRI and dynamic causal modeling. *Neuroimage, 50*(1), 233-242. doi: 10.1016/j.neuroimage.2009.12.029

Hamzei, F., Lappchen, C. H., Glauche, V., Mader, I., Rijntjes, M., & Weiller, C. (2012). Functional plasticity induced by mirror training: the mirror as the element connecting both hands to one hemisphere. *Neurorehabil Neural Repair, 26*(5), 484-496. doi: 10.1177/1545968311427917

Heller, A., Wade, D. T., Wood, V. A., Sunderland, A., Hewer, R. L., & Ward, E. (1987). Arm function after stroke: measurement and recovery over the first three months. *Journal of Neurology, Neurosurgery & Psychiatry, 50*(6), 714-719.

Hendricks, H. T., Hageman, G., & van Limbeek, J. (1997). Prediction of recovery from upper extremity paralysis after stroke by measuring evoked potentials. *Scand J Rehabil Med, 29*(3), 155-159.

Johnston, S., Mendis, S., & Mathers, C. (2009). Global variation in stroke burden and mortality: estimates from monitoring, surveillance, and modeling. *Lancet Neurol*(8), 345–354.

Jorgensen, H. S., Nakayama, H., Raaschou, H. O., Vive-Larsen, J., Stoier, M., & Olsen, T. S. (1995). Outcome and time course of recovery in stroke. Part I: Outcome. The Copenhagen Stroke Study. *Arch Phys Med Rehabil, 76*(5), 399-405.

Kang, Y. J., Ku, J., Kim, H. J., & Park, H. K. (2011). Facilitation of corticospinal excitability according to motor imagery and mirror therapy in healthy subjects and stroke patients. *Ann Rehabil Med, 35*(6), 747-758. doi: 10.5535/arm.2011.35.6.747

Koch, S. P., Werner, P., Steinbrink, J., Fries, P., & Obrig, H. (2009). Stimulus-induced and state-dependent sustained gamma activity is tightly coupled to the hemodynamic response in humans. *J Neurosci, 29*(44), 13962-13970. doi: 10.1523/JNEUROSCI.1402-09.2009

Mahoney, F. I., & Barthel, D. W. (1965). Functional Evaluation: The Barthel Index. *Md State Med J, 14*, 61-65.

Marconi, B., Genovesio, A., Battaglia-Mayer, A., Ferraina, S., Squatrito, S., Molinari, M., . . . Caminiti, R. (2001). Eye-hand coordination during reaching. I. Anatomical relationships between parietal and frontal cortex. *Cerebral Cortex, 11*, 513-527.

Matthys, K., Smits, M., Van der Geest, J. N., Van der Lugt, A., Seurinck, R., Stam, H. J., & Selles, R. W. (2009). Mirror-induced visual illusion of hand movements: a functional magnetic resonance imaging study. *Arch Phys Med Rehabil., 90*(4), c675-681.

Mehnert, J., Brunetti, M., Steinbrink, J., Niedeggen, M., & Dohle, C. (2013). Effect of a mirror-like illusion on activation in the precuneus assessed with functional near-infrared spectroscopy. *J Biomed Opt, 18*(6), 066001. doi: 10.1117/1.JBO.18.6.066001

Michielsen, M. E., Smits, M., Ribbers, G. M., Stam, H. J., van der Geest, J. N., Bussmann, J. B., & Selles, R. W. (2011). The neuronal correlates of mirror therapy: an fMRI study on mirror induced visual illusions in patients with stroke. *J Neurol Neurosurg Psychiatry, 82*(4), 393-398. doi: 10.1136/jnnp.2009.194134

Morkisch, N., Lommack, R., Kadow, L., Rietz, C., & Dohle, C. (2012). *Evaluation eines standardisierten Therapieprotokolls zur Spiegeltherapie*. Paper presented at the 22. Jahrestagung der Deutschen Gesellschaft für Neurorehabilitation e.V. (DGNR), Fürth, Germany.

Muehlschlegel, S., Selb, J., Patel, M., Diamond, S. G., Franceschini, M. A., Sorensen, A. G., . . . Schwamm, L. H. (2009). Feasibility of NIRS in the neurointensive care unit: a pilot study in stroke using physiological oscillations. *Neurocrit Care, 11*(2), 288-295.

Murray, C. (1996). *The global burden of disease. A comprehensive assessment of mortality and disability from diseases, injuries, and risk factors in 1990 and projected to 2020*. Cambridge: Harvard Univ. Press.

