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Viewing own movements through the mirror: the effects of sensorimotor conflict on the motor system

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Declaration

I declare that the work presented in this thesis is my own.

Where information has been derived from other sources, I confirm that this has been reported in the thesis.

Cristina Russo

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List of abbreviations and acronyms

MVF Mirror Visual Feedback

MBT Mirror Box Therapy

REMIT Reversed Mirror Therapy

Summary

The present dissertation comprises three studies exploring the impact of the incongruence between sensory and motor information (i.e. sensorimotor conflict) provided by the Mirror Visual Feedback (MVF), a manipulation during which subjects are required to perform movements while observing their reflection through a mirror placed perpendicular to their body midline. Together, results converge in showing the importance of sensorimotor conflicts for influencing the impact of the MVF at the level of illusory experiences, cortical excitability and post-stroke motor recovery.

The first study offers an overview of the illusory sensation (the so-called Mirror Illusion) brought about by the MVF in stroke patients with motor deficit (upper-limb hemiparesis). Results evidence that stroke patients show a reliable Mirror Illusion, similar to that experienced by neurologically healthy participants, demonstrating the pervasiveness of the MVF effects. However, different factors impact the susceptibility of stroke patients to the Mirror Illusion. First, residual motor functions are necessary to generate the illusion, while unreliable tactile sensation may even increase it. Second, cortical damages to low- and high-level motor areas are associated to a larger illusory effect, while parietal lesions differentially affect the Mirror Illusion, likely disrupting or facilitating multisensory, body- and self-related, processing.

The second study, in healthy participants, aims at experimentally exaggerating the degree of visuo-motor incongruence to assess its modulatory effect on motor cortex excitability, measured through Transcranial Magnetic Stimulation. Results show that the primary motor cortex (M1) ipsilateral to the moving hand is differentially influenced by the speed of the observed movement: the greater the mismatch with the performed movements, the higher the increase of M1 excitability. Specifically, the utmost modulatory effect is registered during the observation of a slower pace. This result indicates that the magnitude of the mismatch between performed (motor output) and observed (visual input) movement can be used to adjust the activity of the observer's motor system.

Based on such evidence, the third study develops a novel strategy to rehabilitate post-stroke hemiparesis, which takes advantage of a reversed MVF: at variance with standard Mirror Box Therapy, that requires to watch the mirror reflection of the intact limb's movements, patients are asked to observe the reflection of the paretic limb (here called Reversed Mirror Therapy - REMIT). Results show that the REMIT has comparable effects of the standard version, as both lead to motor improvements in stroke patients in a chronic stage of illness. This evidence supports the hypothesis that the critical factor at the basis of the clinical efficacy of the MVF is represented by the sensorimotor conflict, rather by the mere motor observation or imagery, or the removal of a learned component of hemiparesis.

In conclusion, this set of experiments documents the key role of a two-fold conflict inherent in MVF conditions: the sensorimotor mismatch and incongruent multisensory inputs interact to generate illusory experiences boosting behavioral, neural and clinical effects.

Chapter 1

1.1. Mirror Visual Feedback: behavioral effects

A mirror placed perpendicularly to the body midline gives the image of what is in front of it, but due to the almost perfect symmetry of the human body, this reflection represents a good substitution of what it is hidden (Holmes and Spence, 2005). To exemplify, whether a reflective surface is located along the midsagittal plane of a participant who places the left arm in front and the right limb behind it, the mirror image provided by the reflection (of the left part of the body), evokes the illusion of the other, unseen, limb. In last decades, this mirror manipulation has been widely used in experimental settings with both neurological and healthy participants. Specifically, in the clinical context, it has been applied with rehabilitative purposes for a wide range of neurological conditions (see below). On the other hand, it has been used as a tool to investigate the consequences of viewing a superimposed mirror reflection of one limb on the other contralateral unseen one.

In particular, a significant number of studies on healthy subjects have demonstrated physiological and behavioral alterations related to this arrangement. For instance, the mere view through the mirror of the body part being stimulated leads to a decrease in pain (Longo et al., 2009), and affects the perceived size of tactile stimuli (Longo and Sadibolova, 2013). In addition, when a sensorimotor conflict is experimentally induced, the same setting has been proven capable of eliciting pain and discomfort sensations (McCabe et al., 2005), and modulating the physiological regulation of skin temperature in the hidden limb (Sadibolova and Longo, 2014). Necessary for those effects to arise, generally called "Mirror Illusion", is the reflection provided. In fact, participants are continuously required to watch their own limb reflection through the mirror. Hence the name "Mirror Visual Feedback", MVF (Altschuler et al., 1999).

Other experiments have manipulated the visually estimated location of the hidden arm, inducing behavioral effects such as pointing errors (Holmes and Spence, 2005) and reaching bias (Medina et al., 2015; Snijders et al., 2007) toward the limb in the mirror. Recently, it has been demonstrated that those effects could be further increased through cortical modulation via transcranial Direct Current Stimulation (tDCS). Specifically, active tDCS with bi-hemispheric montage, with the anodal electrode over the primary motor cortex (M1) ipsilateral to the hand in front of the mirror, further affects the reaching bias (Jax et al., 2015). Illusory dislocations of the static, unseen, forearm have been registered when looking at the mirror reflection of both active (Romano et al., 2013) and passive (Metral et al., 2015) displacement of the seen hand (i.e. a mirror kinaesthetic illusion). ether, this evidence points at the significant involvement of visual afferents in kinaesthesia (Metral et al., 2015). Recent compelling proofs demonstrate how these effects are not consequences of a purely visual phenomenon; rather, they emerge from a combination of signals from the two arms, i.e. visual afferents from the virtually moving arm and proprioceptive afferents from the contralateral, moving arm (Chancel et al., 2016).

A plethora of different studies has also explored the effects of MVF on motor learning with the limb positioned behind the mirror. For instance, it has been demonstrated that the hand out of the sight is able to learn behavioral tasks such as rotating balls counterclockwise when reassessed after the training of the other hand (Nojima et al., 2012). Also the performance of fine motor exercises, such as moving pegs and marbles, can be enhanced when the training of the other hand is under MVF conditions (Hamzei et al., 2012). Interestingly, upregulating M1 ipsilateral to the hand in front of the mirror through anodal tDCS further improved MVF-related performance of the hidden limb (von Rein et al., 2015). Similarly, the same arrangement is able to improve hand dexterity in the elderly, as compared to both sham stimulation and active tDCS without MVF training (Hoff et al., 2015). As a whole, by exploring many aspects related to the multisensory representation of the body, those pieces of evidence demonstrate how the simple MVF manipulation is able to induce changes in sensory and motor systems.

1.2. Mirror Visual feedback as a rehabilitation tool

In clinical settings, Mirror Visual Feedback was first introduced in the form of Mirror Box Therapy (MBT) to alleviate Phantom Limb Pain (Ramachandran et al., 1995) and then successfully translated to the treatment of hemiparesis (Altschuler et al., 1999). During the Mirror Box Therapy, patients are invited to put both their arms at the two sides of the mirror placed perpendicular to their body midline, with the impaired (affected or absent) limb hidden to the sight. In addition, the intervention may require to perform uni- or bi-manual movements watching the mirror reflection of the moving hand. In this way, the illusion that the hidden, impaired, hand is moving arises. This illusion has been proven to be effective in ameliorating a wide range on symptoms, such as Phantom Limb Pain, hemiparesis from stroke and complex regional pain syndrome (Ramachandran and Altschuler, 2009). Recently, the beneficial effects of MVF have been further exploited, and cumulative reports showed promising results in alleviating many other conditions characterized by impaired motor control, such as alien hand (Romano et al., 2014), cerebral (Park et al., 2016), and idiopathic facial (Barth et al., 2014) palsy. In addition, the Mirror Box Therapy has been applied to treat other conditions that affect upper limbs, such in the rehabilitation after hand surgery (Rosèn and Lundborg, 2005), wrist (Altschuler and Hu, 2008) and distal radial fracture (Bayon-Calatayud et al., 2016). Moreover, given the promising results in healing conditions such as phantom pain after amputation [for a review, see (Barbin et al., 2016)] and complex regional pain syndromes (Cacchio et al., 2009) the beneficial effects of MVF have been investigated also for other aching conditions, such as causalgia (Selles et al., 2008). With respect to post-stroke motor rehabilitation, following the original protocol

(Altschuler et al., 1999), bimanual exercises are performed during the therapy, namely also the paretic limb has to be moved as better as possible. In the case of severe hemiplegia, uni-manual movements are required to be performed with the unimpaired limb only. The beneficial effects of the Mirror Box Therapy on motor recovery have been proven by several studies. Indeed, this intervention was shown to promote functional improvements of upper-limb and lower-limb hemiparesis/plegia, both in subacute and chronic phases of illness, ameliorating motor functions at different levels, including the range of motion, speed and accuracy of the movement performed with the affected limb (Altschuler et al., 1999; Sütbeyaz et al., 2007; Yavuzer et al., 2008). In addition, other components of the motor control can undergo beneficial improvements after MVF training, such as motor planning, spatial efficiency in movement execution as well as multi-joint coordination [for a review, (Thieme et al., 2013)]. Moreover, changes in surface and temperature sensitivity (Colomer et al., 2016), and even an amelioration of spatial hemi-neglect, have been registered (Dohle et al., 2009; Radajewska et al., 2013; Wu et al., 2013). Importantly, improvements of motor functions generalize to everyday life activities (Park et al., 2015).

The Mirror Box Therapy seems not to be equally effective in all stroke patients, and the variability in the clinical outcome is so high that it has been suggested to classify patients as responders and not responders (Dohle et al., 2009). However, which factors determine differences in the clinical outcome are matter of debate. For instance, it has been proposed that the clinical effects of the

Mirror Box Therapy are most prominent when the difference between the real and the mirrored visual feedback is greatest (Dohle et al., 2011), i.e., with densely hemiplegic patients who have no distal function at the beginning of the therapy (Dohle et al., 2009). It follows that the MVF should be more effective in patients with no motor function at all. Other evidence points out that it is the severity of motor disorder, together with the baseline pattern of functional bilateral activation of the precuneus to be related to the improvements brought about by MVF (Brunetti et al., 2015). The stage of illness is also relevant, with the Mirror Box Therapy being effective in both subacute and chronic stroke (Michielsen et al., 2011; Yavuzer et al., 2008). Conversely, MVF interventions applied in acute patients seem not effective in providing additional functional improvement (Yeldan et al., 2015). This uncertainty about the factors contributing to the clinical efficacy of the Mirror Box Therapy is also related to the fact that, on a broader perspective, the mechanisms of action of MVF are still unknown.

1.3. Neural correlates of MVF effects

A plethora of different investigations, both in healthy and stroke participants, has tried to shed light on the neural correlates of MVF interventions, focusing on acute (namely, after a single session) and long-term (namely, after MVF and MBT training) effects. In the following section, a quick overview of the most updated evidence, coming from Transcranial Magnetic Stimulation (TMS) and functional Magnetic Resonance Imaging (fMRI) studies, will be presented.

TMS has been widely used as a tool to measure the cortical excitability concomitant the observation of movements through the mirror. It has been suggested that watching uni-manual movements in a mirror increases the excitability of the primary motor cortex (M1) ipsilateral to the moving hand. Evidence comes from studies both in healthy participants (Garry et al., 2005; Carson and Ruddy, 2012; Fukumura et al., 2007; Funase et al., 2007) and in stroke patients (Kang et al., 2012; Saleh et al., 2014). Specifically, this activation has been found greater than direct vision of the moving limb from some Authors (Garry et al., 2005; Carson and Ruddy, 2012), but not from others (Funase et al., 2007; Reissing et al., 2014). Those discrepancies may be ascribed to methodological differences across studies (Kumru et al., 2016).

On the other hand, evidence regarding neuroplasticity changes due to MVF training are less controversial. After a MVF-based training, in comparison with control training, TMS studies demonstrated that the excitability of M1 increases in the affected and decreases in the non-affected hemisphere (Läppchen et al., 2012; Nojima et al., 2012). This effect is suggested to be based on disinhibition, including intracortical inhibition and facilitation and not on interhemispheric inhibition (Läppchen et al., 2012). Specifically, TMS studies aimed at directly assessing changes in the interhemispheric inhibitory balance due to MVF pointed out either a reduction (Carson and Ruddy, 2012; Avanzino et al., 2014), or no change (Hamzei et al., 2012; Nojima et al., 2012).

With respect to the great amount of fMRI data regarding MVF conditions, a remarkable effort toward a unitary view has been recently done with an interesting review that tried to bring together current knowledge (Deconinck et al., 2014). Three possible functional networks though which MVF could influence the brain, both in patients and in healthy subjects, have been pointed out: 1) Attentional Network; 2) Mirror Neuron System Network; 3) Motor Network (Deconinck et al., 2014). Briefly, compared to control conditions, performing movements observing through the mirror seems to lead to increased activity in: i) Primary and secondary visual and somatosensory areas: MVF conditions appear associated with conscious awareness of sensory feedback or control of agency; ii) Parts of the Mirror Neuron System (Rizzolatti and Craighero, 2004): MVF conditions activate the Superior Temporal Gyrus (STG) (Matthys et al., 2009); iii) Areas in motor network. Specifically, M1 ipsilateral to the reflected hand has been considered the final hub for the positive MVF effects (Deconinck et al., 2014).

Crucially, the global MVF effect on brain activation seem strongly dependent upon the specific type feedback. In particular, the effects on primary and secondary visual processing areas are primarily due to unilateral MVF (namely, when only the hand in front of the mirror is required to move, while the other is maintained still behind the mirror). On the other hand, bimanual MVF conditions (namely, when also the hand behind the mirror is moved as better as possible), lead to an increased activation of higher order areas involved with attentional processes (precuneus and posterior cingulate cortex) and the ipsilateral M1 (Michielsen et al., 2010).

To sum up, bilateral MVF conditions in stroke patients with motor impairment are featured by important involvement of frontal and parietal regions related to higher cognitive functions like attention and monitoring (Deconinck et al., 2014; Michielsen et al., 2011). After MVF training, important plastic changes seem involve the primary motor cortex ipsilateral to the moving hand (Läppchen et al., 2012; Nojima et al., 2012). Recently, also the M1 contralateral to the moving limb has received increasing attention. Particularly, a magnetoencephalography (MEG) study on chronic stroke patients found movement-related beta desynchronization between motor cortices to be less lateralized (namely, more similar to controls) during bilateral hand movement performed with MVF (Rossiter et al., 2015). In addition, a recent model depicts a significant role of the contralesional parietal cortex in determining MVF modulation of the ipsilesional M1 (Saleh et al., 2017). This new intriguing proposal, that arises from a dynamic causal study of fMRI activation under virtual reality MVF conditions, deserves further investigation in future studies.

1.4. Theories regarding the mechanisms of action of the MVF

Whereas the role of visual information is recognized as pivotal in driving the clinical effects of the MVF, the specific mechanism of action of MVF has been delineated according to different theories. For instance, the reflection given by the mirror has been thought capable of solving the learnt component of the motor impairment (Ramachandran and Altschuler, 2009), key in exploiting Mirror Neuron System's resonance mechanisms (Yavuzer et al., 2008), or pivotal in sustaining motor imagery (Stevens and Stoykov, 2003). In the following sections, a description of the two most accredited hypotheses regarding the mechanism of action of the MVF will be provided.

MVF and the learnt component of the paralysis. The hypothesis originally put forward by Ramachandran focuses on the possible role of the MVF acting as to 'unlearn' the learnt component of the paralysis (Ramachandran and Altschuler, 2009). Specifically, the learnt component of the paralysis is a putative mechanism that hinders recovery and rehabilitation. This type of stroke sequela is conceptualized as the consequence of no visual feedback to the motor output sent by the brain (Ramachandran et al., 1995). Following this proposal [see also (Ramachandran and Altschuler, 2009)], soon after stroke, any attempt to move the most affected limb is not coupled with the corresponding visual feedback, due to the paralysis. Every time patients attempt to move the paralyzed limb, they receive sensory feedback (through vision and proprioception) that the limb did not move. This negative feedback becomes “stamped” in the brain, so that, the brain learns that the limb is not able to move. This “learnt” component of the paralysis, may, in turn, worsen the motor impairment and even hamper the possibilities of obtaining substantial recovery. Thus, the lack of feedback itself, by becoming “stamped” in the brain, plays a deleterious function. Ramachandran’s concept of learnt paralysis recalls another seminal concept, namely “learned non use” (Taub and Berman, 1968). This concept, put forward following studies both in primates and in men, suggests the role of non-use as key in the genesis of certain motor disorders. Specifically, non-use induced by the paralysis generates a learned non-use phenomenon that prevents or limits the expression of the motor recovery, thus compromising the possibilities of recovery itself. The learned non-use described by Taub probably corresponds to what Henry Meige described in hemiplegics using the expression ‘functional motor amnesia’ (Meige, 1905). Despite the fact that Ramachadran considers its concepts as different from Taub’s (Ramachandran and Altschuler, 2009), both processes are thought to be consequence of non-adaptive mechanisms, which result from the initial phase of stroke and then are maintained due to pathological plasticity. Therefore, following Ramachandran’s proposal, under MVF conditions, the visual information given by the mirror, of the impaired arm moving properly, could finally give a coherent feedback to the output sent by the brain, disentangling the above-mentioned pathological loop and, in turn, restoring the 'learnt' component of the motor impairment (Ramachandran and Altschuler, 2009).

MVF and Mirror Neurons System. Others authors have attributed MBT effects to the activity of the Mirror Neurons System, namely, a set of cells of both monkeys and humans that discharges not only when performing an action, but also when observing the same movement being performed by others (Rizzolatti and Craighero, 2004). This network, including the premotor cortex, supplementary motor area, inferior frontal gyrus, and inferior parietal lobule, is thought to play a key role in action recognition, motor learning and rehabilitation (Buccino et al., 2006). In this view, the illusionary image of a normal movement of the affected hand may help to recruit the motor system through the intimate connection between visual input and premotor areas, in a similar way to action observation (Yavuzer et al., 2008). In line with this, the MBT has been also related to a visually-guided motor imagery, namely a dynamic state of internal action representation without overt motor output (Jeannerod, 1994). Motor imagery itself could be considered as a way to trigger the above-mentioned mechanisms. In fact, following the *simulation hypothesis* it has been proposed that movement execution, motor imagery and action observation are all driven by the same basic mechanism (Jeannerod, 2001). Indeed, motor imagery, by activating the same network engaged in actual execution, has also been proven a promising tool in stroke (Braun et al., 2006). Here, motor imagery may offer another route to access to the motor system in stroke patients with poor voluntary motor ability. Specifically, the mirror would create the visual feedback of successful performance of the imagined action with the impaired limb (Stevens and Stoykov, 2003). Under MVF conditions, the recall to Mirror Neurons System (Rizzolatti and Craighero, 2004) is clear: the observation through the mirror of the correct movement would give the visual information necessary to the activation of motor areas. Evidence indicates the immediate activation of the superior temporal gyrus (Matthys et al., 2009 and elevated engagement of the premotor cortex (Hamzei et al., 2012) after MVF training. Both areas have been associated with the mirror neuron system. The superior temporal gyrus is involved in the visual identification of biological motion (Schultz et al., 2004). Together with the PMC, it forms a network that sustains biological motion imitation and motor skills acquisition (Buccino et al., 2006).

Notwithstanding, a recent fMRI investigation in stroke patients did not find MVF-related activity in areas of the mirror neuron system, thus questioning the above-mentioned hypothesis. Specifically, this study found increased neural activity in the precuneus and in the posterior cingulate cortex (Michielsen et al., 2010), areas with multisensory functions associated with self-awareness and spatial attention (Cavanna and Trimble, 2006). This evidence suggests a key role of the sensorimotor conflict inherent in MVF conditions in driving MVF effects.

1.5. The role of the multisensory conflict induced by the MVF

Another mechanism that may be relevant for explaining the effect of MVF is related to the phenomenon of multisensory integration, which is the ability of the brain to integrate congruent and incongruent sensory information from different modalities. As previously mentioned, we have seen that neuroimaging evidence points to the pivotal function of the sensorimotor mismatch between performed and observed actions in mediating MVF effects (Michielsen et al., 2010). In fact, rather than recruiting mirror motor areas in stroke patients, the Mirror Box Therapy has been shown effective in increasing neural activity in the precuneus and in the posterior cingulate cortex (Michielsen et al., 2010), areas with multisensory functions associated with self-awareness and spatial attention (Cavanna and Trimble, 2006). Similarly, under MVF conditions, activation of areas normally involved in monitoring sensory inputs to guarantee the correspondence between motor intentions and outputs has been found (Fink et al., 1999). In line with this evidence, it is plausible that during MVF conditions, the mismatch between visual, motor and somatosensory input, together with the conflict between expected and performed movements, might influence both behavioural and cortical functioning, driving the well-known effects.

Starting from those intriguing suggestions, while taking into account all the above-mentioned pieces of evidence, I focus my attention on the conflict inherent in MVF conditions when bimanual movements are required, delineating two different but overlapping components: the integration of conflicting input coming from different sensory modalities and the binding of expected and actual sensorimotor feedback.

Multisensory integration

In everyday life, we are always surrounded by a plethora of different stimuli that are analyzed by our senses; some of them give information about different aspects of the external world and need to be processed separately; most of them, however, are in accordance with each other and concern the same environmental object or event. In the latter case, each sensory modality decodes distinct stimuli following specific sensory modality rules, but in order to have a coherent and reliable representation of the external world, at some level of elaboration, complementary information about the same referent need to be combined into a single unitary percept to orient behavior. In fact, the brain is able to evaluate the importance of input coming from different sources and sensory channels and, if necessary, to synthetize all the information provided in a unique representation; this brain function, known as multisensory integration, has clear evolutionary basis, promotes sensory system interaction, and guarantees adaptive behavioral responses (Ghazanfar and Schroeder, 2006). From a behavioral point of view, this brain capability helps in disambiguating the detection and discrimination of stimuli, speeds responsiveness accuracy and reaction times (Stein and Stanford, 2008) and plays a fundamental role in perception, cognition and behavior

(Ghazanfar and Schroeder, 2006). Notwithstanding, the brain forces this structure even in cases of conflicting information, giving rise to cross-modal perceptual illusions, the importance of which have been delineated in neurorehabilitation (Bolognini et al., 2015).

Under MVF conditions, different sensory information need to be integrated into a single percept: the visual information from the mirror has to be combined with the motor command from the brain and the somatosensory signals from the limbs.

In this respect, an interesting study in healthy subjects, where the MVF was used to investigate the role of visual and proprioceptive information concerning the location of the unseen hand, showed that the felt position of the hidden hand depends upon an integrated, weighted sum of visual and proprioceptive information (Holmes and Spence, 2005). Specifically, the visual information seems to be weighted more strongly under active visuo-motor experience, compared to passive visual exposure only (Holmes and Spence, 2005).

Similarly, the integration of visual and proprioceptive information was recently investigated during a kinaesthetic illusion (Chancel et al., 2016). In this study, healthy participants were required to observed in the mirror the reflection of their left limb, whose forearm was passively moved. The illusory displacement of the right unseen hand, was assessed through both subjective reporting and a behavioural measure (namely, participants had to press a button with the foot to signal the onset of the illusion). Results confirmed that MVF conditions are not merely visual. In fact, both the visual afferents related to the virtually moving arm and the somaesthetic afferents of the contralateral arm are necessary. Significant illusory effects persist despite progressively impoverishment of the visual information from the mirror (by covering the mirror from 0% to 100%) and are influenced by alteration of the proprioceptive information from the hand in front of the mirror through the co-vibration of antagonist muscles. In fact, it was shown that even a limited amount of visual information is enough to provide cues for kinaesthetic purposes, i.e. illusory effects arose even with mirror covered for 84%. In addition, the masking of the somaesthetic afferents of the arm reflected in the mirror significantly affected MVF effects, namely this manipulation was associated with a significantly lower velocity of illusory displacement of the other arm (Chancel et al., 2016).