Obrig, H., & Villringer, A. (2003). Beyond the visible--imaging the human brain with light. *J Cereb Blood Flow Metab, 23*(1), 1-18.

Oldfield, R. C. (1971). The assessment and analysis of handedness: The Edinburgh Inventory. *Neuropsychologia, 9*, 97-113.

Oswald, W. D. (1986). Der Zahlen-Verbindungs-Test im höheren Lebensalter. In K. Daumenlang & J. Saue (Eds.), *Aspekte psychologischer Forschung*. (pp. 377-388). Göttingen, Germany: Hogrefe.

Platz, T., & Roschka, S. (2009). Rehabilitative Therapie bei Armparese nach Schlaganfall. *Neurol Rehabil, 15*(2), 17-42.

Praamstra, P., Torney, L., Rawle, C. J., & Miall, R. C. (2011). Misconceptions about mirror-induced motor cortex activation. *Cereb Cortex, 21*(8), 1935-1940. doi: 10.1093/cercor/bhq270

Ramachandran, V. S. (1994). Phantom limbs, neglect syndromes, repressed memories, and Freudian psychology. *Int Rev Neurobiol, 37*, 291-333.

Rizzolatti, G., Fabbri-Destro, M., & Cattaneo, L. (2009). Mirror neurons and their clinical relevance. *Nat Clin Pract Neurol, 5*(1), 24-34. doi: 10.1038/ncpneuro0990

Saleh, S., Adamovich, S. V., & Tunik, E. (2013). Visual Feedback Discordance Mediates Changes in Brain Activity and Effective Connectivity: A Stroke fMRI Dynamic Causal Modeling Study. *2nd International Conference on Advances in Biomedical Engineering*.

Singh, A. K., Okamoto, M., Dan, H., Jurcak, V., & Dan, I. (2005). Spatial registration of multichannel multi-subject fNIRS data to MNI space without MRI. *Neuroimage, 27*(4), 842-851.

Strangman, G., Goldstein, R., Rauch, S., & Stein, J. (2006). Near-infrared spectroscopy and imaging for investigating stroke rehabilitation: test-retest reliability and review of the literature. *Arch Phys Med Rehabil, 87*(12 Suppl 2), S12-19.

Taub, E., Crago, J. E., & Uswatte, G. (1998). Constraint-induced movement therapy: a new approach to treatment in physical medicine. *Rehabil Psychol, 43*, 152-170.

Thieme, H., Bayn, M., Wurg, M., Zange, C., Pohl, M., & Behrens, J. (2013). Mirror therapy for patients with severe arm paresis after stroke--a randomized controlled trial. *Clin Rehabil, 27*(4), 314-324. doi: 10.1177/0269215512455651

Thieme, H., Mehrholz, J., Pohl, M., Behrens, J., & Dohle, C. (2012). Mirror therapy for improving motor function after stroke (Review). *The Cochrane Library*(3).

Tominaga, W., Matsubayashi, J., Deguchi, Y., Minami, C., Kinai, T., Nakamura, M., . . . Mitani, A. (2009). A mirror reflection of a hand modulates stimulus-induced 20-Hz activity. *Neuroimage, 46*(2), 500-504.

Wang, J., Fritzsch, C., Bernarding, J., Holtze, S., Mauritz, K. H., Brunetti, M., & Dohle, C. (2013). A comparison of neural mechanisms in mirror therapy and movement observation therapy. *J Rehabil Med, 45*(4), 410-413. doi: 10.2340/16501977-1127

Wang, J., Fritzsch, C., Bernarding, J., Krause, T., Mauritz, K. H., Brunetti, M., & Dohle, C. (2013). Cerebral activation evoked by the mirror illusion of the hand in stroke patients compared to normal subjects. *NeuroRehabilitation*. doi: 10.3233/NRE-130999

Wohrle, J. C., Behrens, S., Mielke, O., & Hennerici, M. G. (2004). Early motor evoked potentials in acute stroke: adjunctive measure to MRI for assessment of prognosis in acute stroke within 6 hours. *Cerebrovasc Dis, 18*(2), 130-134. doi: 10.1159/000079265

Woodbury, M. L., Velozo, C. A., Richards, L. G., Duncan, P. W., Studenski, S., & Lai, S. M. (2007). Dimensionality and construct validity of the Fugl-Meyer Assessment of the upper extremity. *Arch Phys Med Rehabil, 88*(6), 715-723. doi: 10.1016/j.apmr.2007.02.036