Sensorimotor processes

Sensorimotor integration is defined as the capability of the central nervous system to integrate different sources of sensory input, and to transform them into motor actions. The motor system is closely coupled to sensory feedback systems and is always monitored to detect deviations from what it is expected. In fact, the function of the motor control system is to allow an individual to act in a smooth and coordinated manner, as well as to prepare for the consequences of the planned movements. The human brain possesses sophisticate mechanisms for integrating visual and motor information, as well as for evaluating the causal and functional links between actions and their sensory consequences, and for detecting mismatches between the predicted and the actual sensory feedback (Frith and Wolpert, 2000). Indeed, movement-related mechanisms could be seen as a simple coupling of twofold transformations: from motor commands to sensory consequences and from sensory feedback to motor commands. This sensorimotor loop is represented as an internal model of movements, where multimodal sensory input, contributes to the integration (Wolpert and Ghahramani, 2000). Following an influent computational model (Frith and Wolpert, 2000), the motor system, on the bases of internal (egocentric) and external (allocentric) variables, predicts a certain response from the sensory system. In parallel, a control system compares the achievement of this desired state with the motor command necessary to achieve it. Subsequently, the "controllers" implement the appropriate motor commands to achieve the desired movement. This prediction (efferent copy) is compared with the actual sensory feedback and modified accordingly (Frith and Wolpert, 2000). For a visual representation of the abovementioned process, see Figure 1.

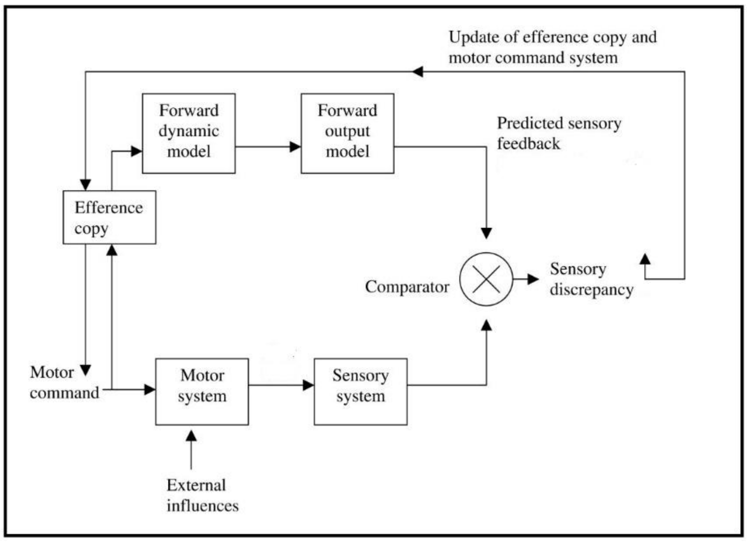

Figure 1. Schematic representation of the role of the "efference copy" in the motor control system. The information in the system of a certain moment is used to predict the sensory consequences of the motor command. This prediction, or efferent copy, is compared with the effective sensory consequences of a certain activity. In case of discrepancy, this information is necessary for updating the given information and restore new efferent copies. Modified from (McCabe and Blake, 2007).

Therefore, the visual system plays the key role of updating the motor system on the position of limbs in the space and gives indications of next movements from (McCabe and Blake, 2007). Due to external perturbations and acquired alterations, the sensorimotor loop could encounter difficulties; in turn, its outcome could become incongruent. Crucially, it has been proposed that the sensorimotor incongruity could be responsible of provoking pain (Harris, 1999; McCabe et al., 2005) and also may represent the leading mechanism of the 'learnt' component of the motor impairment following brain lesion (Ramachandran and Altschuler, 2009).

Notwithstanding, the same model could be exploited to interpret MVF effects, focusing on the inherent sensorimotor mismatch, especially under bimanual conditions. In fact, for any self-generated movement, the central nervous system creates an internal copy of the motor command. This efference copy has the function of signaling motor intention and permits the discrimination between acts that are internally generated and those who are driven by external sources (Blakemore and Frith, 2003). In particular, monitoring systems could be altered by external conflicts, such as those induced by the MVF. For example: under MVF conditions, when the motor output for moving the hidden hand is sent, an efference copy is also created to predict the consequences of the command. During the movement, the proprioceptive feedback from the hand, hidden to the sight, confirms the predicted proprioceptive correlates of the action. Given that the limb is hidden to the sight, the predicted sensory consequences of movement lack direct visual feedback. Conversely, the MVF replaces the lacking visual information with one image that is coherent with the expected sensory feedback. Indeed, since the bimanual movement is synchronous, the reflected visual feedback corresponds to the information specified by the hidden hand's command. Under those circumstances, the incongruent movement provided by the mirror would augment the normal background noise of the motor control. Consequently, the motor system would require a higher number of motor commands to execute the intended movement. This augmented background noise would be responsible of the subjective difficulties that healthy subjects may experience during motor tasks under MVF conditions (Harris and Wolpert, 1998). Similarly, the augmented noise could partly explain the recovery of the motor function in patients with motor impairments (Ramachandran and Altschuler, 2009).

In addition, this motor control framework has been recently taken into account with the aim of examining which estimates of the body's configuration are affected by MVF conditions (Soliman et al., 2016). It has been proposed that the illusory feedback given by the mirror is able to influence both the desired and the predicted state of the motor system. This view is supported by both behavioral and neuroimaging evidence that shows the involvement the cortical V6A, located in the posterior parietal lobe. This multisensory area, a node in a dorso-medial circuit comprising the medial intraparietal sulcus and the dorsal premotor cortex, not only is critically engaged in the mirror illusion and limb state estimation (Soliman et al., 2016), but it is also thought to be involved in both sensorimotor integration and motor planning (Gallivan et al., 2011).

Following this line of reasoning, the MVF effects would be primarily consequences of the twofold conflicts between incoming information: from one hand, a multisensory discrepancy between visual, somatosensory and motor information and, from the other, a cognitive mismatch between expected and actual feedback.

1.6. Aims of the experimental studies

Under multisensory MVF conditions, there are different incoming sensory and motor inputs that need to be unified into a single percept: the visual feedback given by the mirror has to be coupled with the motor output sent by the brain and the proprioceptive and somatosensory information from the hidden arm. In order to maintain coherence, the brain attempts to solve this multifaceted conflict creating an illusion; i.e., the movement of the (paretic) arm behind the mirror, namely Mirror Illusion. From a clinical point of view, this arrangement has been shown promisingly effective in ameliorating motor deficits due to stroke, albeit the underpinning mechanisms are still under investigations.

The next chapters will give a characterization of the effects of MVF conditions in both healthy and brain-damaged patients with motor impairment following cerebrovascular accidents, with the aim of providing evidence that supports the role of sensorimotor conflicts in the characterization of MVF effects. Through a series of studies, I will provide evidence of MVF effects in terms of subjective experience both in healthy and stroke participants (Chapter 2), cortical activity in healthy individuals (Chapter 3), and on motor recovery in post stroke patients (Chapter 4).

Specifically, in Chapter 2, I will deeply evaluate the subjective sensations associated to a single session of MVF in brain-damaged patients with motor impairment following cerebrovascular attack. Individual differences in the illusory sensations brought about by MVF, assessed through an ad-hoc questionnaire, will be further studied by taking into consideration the role of clinical and motor characteristics of the sample. The final aim is to provide evidence about whether and to what extent brain damages and related impairments influence the MVF experience.

In Chapter 3, I will use a TMS paradigm to investigate which are the consequences of an altered visual feedback on motor cortical excitability. I will assess the cortical excitability of healthy individuals exposed to an alteration of visual feedback, in a mirror-box-like paradigm, where the conflict inherent in MVF conditions has been further exacerbated.

Finally, in Chapter 4, I will investigate the effects on motor recovery of a modified version of the Mirror Box. This novel arrangement, here called REMIT, that is Reversed Mirror Therapy, is based on the mirror reflection of the impaired, rather than the intact, upper-limb. The aim is to assess whether the cortical changes found in healthy subjects concurrent with altered feedback are mirrored by clinical gains in post stroke patients with motor impairment. By doing so, I will complement my investigation about the role of MVF in patients through a rehabilitative protocol characterized by increased sensorimotor conflict.

Chapter 2

Mirror Illusion in post stroke patients with motor deficit

2.1. Aim of the study

As discussed in Chapter 1, MVF conditions could be associated with subjective feelings related to the hand in the mirror and to the one hidden to the sight. In addition, when concomitant movements are required, those sensations could come along with the retrieval of motor and somatosensory feelings. This phenomenon, known as Mirror Illusion, may be ascribed to the multisensory conflict provided by the mirror and to the related sensorimotor mismatch. Specifically, in patients with unilateral motor deficit this arrangement creates a conflict between the defective motor performance and the sensory feedback from the unaffected limb (Michielsen et al., 2010). This incongruence may force the activity of the observer's motor system to re-adjust for overcoming incongruent sensory inputs. However, up to now, the incidence of illusory sensations brought about the MVF has been not yet systematically assessed in stroke patients: whether and to what extent stroke patients experience a reliable Mirror Illusion is still unknown. In fact, while almost all MVF studies report a general feeling of illusion experimented by participants, what seems lacking is a deep evaluation of the individual sensations associated with the Mirror Illusion and the influence of subjective variables. Indeed, almost all the rehabilitation studies with the Mirror Box Therapy describe only anecdotally the emergence of illusory sensations, sometimes by reporting patients' spontaneous claims such as "It looks like my bad arm is moving normally" (Altschuler et al., 1999). Crucially, the occurrence of the Mirror Illusion may represent a key component of the Mirror Box Therapy (Bolognini et al., 2015), likely being able of acting as predictor of the clinical outcomes. It could be possible that clinical and lesional features of brain-damaged patients with motor deficit play a role in characterizing the illusionary experience. Similarly, given the multisensory nature of MVF conditions, different factors may affect the illusion in different ways. In this theoretical framework, the aim of the present study was to characterize the clinical and the lesion profiles associated to the illusory response to the MVF. Here, I aimed to identify the susceptibility of stroke patients with upper-limb hemiparesis to the Mirror Illusion (measured through a systematic self-report questionnaire), comparing the responses of stroke patients with left and right hemispheric lesions to those of age-matched neurologically healthy controls. Then, I looked for the identification of the demographic and clinical factors that may predict the emergence of the Mirror Illusion in stroke patients, also considering the role of the lesion profile and of the motor and visual imagery abilities. In particular, a group of 28 stroke patients with upper limb hemiparesis underwent a single session of MVF. Their experienced illusion, as assessed with a questionnaire [modified version of (Longo et al., 2009), was compared to that of a group of 18 neurological healthy individuals. I explored which different factors are associated with the induction of the Mirror Illusion: namely: age, gender, length of illness, etiology, (upper limb) motor evaluation, mental imagery and mental rotation ability, lesion size and locations.

2.2. Materials and Methods

Participants

Twenty-eight brain-damaged (BD) stroke patients with a unilateral hemispheric lesion, and a contralateral upper limb motor impairment were recruited from the in- and out-patient population of the rehabilitation units of the IRCCS Istituto Auxologico Italiano (Milan, Italy) and the Azienda Ospedaliera Carlo Poma (Bozzolo, MN, Italy). Participants gave their informed consent to the protocol, which obtained the approval from local Ethical Committees, and was conformed to the ethical standards of the Declaration of Helsinki (World Medical Association, 1991).