Figures and Tables

Table 1

	Total (n=11)	Responders (n=6)	Non-responders (n=5)
Days since stroke	26 [15-92]	28 [15-92]	26 [16-62]
Age	66 [49-74]	66.5[49-68]	62 [53-74]
Female / Male	4 / 7	2 / 4	2 / 3
Left / Right hemishere lesion	7 / 4	5 / 1	2 / 3
BI	50 [20-75]	52.5 [20-65]	40 [25-75]
FMA-total	5 [0-13]	10 [0-13]	1 [0-5]
FMA-finger	0 [0-3]	2 [0-3]	0 [0-0]
TMT (sec)	29.5 [18-82]	29.5 [20-82]	29.5 [18-49]
PIA	0.81 [0.32-0.98]	0.89 [0.73-0.98]	0.63 [0.32-0.81]
MEPs (present / not present / not recorded)	2 / 6 / 3	1 / 4 / 1	1 / 2 / 2
SSEPs (present / not present / not recorded)	5 / 3 / 3	3 / 1 / 2	2 / 2 / 1
MI-PC	-0.07 [-0.49-1.46]	0.10 [-0.15-1.46]	-0.11 [-0.49- (-0.03)]
MI-M1	0.06 [-0.53-3.46]	0.03 [-0.53-0.44]	0.15 [-0.10-3.46]
CI-BI	25 [5-40]	30 [25-40]	15 [5-20]
CI-FMA-total	3 [-1-26]	11 [1-26]	0 [-1-3]
CI-FMA-finger	1 [0-8]	5 [1-8]	0 [0-0]
CI-TMT (sec)	2.4 [-6-34.4]	1.6 [-6-34.4]	2.6 [0.3-6]

	Dominant hand (n=4)	Non-dominant hand (n=7)
TMT (sec)	25.7 [18-32]	31.0 [20-82]

Abbreviations: TMT: trail making test; sec: seconds; FMA: Fugl-Meyer assessment; PIA: Percentage of ideal attention; MEPs: motor evoked potentials; SSEPs: somatosensory evoked potentials; MI: mirror index; PC: precuneus; M1: primary motor cortex; CI: clinical improvements.
Median values and range [in bracket] of the days since stroke at the time of the pre-FMA, age and pre-assessments of BI, FMA-finger, FMA-total, TMT, PIA, MI-PC and MI-M1 as well as the clinical improvements of BI, FMA-total, FMA-finger and TMT.
Frequencies of females, left hemisphere lesion, present / not present and not recorded MEPs and SSEPs.
Total sample: left column; Responders: middle column; Non-responders: right column.
Bottom row: TMT values separated for dominant and non-dominant hand performance.

Table 2

TIME	HEMISPHERE	MIRROR	PC-ROI HBO	PC-ROI HBR	M1-ROI HBO	M1-ROI HBR
Pre-measurement	ipsi	MIR	0.84 (0.40)	0.11 (0.13)	0.27 (0.22)	- 0.09 (0.02)
		NOR	0.63 (0.42)	- 0.07 (0.06)	0.25 (0.16)	- 0.06 (0.03)
	contra	MIR	0.61 (0.32)	0.06 (0.06)	0.30 (0.21)	- 0.04 (0.06)
		NOR	0.53 (0.28)	- 0.02 (0.05)	0.61 (0.28)	- 0.26 (0.17)
Post-measurement	ipsi	MIR	0.40 (0.21)	- 0.02 (0.01)	0.26 (0.16)	- 0.04 (0.01)
		NOR	0.20 (0.20)	- 0.07 (0.05)	0.21 (0.15)	- 0.06 (0.02)
	contra	MIR	0.55 (0.24)	0.06 (0.06)	0.35 (0.17)	- 0.07 (0.02)
		NOR	0.36 (0.16)	0.03 (0.04)	0.14 (0.15)	- 0.09 (0.03)
RESPONDER						
Pre-measurement	ipsi	MIR	0.76 (0.42)	0.20 (0.23)	0.16 (0.40)	- 0.08 (0.04)
		NOR	0.20 (0.19)	- 0.16 (0.08)	0.13 (0.28)	- 0.02 (0.04)
	contra	MIR	0.32 (0.38)	0.07 (0.10)	0.38 (0.36)	- 0,12 (0.05)
		NOR	0.19 (0.17)	- 0.07 (0.08)	0.34 (0.34)	- 0.09 (0.08)
Post-measurement	ipsi	MIR	0.35 (0.18)	- 0.03 (0.02)	0.32 (0.18)	- 0.03 (0.02)
		NOR	0.29 (0.23)	- 0.03 (0.02)	0.33 (0.19)	- 0.04 (0.02)
	contra	MIR	0.25 (0.14)	- 0.01 (0.02)	0.35 (0.13)	- 0.05 (0.02)
		NOR	0.19 (0.20)	- 0.01 (0.03)	0.25 (0.18)	- 0.07 (0.04)
NON-RESPONDER						
Pre-measurement	ipsi	MIR	0.94 (0.78)	- 0.001 (0.07)	0.40 (0.15)	- 0.10 (0.03)
		NOR	1.15 (0.88)	0.05 (0.07)	0.38 (0.11)	- 0.10 (0.03)
	contra	MIR	0.96 (0.53)	0.04 (0.07)	0.21 (0.19)	0.06 (0.12)
		NOR	0.93 (0.57)	0.05 (0.06)	0.93 (0.46)	- 0.46 (0.37)
Post-measurement	ipsi	MIR	0.45 (0.44)	- 0.003 (0.02)	0.19 (0.29)	- 0.04 (0.01)
		NOR	0.09 (0.37)	- 0.11 (0.12)	0.07 (0.24)	- 0.09 (0.04)
	contra	MIR	0.90 (0.47)	0.15 (0.14)	0.35 (0.37)	- 0.09 (0.01)
		NOR	0.56 (0.24)	0.07 (0.06)	0.01 (0.26)	- 0.11 (0.04)