The sample included 5 females and 23 males with a mean age of 66.7 years (Standard Deviation, SD = ± 10.1), and a mean education level of 12.1 years (SD = ± 4.6). Patients had suffered a cerebrovascular disease and were tested in a subacute or chronic stage of illness. The 50% of patients had a damage affecting the right cerebral hemisphere. Demographical and clinical details of BD patients are reported in Table 1.

Adult patients with brain lesion entered in the study if presenting with an upper-limb hemiparesis following a unilateral ischaemic or haemorrhagic cerebrovascular accident. Exclusion criteria were: 1) history of previous neurological or psychiatric disorders; 2) sign of cognitive decline; 3) in cases of right-hemisphere lesions: presence of severe Unilateral Spatial neglect; in cases of left-hemisphere lesions: presence of severe language comprehension impairment. The last deficit could compromise the understanding of the experimental instructions, while neglect may impair the direction of attention toward the mirror.

A group of 18 neurologically unimpaired subjects, matched for age (66.2 ± 9.6 years, range = 50 - 81), sex (9 females, 9 males), and years of education (12 ± 4 years, range 5 - 18) served as controls. All healthy controls were right-handed with no history of neurological or psychiatric diseases.

ID		Age / Gender	Aetiology	Education (years)	Stroke duration (months)
Right-Hemisphere BD patients	**P1**	75, M	I	6	3
	P2	49, M	I	18	6
	P3	63, F	I	13	41
	P4	78, M	I	5	1
	P5	83, F	I	13	2
	P6	57, M	I	13	10
	P7	77, M	I	13	3
	P8	60, M	H	8	1
	P9	77, M	I	13	4
	P10	57, M	I	17	1
	P11	71, M	H	17	3
	P12	60, M	I	5	1
	P13	70, M	I	13	2
	P14	49, M	H	23	2
Left-Hemisphere BD patients	**P15**	56, M	I	13	39
	P16	70, F	I	9	2
	P17	69, M	I	13	1
	P18	87, F	H	18	3
	P19	46, M	H	13	2
	P20	61, M	I	18	2
	P21	72, M	H	5	3
	P22	65, F	H	16	1
	P23	63, M	H	13	14
	P24	68, M	I	5	84
	P25	70, M	H	10	15
	P26	75, M	I	10	16
	P27	68, M	H	11	3
	P28	73, M	H	10	22

Table 1. Demographical and clinical data of the patients. ID: Identification number**.** Aetiology: I = Ischaemic stroke, H = Haemorrhagic stroke. Gender: M = Male, F = Female.

Clinical Assessment

Prior the experimental session, patients underwent a clinical assessment including the following tests (see Table 2):

1) NIH stroke scale (Brott et al., 1989), a 15-item scale evaluating the effect of the stroke on: consciousness, language, neglect, vision, motor strength (in this study only the upper limb was evaluated), ataxia, dysarthria, and sensory loss. Ratings for each item are scored with 3 to 5 grades with 0 as normal (Maximum score, signifying severe stroke = 34).

2) Assessment of asymmetric visual and somatosensory deficits (Bisiach et al., 1983) including extinction to bilateral stimuli, which are assessed by manual confrontation. At this standard neurological exam, the presence of a visual-field or somatosensory deficit is indexed by the lack of report of ≥30% of contralesional single stimuli. For each function tested (i.e., visual and somatosensory), the score range is: 0 = unimpaired performance; 3 = maximum deficit.

3) 'Pinch Grip' subtest of Motricity Index (Demeurisse et al., 1980): a brief means of assessing motor impairment by examining one movement, namely grip a 2.5 cm cube between thumb and forefingers. The movement is given a score according to the strength. The scores are between 0 = no movement to 33 = normal movement.

4) Motor activity Log scale, MAL (Uswatte et al., 2006): In this semi-structured interview, patients are requested to record and evaluate the amount (subscale

MAL-A) and quality (subscale MAL-Q) of daily life activities of the paretic arm, using a 6-point ordinal scale. Higher scores indicate better performance.

5) Mental Imagery Questionnaires, Vividness of Visual Imagery Questionnaire (VVIQ) (Marks, 1973) and Vividness of Motor Imagery Questionnaire (VMIQ) (Isaac et al., 1986). The VVIQ comprises 16 items divided in four sessions of scenes and situations and measures the ability to form visual mental images; the VMIQ comprises 24 items related to movement (e.g., walking, jumping) and measures the capability to recreate motor mental images. Both questionnaires require to mentally recreate images and to judge their vividness along a 5-point scale, with lower scores indicative of low vividness: 1= No image at all; 2= Vague and dim; 3= Moderately clear and vivid; 4= Clear and reasonably vivid; 5= Perfectly clear and as vivid as normal vision.

ID	NIH	VID	SOD	MI	MAL-A	MAL-Q	VVIQ	VMIQ
P1	4	1	1	22	2	2	75	73
P2	6	0	2	0	2	1	72	99
P3	0	0	0	33	4	3	79	120
P4	2	0	0	22	3	2	73	119
P5	2	1	0	22	1	2	80	120
P6	8	1	2	11	0	0	76	117
P7	6	0	3	0	0	0	80	120
P8	7	3	3	0	2	2	66	109
P9	4	0	0	0	0	0	49	96
P10	2	0	0	0	0	0	78	65
P11	5	0	3	0	0	0	73	108
P12	2	0	2	0	2	1	56	71
P13	2	0	0	33	5	2	80	93
P14	2	0	0	22	2	1	70	86
P15	1	0	0	33	0	0	60	113
P16	3	0	0	33	4	3	65	69
P17	5	0	0	33	4	4	62	80
P18	5	0	0	11	5	2	75	70
P19	3	0	0	26	3	3	72	83
P20	2	0	0	33	3	3	62	79
P21	2	3	0	22	0	0	67	91
P22	7	0	3	11	1	1	71	111
P23	6	0	0	0	0	0	62	103
P24	3	0	0	33	2	5	75	109
P25	2	0	0	33	4	4	80	114
P26	4	0	0	0	1	2	35	47
P27	0	0	0	33	3	3	70	88
P28	2	0	0	26	4	2	54	48

Table 2. Neurological and motor data for patients. ID: Identification number. NIH = National Institute of Health Stroke Scale: individual total score (score range = 0 - 34). ViD = Visual Deficit, and SoD = Somatosensory Deficit (score range = 0 - 3). MI = Motricity Index: scores of Pinch Grip subtest (score range = 0 - 33). MAL = Motor Activity Log Rating Scale: MAL-A = Amount of movements, quantitative subscale (score range = 0 - 5); MAL-Q = Quality of movements, qualitative subscale (score range = 0 - 5). VVIQ = Vividness of Visual Imagery Questionnaire (score range = 0 - 80). VMIQ = Vividness of Motor Imagery Questionnaire (score range = 0 - 120).

Lesion Data

MRI or CT scans were available for 27 out of 28 patients (P26's scan was not available). Regions of Interest (ROIs) defined the location and the size of the lesion for each patient (Figure 1). These were reconstructed by means of a template technique, by manually drawing the lesion on the standard template from the Montreal Neurological Institute (Rorden and Brett, 2000), on each 2D slice of a 3D volume. Figure 1 shows the overlay lesion plot of all patients. Mean lesion volumes were 65.34 cc^3 (± 89.65 cc^3, range = 4.7 - 242.5 cc^3) for right-hemisphere damaged patients, and 11.05 cc^3 (± 18.66 cc^3, range = 0.6 - 70.4 cc^3) for left-hemisphere damaged patients.

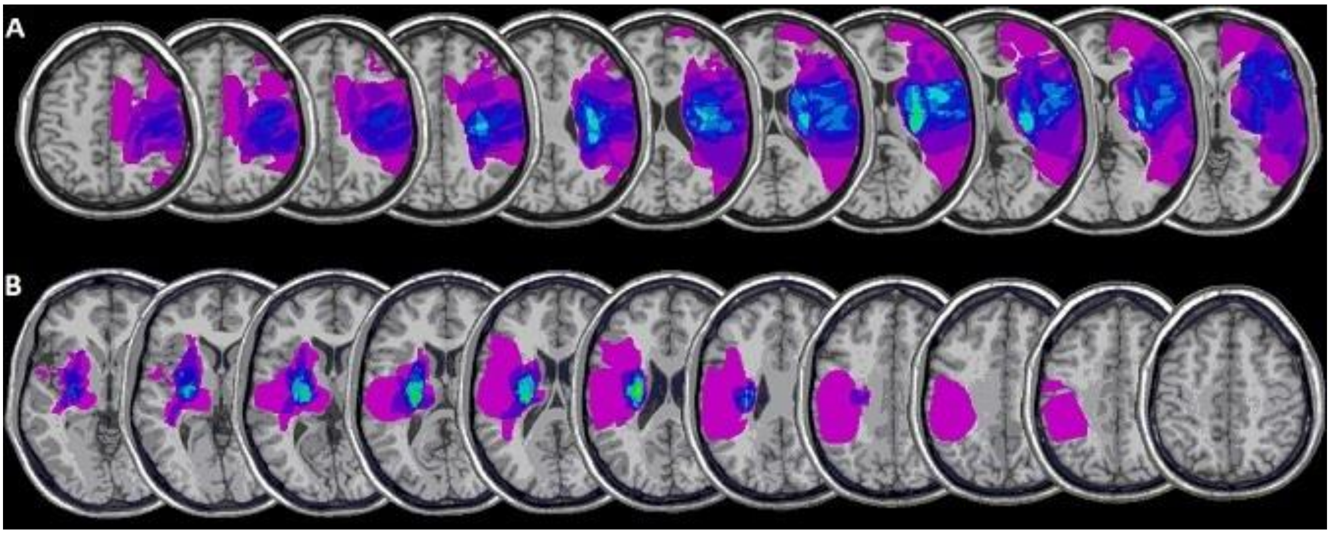

Figure 1. Lesions of patients. Overlay lesion plots for patients with (A) a right-hemisphere lesion (N = 14) and with (B) a left-hemisphere lesion (N = 13). Each colour represents 20% increments, from green areas indicating maximum overlap, to pink areas indicating minimum overlap.

Procedure

Induction of the Mirror Illusion

Patients sat at a table with their arms on the desk at the two sides of a mirror placed perpendicular to their body midline, with the paretic arm behind (see Figure 2A). They practiced 15 arm exercises, each lasting 1 minute, while continuously asked to observe the mirror. The movements had to be performed moving both hands or arms symmetrically while watching the reflection of the mirror. The instruction emphasised to move the paretic limb as best they could (Altschuler et al., 1999). Each new movement was explained and showed by the experimenter who was sat in front of the patient and checked whether they were performing watching through the mirror. Healthy subjects underwent the same MI session, performing the movements with their non-dominant left hand behind the mirror (See Figure 2B). At the end of the session, each participant filled the Mirror Illusion Questionnaire (MIQ) [adapted from (Longo et al., 2009)], which comprised the following items (translated from Italian): 1) "It felt like I was looking directly at my hand rather than at a mirror image"; 2) "It felt like the hand I was looking at was my paretic/*left* hand"; 3) "It felt like the hand behind the mirror was moving"; 4) "It felt like the hand behind the mirror would move together with the other hand"; 5) "It felt like I perform well with both hands"; for healthy controls: "I had difficulty in maintaining the hand behind the mirror still". Participants rated each item using a 7-point Likert scale, with 6 = strongly agree, 3 = neither agree, nor disagree, 0 = strongly disagree. The sum of scores given to each item has been considered indicative of the amount of illusion.

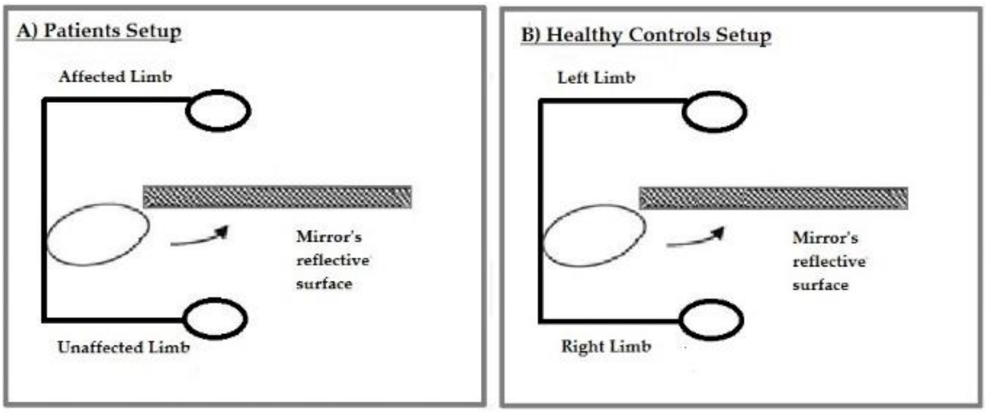

Figure 2. Experimental setup. Patients (A) place their paretic arm behind the mirror, out of the sight; the mirror reflects the image of the unaffected arm. Right-handed neurologically healthy control participants (B) place their left arm behind the mirror, out of the sight; the mirror reflects the image of the right arm. Modified from (McCabe, 2011).

Statistical Analyses

Statistical analyses were performed using IBM SPSS Statistics, Version 22.0 (Armonk, NY: IBM Corp.). In order to assess the difference in the illusion between brain damaged patients and healthy controls, Mirror Illusion Questionnaire scores (log-transformed) were submitted to an Analysis of Variance (ANOVA) with Group (Controls, Right-hemisphere BD and Left-hemisphere BD patients) as between-subjects factor. Moreover, the MIQ total score of each patient was compared with that of healthy controls by means the Crawford & Garthwaite's Test (Crawford and Garthwaite, 2002), a statistical method used in single-case studies to perform case-control comparisons aimed at estimating the abnormality of the patient's score at each test (i.e., the estimate of the percentage of the control population that would obtain a lower score).

Finally, regression analyses were run to identify predictors of the Mirror Illusion from clinical, demographic, and lesion data.

2.3. Results

With respect to the MIQ, the ANOVA failed to show a significant main effect of Group [$F_{(1, 27)} = 1.5$, $p = 0.22$], hence indicating that overall stroke patients showed a reliable Mirror Illusion comparable to that experienced by healthy individuals (see Figure 3). However, single case analyses showed that 4 out of 28 patients had a significant lower MIQ total score, as compared to healthy controls (24.1): P6 (score = 16, t = -2.32, $p = 0.03$), P9 (0, t = -6.9, $p < 0.01$), P14 (16, t = -2.32, $p = 0.03$), P23 (16, t = -2.32 $p = 0.03$).

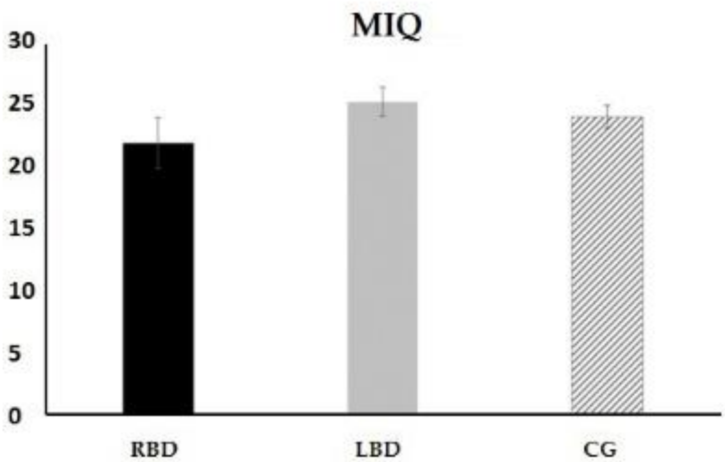

Figure 3. MIQ. Mean total MIQ score of stroke patients, with a right-hemisphere lesion (RBD, black bar) and Left-hemisphere lesion (LBD, light grey bar), and of healthy controls (CG, patterned black and white bar). MIQ: Mirror Illusion Questionnaire. Error bars = Standard Error of the Mean, SEM.

Multiple regression analysis with the dependent variable MIQ total score and Age (β coefficient = -0.11, t = -.57, $p = 0.57$), Disease duration (β coefficient = 0.66, t = 0.32, $p = 0.75$) and NIH total score (β coefficient = -0.15, t = -0.74, $p = 0.46$) as predictors did not show any significant association of these factors to the MIQ score. Considering the motor and sensory deficits as predictors, the analysis did show the association between the MIQ score and the Pinch Grip subtest of the Motricity Index (β coefficient = 0.66, t = 3.15, $p < 0.01$), indicating that the less severe motor deficit (i.e., higher the motor score), the larger the experienced MI; also the somatosensory deficits predicted the illusion (β coefficient = 0.45, t = 2.09, $p = 0.05$), with a more severe tactile defect (higher score) being associated with a higher MIQ score. The visual deficit (β coefficient = 0.02, t = 0.12, $p = 0.9$) was not associated to the MIQ score. Similarly, the amount and quality of use of the paretic hand in daily living was not associated with the MIQ score (MAL-A, β coefficient = 0.19, t = 0.7, $p = 0.5$; MAL-Q, β coefficient = 0.25, t = 0.92, $p = 0.4$).

Finally, visual imagery abilities (VVIQ, β coefficient = 0.60, t = 0.289, $p < 0.01$), but not motor imagery abilities (VMIQ, β coefficient = -0.30, t = -1.45, $p = 0.16$), were positively associated with the illusory sensations. With respect to the lesion profile, as reported in detail in Table 3, the size (number of voxels) of the lesion affecting middle and superior frontal cortices, the precentral gyrus and the inferior parietal cortex, was positively associated to the MIQ score (all Ps < 0.05), hence the larger the lesion affecting these areas, the larger the illusory effects. Conversely, the size of the lesion involving the superior parietal cortex and the postcentral gyrus was negatively related to the MIQ score (*Ps* < 0.05, see Table 3), that means the larger the lesion affecting these areas, the smaller the illusory effects.

	β coefficient	**T**	***p*-level**
Basal ganglia	0.09	0.68	0.51
Insula	-0.21	-0.49	0.63
Thalamus	-0.02	-0.30	0.77
Ippocampus	-0.03	-0.23	0.82
Inferior Frontal	-1.87	-1.81	0.10
Middle Frontal	8.20	4.47	**0.001**
Superior Frontal	16.50	3.39	**0.005**
Precentral Gyrus	18.92	7.90	**0.001**
Superior Temporal	-0.60	-0.17	0.87
Middle Temporal	-1.61	-1.18	0.26
Inferior Temporal	0.68	0.20	0.84
Inferior Parietal	29.66	7.99	**0.0001**
Superior Parietal	-21.11	-4.16	**0.001**
Postcentral Gyrus	-40.50	-8.84	**0.0001**

Table 3. Results from the multiple regression analysis. The dependent variable was the MIQ and the independent variables were the amount of lesion (number of voxels of the damaged area) affecting the different brain regions. Bold number indicates *p*-values < 0.05.

2.4. Discussion

The present results demonstrated that, overall, brain damaged patients with upper-limb hemiparesis due to stroke experienced the Mirror Illusion in a similar manner to healthy individuals, regardless of the side (left or right) of their hemispheric lesion. This evidence indicates that the illusion is a pervasive cross-modal phenomenon largely resistant to brain injuries (Bolognini et al., 2015). However, single case analysis also showed that 4 out 28 patients did not experience a reliable Mirror Illusion, a finding that suggests that a specific clinical or lesion profile may impact the illusory effects of the Mirror Visual Feedback.

This proposal is supported the finding of regression analyses. Those results, in fact, showed that the severity of motor and somatosensory deficits influences the illusion, with the motor deficit being negatively associated with the illusory effects (the less severe motor deficit, the greater the Mirror Illusion), and the tactile defect being positively associated with them (greater the tactile defect, the higher MIQ score). Instead, the time elapsed from stroke, the stroke severity (as indexed by the NIH stroke scale) and the patients' age did not influence the Mirror Illusion. Mental imagery abilities also predict the susceptibility to this illusion, but it is the visual, rather than the motor, imagery that is relevant. Finally, the lesion profile modulates the illusory effects: the extension of the lesion affecting superior and middle frontal cortices, the precentral gyrus and the inferior parietal cortex predicts a stronger Mirror Illusion, while a damage of the superior parietal cortex and of the postcentral gyrus is associated with a weaker illusion.

With respect to the clinical signs, the amount of residual motor capacity of the paretic arm is positively associated with the amount of illusory effects: the lower the motor deficit (as indexed by 'the Pinch Grip' subtest of the Motricity Index), the higher the Mirror Illusion. Hence, some residual motor functions are necessary to allow the incongruent Mirror Visual Feedback to induce an illusionary feeling of moving the (out-of-view) paretic arm, allowing a multisensory reactivation of the motor, maybe latent, representation of the paretic limb. To note, the four patients that experienced a significant lower illusion as compared to control group, reported little to no beneficial qualitative contribution and little to no use of the paretic limb (as assessed through MAL questionnaire). Instead, the tactile deficit influences the Mirror Illusion in the opposite direction: the greater the tactile impairment, the greater the illusion. Cross-modal illusions typically emerge when the information provided by one sense (vision in the Mirror Illusion) dominates over another sense (here the impaired touch), such as it can bias the processing within unreliable, weaker, sensory modality. Therefore, the mirror-induced illusory sensations need to overcome and dominate the unreliable, real, tactile sensations in order to be able to alter them, in turn promoting the feeling of moving the paretic arm. Instead, reliable, tactile sensations reduce the Mirror Illusion because in this case the visual feedback cannot overcome and dominate over the real touch. Brain diseases, by altering the acuity of one sense, may render such weakened sense more vulnerable to cross-modal interferences from the intact senses (Bolognini et al., 2015).

The visual modality is also relevant, but it is not the integrity of visual processing to affect the illusion; rather it is the visual imagery. Although visual perception and visual imagery have common neural substrates, the subjective experiences of imagining and seeing are clearly different, and at least some sensory processes may be engaged differently by visual imagery and perception (Ganis et al., 2004). More importantly in the present context, visual imagery and perception differently interact with multisensory integration (Amedi et al., 2005).

With respect to the neural underpinnings of the Mirror Illusion, our findings indicates that both the frontal and the parietal lobes are involved in such illusion, with interesting dissociations: damages to the precentral gyrus, middle and superior frontal regions and the inferior parietal cortex are positively associated with it, whereas damages to the postcentral gyrus and to superior parietal regions are negatively related with the illusion.

The positive association between the amount of the damage to the precentral gyrus and the Mirror Illusion indicates that a damaged to the motor cortex increases the susceptibility to the illusion, likely because the patient remains deprived of a reliable motor input necessary to discriminate between real and illusory movements; under this condition, the visual modality can easily dominate, biasing movement sensations. Moreover, prefrontal self-related processes, and in particular self-reflective introspection, which activates superior and middle frontal regions, are not necessarily engaged during sensory perception and can be actually suppressed (Goldberg et al., 2006). Therefore, a lesion disrupting such self-related prefrontal activity may facilitate the so-called phenomenon of 'losing oneself' into the illusory sensory experience created by the mirror reflection.