Abbreviations: ipsi: ipsilateral; contra: contralateral; MIR: mirrored visual feedback; NOR: normal visual feedback; PC: precuneus; M1: primary motor cortex; ROI: region of interest.

Estimated marginal means of the beta values from fNIRS for the PC-ROI and the M1-ROI for HBO and HBR, respectively. Corresponding standard errors are depicted in brackets.

Table 3

Standardized canonical discriminant function coefficients	
MI-PC	0.88
FMA-finger	1.06

Structure matrix	
FMA-finger	0.64
days since stroke	0.45
MI-PC	0.37
Age	0.19
PIA	0.10
BI	-0.04
MI-M1	0.04
TMT	-0.003

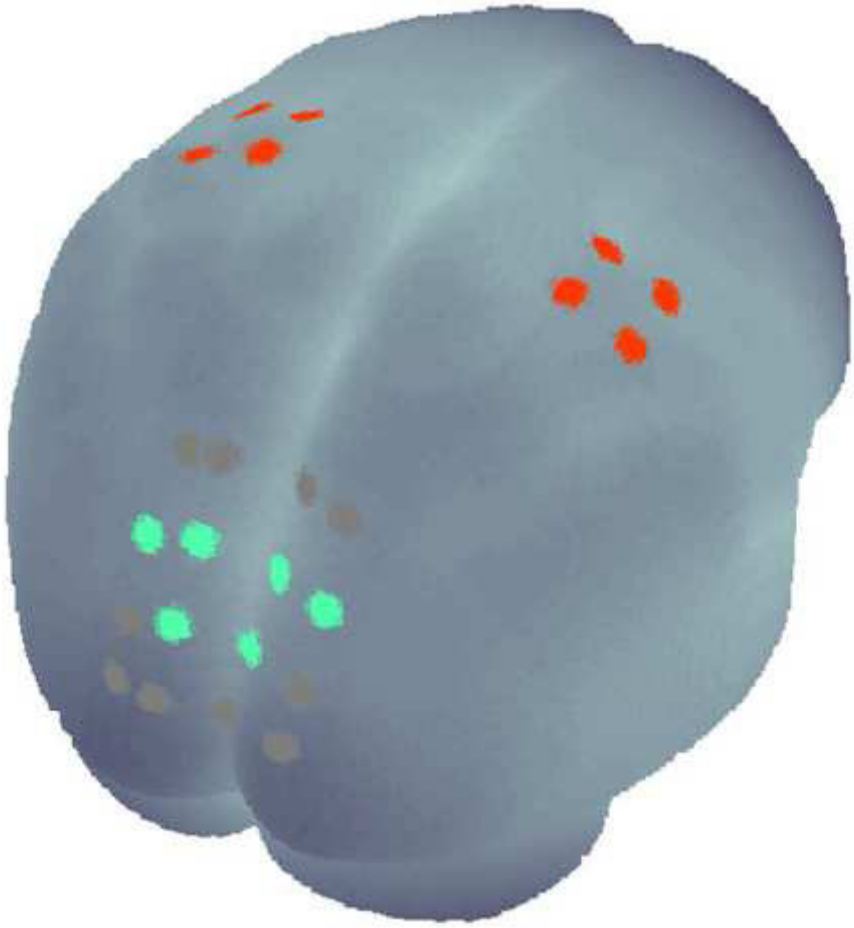
Results of the discriminant analysis in detail. Upper row: Standardized canonical discriminant function coefficients of FMA-finger and MI-PC. Lower row: Structure matrix with pooled within-groups correlations between discriminating variables and standardized canonical discriminant functions.