In healthy individuals the sense of agency, namely the feeling that leads us to attribute an action to ourselves, activates the inferior parietal cortex (Yomogida et al., 2010), which is also involved, in amputees, in the self-attribution of the mirror image of the intact arm (Foell et al., 2014). This suggests that a damage to this area may fool the brain about the sense of agency of the illusionary movement reflected by the mirror, in turn explaining the increased Mirror Illusion. The association between the inferior parietal damage and increased Mirror Illusion is also reminiscent of the hyper-binding parietal model proposes to explain synaesthesia: accordingly, the increment of the illusion related to an inferior parietal lesion could reflect a sort of lesion-induced disinhibition of parietal mechanisms regulating and controlling cross-modal binding in normal conditions (Hubbard, 2007). In this regard, it is noteworthy that a number of cross-modal illusions are largely spared, and even enhanced, in patients with neglect whose main neural underpinning is the right inferior parietal lobule (Bolognini et al., 2015).

Instead, the extension of the lesion affecting the superior parietal cortex and the postcentral gyrus predicts a reduction of the Mirror Illusion. The superior parietal cortex plays a major role in cross-modal attention, visuo-motor coordination and visual-proprioceptive integration (Fink et al., 1999; Yomogida et al., 2010). Using positron emission tomography, Fink and co-workers showed that the effects of MVF during either in-phase or out-of-phase movements (that are perceived as being in-phase, due to the mirror) are linked to the activity of the superior parietal cortex (Fink et al., 1999). With respect to the postcentral gyrus, substantial visual-somatosensory interaction takes place in the primary somatosensory cortices (Bolognini and Maravita, 2007; Fiorio and Haggard, 2005) and the Mirror Illusion has an immediate effect on the activation of somatosensory areas (Fritzsch et al., 2014). The increase activity of primary and secondary somatosensory regions was proposed to reflect a rise in attentional resources to resolve the perceptual incongruence brought about by the Mirror Illusion (Deconinck et al., 2014). Moreover, since the somatosensory cortex encodes internal references for one's own body representation (Tsakiris et al., 2007), its damage could prevent the cross-modal embodiment of the moving hand into the patient's own body representation, lowering the efficacy of the illusion.

In conclusion, the present work shows that different behavioural and neural factors contribute to the vulnerability to the Mirror Illusion in stroke patients with hemiparesis. The brain possesses an inherent tendency to integrate conflicting multisensory information in order to preserve a coherent representation of body-related signals (Bolognini et al., 2015). Such ability, here indexed by the Mirror Illusion, is influenced in stroke patients by sensory and motor defects and the lesion profile. Some residual motor function are necessary to give rise to the Mirror Illusion, while unreliable tactile sensation may even enhance it; visual imagery is also relevant. On the other hand, damages to low- and high-level areas of the motor cortical system enhance the Mirror Illusion, while lesions to parietal regions may differentially impact it, disrupting or facilitating specific processes involved in multisensory, body- and self-related, processing. This evidence may be useful to improve the rehabilitation of post-stroke hemiparesis, suggesting a potential role of the assessment of the patient's susceptibility to the Mirror Illusion as a predictor of the individual clinical response to the Mirror Therapy. Recent findings are promising in this regard showing that the amputees' ability to associate the arm movement reflected in the mirror to their phantom limb is linked to the phantom limb pain relief induced by the Mirror Therapy (Foell et al., 2014).

Chapter 3

Modulation of primary motor cortex excitability through the observation of altered visual feedback

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3.1. Aim of the study

As discussed in the previous chapters, under MVF conditions, when the hand in the mirror is superimposed on the hand behind the screen, and participants are required to perform movements, the conflicting information provided needs to be unified into a single percept. The mismatch induced by the MVF comprises a cognitive discrepancy between performed and seen movement, and a multisensory conflict between motor output and visual feedback. Previous TMS studies have shown that the observation of self-generated movements through the mirror leads to a greater activation of the M1 ipsilateral to the moving hand, than performing unimanual movements without vision (Funase et al., 2007; Garry et al., 2005). As also observing movements increases the excitability of the observer's M1 (e.g. Fadiga et al., 1995; 2005), likely by the activation of mirror systems involved in action simulation, it has been suggested that similar mechanisms might modulate cortical facilitation under MVF conditions (e.g. Ramachandran and Altschuler, 2009). However, what happened when the mismatch related to MVF conditions is further exacerbated, namely when the feedback deviates even greatly from what it is expected?

In the present study, I have explored the impact of multisensory conflict provided by MVF conditions on corticospinal excitability. To this aim, the sensorimotor mismatch of the MVF was manipulated in order to assess whether, and in which direction, the amount of visual-motor discrepancy could modulate motor cortex excitability. To address this issue, I developed a virtual MVF paradigm, during which subjects had to perform unilateral movements with their left hand, while watching video-clips of a right hand performing the same movements but at different speeds (namely slower, same, and faster). The videos were presented on a flat monitor aligned with participants' sagittal plane, behind which participants had their right hand at rest, similarly to MVF conditions. Single-pulse TMS (sTMS) was used to measure motor cortex excitability in the left hemisphere recorded from different muscles (namely, First Dorsal Interosseous, FDI and Abductor Digiti Minimi, ADM) of the resting hand during the observation of the altered movement.

3.2. Materials and methods

Participants

Fifteen neurological healthy individuals (12 women; mean age ± SD = 25 ± 2.7 years) entered in the study. Inclusion criteria were: 1) Normal or corrected-to normal vision, and 2) Right handeness, assessed through the Oldfield handedness questionnaire (Oldfield, 1971). Exclusion criteria were: 1) Neurological, psychiatric, or other medical problems, and 2) Contraindication to TMS (Rossi et al., 2009). Participants gave written informed consent prior to be enrolled in the study, which was approved by the ethical committee of the University of Milano-Bicocca and was carried out in accordance with the ethical standards of the Declaration of Helsinki (WorldMedical Association, 1991).

Procedure

Prior the starting of the experimental session, participants underwent a training phase during which they were familiarized with the motor task. In this initial part, they were asked to perform cyclic abduction-adduction movements with both the left and the right index fingers for a total duration of 5 minutes. In particular, they were instructed to synchronize the onset of the opening phase of the movement with a metronome beating 1 Hz, to abduct the fingers to near maximum aperture, and to complete one open-close cycle on each beat of the metronome. The same movement, namely cyclic abduction-adduction following the metronome that was beating 1 Hz, was the task of the experimental session, to be done only with the left index. During this phase, participants sat at a table with both their arms at the two sides of a flat computer screen that was placed perpendicular to their body midline. They were asked to find the most comfortable position to be maintained throughout the duration of the tasks with the right arm behind the screen, relaxed and hidden to the sight. During the experimental session, they performed the trained abduction-adduction movement with their left hand only, at the same frequency of 1 Hz paced by the metronome. This task was performed while they had the instruction to watch different videos presented in the flat computer screen that was placed perpendicular to their body midline. The video-clips presented a right hand that was performing the same cycling abduction-adduction index finger movement. In order to recreate the MVF setting, each participant had the position of the screen adjusted, so that his/her right hand was hidden from view, and the hand on the screen appeared roughly the same size and position as his/her right hand (Figure 1).

The right hand in the computer screen performed the same motor task, in four different conditions (namely, *Slower pace, Same pace, Faster pace, Static)*, the order of which was randomized and counterbalanced between participants, with few minutes rest between conditions, to avoid fatigue.

Specifically the four videos were characterized as follows.
1) In the *Slower pace* condition, the seen hand moved slower than the participants'

hand: the hand shown in the video moved at 0.5 Hz, that is, at the half speed of participant's left hand;

2) In the *Same pace* condition, the seen hand and the participant's hand had the same speed: the hand shown in the video performed the movement at a frequency rate of 1 Hz, namely moving at the same speed of the participant's left hand;

3) In the *Faster pace* condition, the seen hand moved faster than the participants' hand: the hand moved at 2 Hz, namely, two times faster than the speed of the participant's hand (2 Hz);

4) In the *Static* condition, the seen hand did not move: the video-clip showed a right hand that was maintained static, with the fingers closed in a natural position, with the palm facing down, for whole duration of the condition; the participant's hand moved at 1 Hz.

Following the gender of each participant, the video-clips presented a male or female right hand, accordingly.

In particular, the "*Same pace*" was made of 7 movements, (1 sec each), presented 21 times in a loop, for a total duration of 147 s. 7 repeated movements were chosen (and not only one) with the aim of creating a video that was smoother and more naturalistic. Conversely, the other videos had the same duration and were realized by halving (0.5 Hz, *Slower pace* condition) or doubling (2 Hz, *Faster pace* condition) the video presentation frame rate. During the *Static* condition, only the first frame of the video-clip, presenting the resting hand, was shown for its whole duration. To note, the last condition served as baseline.

In each video was also included the sound of a metronome, always beating at 1 Hz, regardless the pace of the movements presented in the screen. The metronome started together with the beginning of the movement of the hand in the video. Participants had to move their left index finger, synchronizing the movement to the metronome (that was always 1 Hz), while watching the video clips shown. To note, in the *Same pace* condition, the movement presented through the screen was always synchronous with the movement performed by the participant.

Figure 1. Experimental setting. Participants sat at a table with the two arms at the two sides of a flat PC monitor that was placed perpendicular to their body midline. They performed cyclic abduction-adduction movements with their left index finger at 1 Hz, while watching the monitor. In the screen, video-clips of a right hand, superimposed over their unseen right hand, moving at different paces (0.5 Hz, 1 Hz, 2 Hz) or laying static, were shown. The left M1 was stimulated by means of sTMS. Participants' right hand was static. TMS-induced MEPs of the right hand and active EMG activity of the left hand were recorded from both FDI and ADM muscles.

TMS protocol

During the session, motor-evoked potentials (MEPs) were recorded from the First Dorsal Interosseous (FDI) and the Abductor Digiti Minimi (ADM) of both hands by using surface Ag/AgCl electrodes over belly of each muscle (active electrode) and over the associated metacarpophalangeal joint (Wassermann et al., 2008).

The EMG signal was amplified (gain 1,000) by a Digitimer D360 amplifier (Digitimer, Hertfordshire, UK), bandpass filtered (20 Hz-2.5kHz), digitized (sampling rate: 1kHz) by means of a CED Power 1401 controlled with Spike 2 software (Cambridge Electronic design, Cambridge, UK), and stored for offline analysis. The single pulse TMS (sTMS) was performed using a commercially available 70-mm figure-8 coil connected to a Magstim 200 (Magstim Company, Withland, United Kingdom). An elastic cap was adhered to each patient's head and placed using reference anatomical landmarks according to the nasion−inion line and the interaural line, centered over the vertex. The coil was maintained tangentially to the scalp and 45° from the midsagittal line, in order to optimally activate the corticospinal pathways (Mills et al., 1992). The following procedure was used to obtain cortical excitability parameters (Wassermann et al., 2008). First, the "hot spot", defined as the site where MEPs with the highest amplitudes were elicited with a slightly suprathreshold intensity for the FDI muscles, was determined. Then, the resting motor threshold (rMT), defined as the minimal TMS intensity that elicited MEPs with amplitudes of at least 50 μV in at least 5 out of 10 consecutive stimuli, was obtained (Rossini et al., 1994). The mean (± SD) rMT was 60% (±9) of the maximum stimulator output. The intensity of the pulses was set at 110% of the rMT.

During the session, MEPs induced by sTMS were recorded from participants' right hand while they were observing the video-clips and they were simultaneously performing the movement with the left hand. STMS pulses were randomly delivered every 6 - 7 seconds, leading to a total of 20 pulses for each condition. To note, no sTMS pulses were delivered for the first 7 s of each condition, with the aim of leaving participants enough time to properly acquire the correct pace with their left hand. Sequence and timing of the video-clips and of sTMS pulses were under computer control (E-prime software, Psychology Software Tools, Pittsburgh, PA).