Table 4

Sample	sample size	25th Percentile	Median	75th Percentile
Normal subjects	20	-0.09	0.001	0.92
Patients (Total)	11	-0.15	-0.07	0.22
Responders	6	-0.09	0.10	1.21
Non-Responders	5	-0.46	-0.11	-0.06

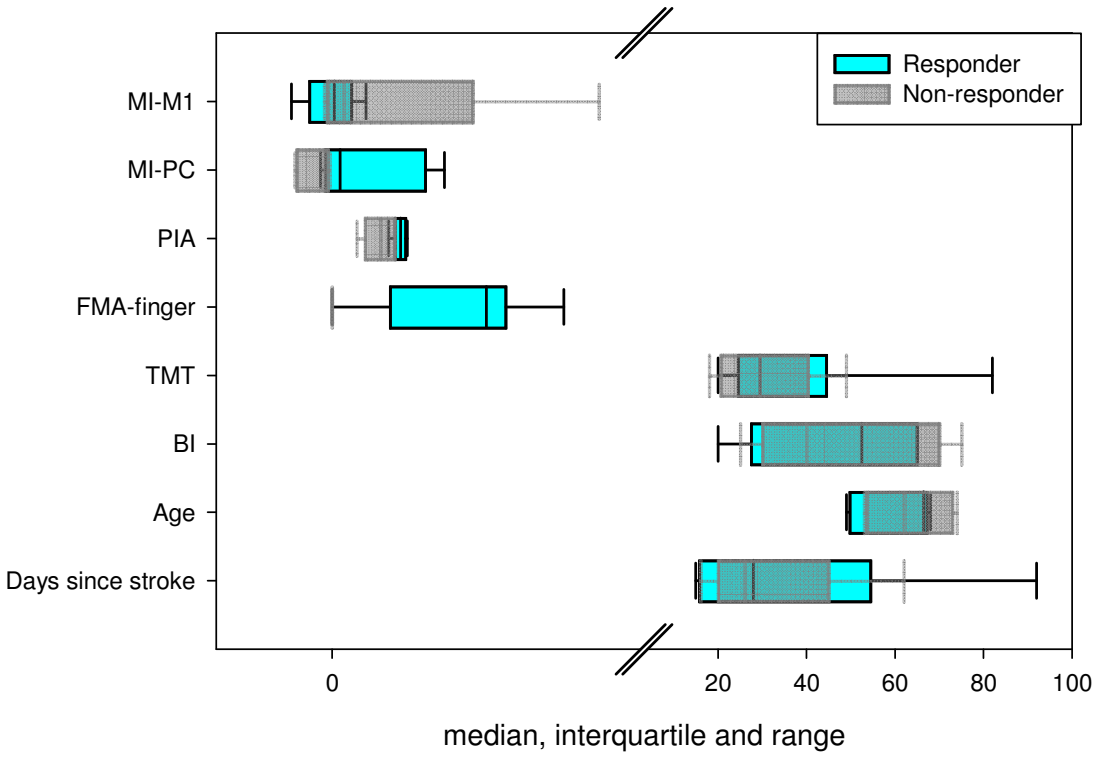
Distribution of the MI-PC (HBO) of the fNIRS pre-measurement in different samples: normal subjects of a former study (Mehnert et al., 2013; not published), patients of the present study in total, responders and non-responders. Sample size, 25th and 75th percentile and median of MI-PC (mirror index measured on precuneus).

Figure 1



Schematic illustration of the channels measured with functional near-infrared spectroscopy. The three PC-ROI channels are marked green and the four M1-ROI channels are marked red.

Figure 2



Box-plots with median values, interquartile range (IQR) and range of following factors: days since stroke, age, BI, TMT, FMA-finger, PIA, MI-PC, MI-M1; for responders (cyan) and non-responders (grey).
Note: For FMA-finger for non-responders there is no box, as they had all 0 points.