Statistical analysis

EMG signals were processed offline. Mean peak-to-peak MEP amplitude values were calculated for each condition and each muscle (FDI, ADM) of the resting right hand. The Kolmogorov-Smirnov test was used to study the distribution of the data. Given the violation of the assumptions, MEP values were log-transformed. Firstly, trials with background EMG activity preceding the TMS pulse or with the MEP amplitude higher or lower than 2 SD of the mean, calculated for each muscle in each condition, were excluded from further analysis (Novembre et al., 2012). Accordingly, 16% of the trials were discarded. Secondly, to reduce individual differences, for each muscle we subtracted the mean MEP amplitude in the *Static* condition from MEP amplitude recorded in each of the other conditions (Avenanti et al., 2007). The effect of the observation of movements performed at different speeds on M1 excitability was assessed by analysing MEPs using a linear mixed effects model (Brown and Prescott, 2006), with Muscle, Movement Pace, and Muscle by Movement Pace interaction as fixed effects, and Subjects as random effect.

Given that MEP amplitude in the hand at rest (here, the right hand) is affected by the contraction of the contralateral, active hand (Liepert et al., 2001), we also analysed the EMG activity of the left, moving FDI. This was done in order to exclude that possible differences in MEP amplitude were simply due to unwanted differences in the execution of the motor task with the left hand in a given condition. To this aim, the mean amplitude of the EMG signal recorded from the left active FDI was measured as the root mean square (RMS), calculated over a 25 ms running window, for the entire duration of each condition. Mean RMS values were then analysed via a repeated measure Analysis of Variance (rmANOVA) with Movement Pace (Same, Faster, Slower, Static) as within-subjects factors.

Finally, to verify whether participants were able to maintain the correct pace in each condition, without being influenced by the rhythm of the observed movements, a Fast Fourier analysis was performed on the EMG signal of the left FDI for each condition. The peaks in this function, denoting the frequency of hand movements over the entire duration of each condition, entered an rmANOVA with Movement Pace as within-subjects factor.

3.3. Results

For right resting hand (which was maintained relaxed and out of the sight), the analysis conducted with a linear mixed model showed a significant main effect of Movement Pace [$F_{(2,30)} = 4.42$, $p = 0.02$]. As shown in Figure 2, for both muscles (namely, FDI and ADM), of the right resting hand, results evidenced a relationship between the pace of the observed movements and motor cortical excitability as indexed by MEPs. In particular, the slower the frequency of the observed movement, the greater the MEP amplitude (mean ± SD = FDI muscle: *Slower pace*: 1.13 ± 0.15 mV, *Same pace*: 1.02 ± 0.18 mV, *Faster pace*: 0.98 ± 0.18 mV; ADM muscle: *Slower* pace: 1.02 ± 0.16 mV, *Same pace*: 0.97 ± 0.14 mV, *Faster pace*: 0.95 ± 0.13 mV).

Instead, the rmANOVA run on the motor activity of the moving hand (RMS values) did not show a main effect of Movement Pace [$F_{(1,14)} = 1.2$, $p > 0.3$], suggesting that the performance of the left hand was not influenced by the observation of movements at different paces.

Finally, EMG activity of the left FDI (Fourier analysis) indicated that participants were able to correctly perform the movement at the required frequency rate of 1 Hz in every experimental condition, regardless the pace shown in the videos (*Slower pace* = 1 ± 0.03 Hz, *Same pace* = 1 ± 0.08 Hz, *Faster pace*

= 1 ± 0.03 Hz; *Static* = 1 ± 0.05), as confirmed by the rmANOVA on the peaks in this function [$F_{(1,14)} = 0.5$, $p = 0.7$].

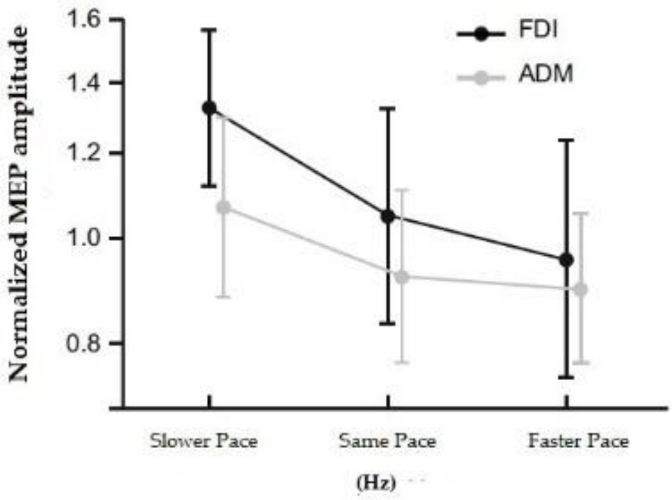


Figure 2. Results. The graph shows MEP amplitude, normalized on the static condition, for FDI and ADM muscles (dark and light grey, respectively), in the three movement conditions (Slower, Same and Faster pace); to note all the conditions were normalized over the static one. Error bars = SEM.

3.4. Discussion

This study evaluated the effects of manipulating the visual feedback of one hand, during the movements of the other, on the excitability of the M1 ipsilateral to the moving limb. The aim was to assess cortical excitability changes that follow an increased MVF conflict. To this aim, I created a modified version of the mirror used in standard MVF paradigms by replacing it with a flat computer screen that was located perpendicular to the body midline of the participants, as in classic MVF conditions. During the experimental session, neurological healthy participants were asked to perform cyclic abduction-adduction of the index finger with the left hand, while watching the screen that presented videos of a right hand that appeared roughly in the same place and position of their own unseen right limb. In fact, this arrangement was created in such way that the videos provided movements of a right hand, superimposed upon participants' right hand. In the four different conditions, the observed hand performed the same movement of the participant's left hand, at the same or different paces. Results show that M1 excitability of the resting hand, indexed by MEPs recorded from the FDI and ADM muscles, is inversely associated to the speed of the altered visual feedback. In particular, the slower the observed movement, the greater the MEPs modulation. Indeed, watching a movement that is slowed down, as compared to the executed one, leads to a greater M1 activation than watching the movement performed at the same pace. Conversely, M1 activation decreases while observing a speeded up movement. The inverse relation that links MEP amplitude and the shown movement speed might be the result of a mechanism that tries to compensate for motor discrepancies: when the observed movement is speeded up, M1 activation decreases, when it is slowed down, M1 activation increases. It is important to say that this effect should not be considered as consequence of unintentional systematic differences across conditions, in terms of contraction strength of the participant's moving left limb (Liepert et al., 2001). In fact, the frequency rate at which the left-hand movements were performed and the mean amplitude of the EMG signal recorded from the left FDI did not differ across conditions. That is, all participants were able to accomplish the task following the given instructions, namely to always perform the trained movement at 1Hz, without being influenced by the pace shown in the screen.

The relation between the (slower) pace of the watched movement and the (greater) M1 activation may indicate that the brain attempt to solve the conflict between the 'successful' motor program of the performing limb, and the visual feedback provided that shows the consequences of what appears as an unsuccessful motor program. In this case, the brain is forced to correct the planned motor output, resulting in a modulation of M1 cortical excitability according to the speed of the observed movement. Interestingly, our intervention influenced not only the cortical excitability of the muscle involved in the observed movement, both also the one that was supposed to serve as a control, in this case ADM, not involved in the viewed task. Thus, results of the present study indicate that altering the visual feedback in MVF conditions lead to unspecific cortical excitability shifts, as MEP amplitude was modulated both for the activity of the muscle directly involved in the observed action (FDI) and for the muscle not involved (ADM). This result appears in contrast with previous evidence that depicts mirror motor mapping occurring following somatotopic rules (Avenanti et al., 2007; Funase et al., 2007). Hence, watching hand movements at different speeds do not lead to a corresponding corticospinal modulation limited to the muscle involved in the observed movement. This evidence indicates that, besides a mere action simulation, other factors might be recruited for the modulation of ipsilateral M1 excitability brought about by the altered speed of the observed movement. A hypothesis is that the increased sensorimotor conflict underpinning MVF conditions, and the attempts of our brain to solve it, may play a crucial role.

In particular, the multisensory mismatch between performed and watched movements, further exacerbated by the present manipulation of the seen movement speed, may have increased the sensorimotor conflict, in turn enhancing its modulatory effect on corticospinal excitability. Indeed, the view of a movement that is slower than the real movement performed by the observer may offer a more conflictual sensory input, which is interpreted by the observer's motor system as less efficient than what expected based on previous motor experience and the online monitoring of the current motor output. This, in turn, may boost cortical mechanisms aimed at correcting the actual motor output accordingly; the result is an increased M1 activity. Notwithstanding, this effect does not represent a simple inner replica in the observer's motor system of the watched action, as not only the muscle involved in the observed action, but also the reference were both influenced by the experimental manipulation. Thus, other mechanisms beyond action simulation are elicited by altered MVF conditions, leading to a more widespread facilitation. Specifically, the mismatch may force the entire motor system to further activate, aiming at providing greater efforts toward the incorrect performance given by the visual feedback. In conclusion, the differential modulation brought about by the altered (slower) MVF seems to imply a mechanism that, by detecting the degree of the sensorimotor conflict, adjusts the activity of the observer's motor system with the aim of overcoming the incongruent visual feedback. This evidence opens up new opportunities in the use of MVF in motor rehabilitation: provide an appropriately altered visual feedback, such as a slowed-down movements, could be more effective in enhancing cortical excitability as the classical '*real-time*' MVF paradigm. An increased sensorimotor conflict may be helpful to enhance the motor output with the aim of compensating for the worsened visual feedback provided.

Chapter 4

Using an altered Mirror Visual Feedback for the rehabilitation of post-stroke hemiparesis

4.1. Aim of the study

In the previous chapters, the conflict inherent in Mirror Visual Feedback conditions has been delineated and explored from a neuropsychological point of view, establishing the degree of susceptibility of stroke patients with motor deficit to the Mirror Illusion, and delineating which clinical, demographic and lesional factors may influence it (Chapter 2). Results showed that in stroke patients with a paretic upper limb, some residual motor abilities are necessary to generate the illusion, while unreliable tactile sensations may even facilitate it. Moreover, cortical lesions affecting motor areas enhance the illusory feelings, while parietal lesions differentially influence them, disrupting or facilitating multisensory, body- and self-related, processing.

The effects of the sensorimotor mismatch brought about Mirror Visual Feedback were further explored in healthy individuals from a neurophysiological perspective: I have demonstrated than an altered visual feedback, mimicking a slower movement than the actual motion performed by the observer, enhances motor cortex excitability in healthy subjects to a greater extent than the view of a movement at the same speed of the observer's hand (Chapter 3).

Taken together, these pieces of evidence pave the way for a modification of Mirror Visual Feedback that couples both the above-mentioned effects in a rehabilitation setting. Therefore, a novel approach was developed with the aim of maximizing the sensorimotor conflict of the Mirror Illusion through an ecological enhancement of the Mirror Visual Feedback: the *'Reversed'* Mirror Therapy (REMIT). Basically, in the REMIT intervention, the setup is the same of the classical Mirror Box Therapy (MBT), with patients required to perform motor exercises while watching through the mirror placed perpendicular to their body midline [for a review, see (Thieme et al., 2013)]. The novelty of the REMIT is that, at variance with the MBT, it is the unimpaired (rather than the paretic) limb that is placed behind the mirror, out of the sight. This strategy simulates, in an ecological mirror setting, the view of a slowed down movement used in the previous study with healthy participants (Chapter 3).

In particular, under REMIT condition, patients put their paretic upper limb in front of the mirror with the healthy arm behind it, out of the vision. In this way, while performing movements, they observe the mirror reflection of their affected limb (see below Figure 2B). This arrangement induces the illusion of watching the intact limb moving badly, as it would be affected by paresis. The hypothesis is that this situation, being featured by a higher degree of sensorimotor conflict than the standard Mirror Illusion paradigm, may increase the motor output to overcome the illusory motor impairment and to re-establish the good functioning of the limb.

To verify this hypothesis, the present study assesses the effects of the REMIT on post-stroke motor recovery in a group of patients with upper limb hemiparesis, which underwent both therapies in their chronic stage of illness (> 6 months from stroke). Given the abovementioned evidence, the novel intervention could be superior to (or, at least, as effective as) the classic Mirror Box Therapy (MBT).

4.2. Materials and methods

Participants

A continuous series of 10 stroke patients with upper limb motor deficits entered this study. Participants were selected from the in- and out-patient population of the rehabilitation unit of the IRCCS Istituto Auxologico Italiano (Milan, Italy). They gave their informed consent to the study, which obtained the approval from local Ethical Committee, and was conformed to the ethical standards of the Declaration of Helsinki (WorldMedical Association, 1991). The sample included 3 females and 7 males with a mean age of 62.7 years (SD = ± 13.6, range: 38 - 77), and a mean education level of 13.5 years (± 4.7, range: 6 - 18). The participants' average length of illness was 33.5 months (± 26.5, range: 8 - 96). All patients were right-handed according to a standard interview (Oldfield, 1971), with no history or evidence of previous psychiatric or neurological diseases, and had a normal or corrected-to-normal vision. Demographic and clinical details of the sample are reported in Table 1. Patients entered in the study following convenience sample if presenting the following inclusion criteria: 1) Upper-limb hemiparesis due to an ischaemic or haemorrhagic cerebrovascular accident; 2) Chronic phase of illness (≥ 6 months); 3) Unilateral cerebral lesion documented by CT and/or MRI; 3) Right handness [Edinburgh Inventory >11, (Oldfield, 1971)]; 4) Brunnstrom stage test: score 3 - 4 (Brunnstrom, 1966); and 5) Unmodified pharmacological therapy in the previous 2 months (4 months in case of Botulin Toxin). Exclusion criteria were: 1) Subarachnoid haemorrhage; 2) Head trauma with a Glasgow Coma Scale < 12; 3) History of previous neurological or psychiatric disorders; 4) Sign of cognitive decline; 5) Visual field defects preventing the complete view of the limb in the mirror reflection; 6) In cases of right-hemisphere lesions: presence of severe Unilateral Spatial neglect (which could impair the direction of attention toward the mirror); in cases of left-hemisphere lesions: presence of severe language comprehension impairment (which could compromise the understanding of the experimental instructions) and/or limb apraxia; 7) Previous participation (in the last 4 months) to another rehabilitative program with non-invasive brain stimulation or with Constrained-induced movement therapy; 8) Cognitive or psychical signs that would prevent them to comprehend or sign the informed consent; and 9) Denial of informed consent.

ID	Age / Gender	Aetiology	Paretic side	Education (years)	DUI (months)
P1	47, M	I	L	13	46
P2	50, M	I	L	18	16
P3	59, F	I	L	13	57
P4	66, M	I	R	8	27
P5	77, M	I	L	6	18
P6	70, M	H	R	18	19
P7	77, F	I	R	8	8
P8	38, F	I	R	16	96
P9	70, M	I	L	18	18
P10	73, M	H	L	17	30

Table 1. Demographic and clinical data of patients. ID: Participants' Identification number; Gender: M = Male, F = Female; Aetiology: I = Ischaemic stroke, H = Haemorrhagic stroke; Paretic side: L = left, R = right. DUI: Duration of illness.

Neuropsychological assessment

In order to assess the presence of cognitive deficits, the following tests were administered:

1) Visual field deficits assessment (Bisiach et al., 1983): visual field defects (upper and lower quadrants) were tested by confrontation. The patient was instructed to fixate the nose of the examiner, who performed a conventional manual confrontation test by ''finger wiggling,'' keeping the hands at about 20 of visual angle to the right and left visual fields. Patients had to report the presence and location (left/right/bilateral) of any perceived movement. They were warned that the stimulation could be unilateral or bilateral and stimuli are administered in 2 consecutive series. First, only 10 unilateral left and 10 unilateral right stimuli (score = 0 - 10 omissions for each side) are presented in a random fixed order. In

the second sequence, which aim at looking for the presence of extinction, 5 unilateral left, 5 unilateral right, and 10 bilateral simultaneous stimuli were delivered (score = 0 - 10 omissions in bilateral trials). The presence of left extinction was indexed by a difference between unilateral left and bilateral stimuli > 20% (Bisiach et al. 1983). Score range is: 3 = maximum deficit; 0 = unimpaired performance, indicative of absence of deficit.

2) Line bisection (Schenkenberg et al., 1980): a test that requires patients to mark with a pencil the midpoint of 6 horizontal black lines (two lines of each of the following lengths: 10 cm, 15 cm, 25 cm; all lines are of 2 mm in width), presented in a random order. Each line is printed centrally on an A4 sheet. The length of the line, i.e. from the left end of the line to the participant's mark, is measured to the nearest millimeter and then converted into a standardized score (percentage of deviation) namely: measured left half *minus* objective half *divided by the* objective half *per* 100 (Rode et al., 2006). This transformation yields positive scores for rightward deviations, and negative numbers for leftward deviations. A percentage deviation score higher than + 8.20% is considered as indicative of left Unilateral Spatial neglect (Fortis et al., 2010).

For patients with lesions affecting the right hemisphere:

1) Letter cancellation task (Diller and Weinberg, 1976): a test that assesses the patient's ability to explore the two halves of a paper sheet and to cross out all the targets embedded into an array of distractors. In particular, the score was the number of "H" letters crossed out by each participant (53 on the left-hand side

and 51 on the right-hand side of the sheet). The cut off is set at 99 targets out of 104 H letters.

2) Line cancellation task (Albert, 1973): a test that assesses the patient's ability to explore and to cross out all the lines displayed in the two halves of a paper sheet. The cut off is set at 19 targets out of 21 lines.

For patients with lesions affecting the left hemisphere:

1) Token Test (De Renzi and Faglioni, 1978): a simple evaluation that assesses auditory comprehension of verbal commands. The Italian validation is comprised of 36 commands divided in 6 parts with increasing complexity, each of which requires the attention and/or the manipulation of one or more of the tokens, which vary in shape, color and size (e.g., "Put the small red square under the white large circle"). The scoring procedure was as follows: for each item, 1 point is given in case of correct answer after the first presentation of the command; 0.5 point is given in case of correct answer after the second presentation of the command; 0 is given in case of wrong answer after the second presentation of the command. The total score is then corrected for age and schooling of each patient. The cut off is set at 26.5 points out of a maximum of 36.

2) Ideomotor apraxia test (De Renzi et al., 1980): a clinical evaluation that assesses the imitation of a set of gestures. It contains gestures demonstrated by the test leader, whom the patient has to imitate (e.g., sticking out the tongue, waving goodbye, saluting and making a fist). The scoring procedure is as follows: for each item, 3 points are given in case the performance is correct and appropriate;

2 points are given in case the performance resembles the correct one, but is somewhat imprecise or the patient uses a body part as object; 1 point is given in case the performance resembles only weakly the correct one but is executed in the correct place, or is correct but carried out in a wrong place (i.e., moving the toothbrush in front of the forehead); 0 point is given in case the performance is not correct or so incomplete that it is not recognizable. The maximum score is 72. The cut-off is set at 53. Patients' individual scores are shown in Table 2.

	Campimetric Deficit	**Bisection**	**Lines Cancellation**	**Letters Cancellation**
P1	-	- 2.6 %	21/21	104/104
P2	-	0.6 %	21/21	104/104
P3	-	- 8 %	21/21	101/104
P5	-	- 5.4 %	21/21	101/104
P9	-	- 4.8 %	21/21	104/104
P10	-	0.8 %	21/21	104/104

	Campimetric Deficit	**Bisection**	**Token Test**	**Ideomotor Apraxia**
P4	**-**	- 6 %	27.25	58/72
P6	**-**	- 3.6 %	31	70/72
P7	**-**	-4.8	32.75	60/72
P8	**-**	-2 %	32.75	70/72

Table 2. **Neuropsychological assessment**. Data of 6 right-brain-damaged patients (Upper Table) and 4 left-brain-damaged patients (Lower Table). MMSE= Mini-mental State Examination; +/-: presence/absence of impairment.

Lesion data

MRI scans were available for all stroke patients. Regions of Interest (ROIs) defined the location and the size of the lesion for each patient (Figure 1). They were reconstructed through a template technique, by manually drawing the lesion on the standard template from the Montreal Neurological Institute (Rorden and Brett, 2000), on each 2D slice of a 3D volume. Figure 2 shows the overlay lesion plot of all patients.

The mean lesion volume (cc^3) of the patients was 75.7 cc^3 (± 83.9 cc^3, range = 2.4 – 221.1 cc^3). In particular, mean lesion volume was 67.58 cc^3 (± 81.5 cc^3, range = 2.4 – 194.4 cc^3) for right-brain-damaged patients, and 87.9 cc^3 (± 98.7 cc^3, range = 6.5 – 221.1 cc^3) for the left-brain-damaged patients.

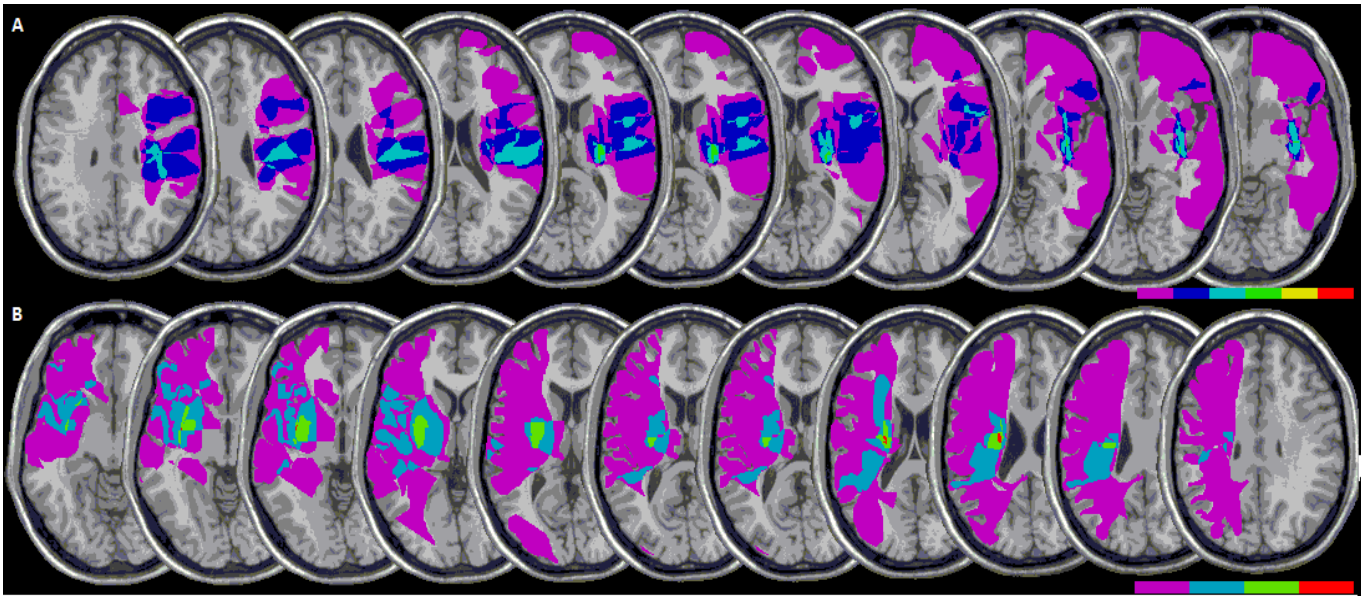


Figure 1. Lesions of patients. Overlay lesion plots for right- (A) and left- (B) damaged participants. Each colour represents 20% increments, from red areas indicating maximum overlap, to pink areas indicating minimum overlap.

Baseline clinical assessment

The week before the beginning of the treatment, patients underwent a clinical assessment including the following tests:

1) NIH stroke scale (Brott et al., 1989): a 15-item scale evaluating neurologic outcome and the degree of recovery for patients with stroke. In particular, stroke effects are assessed on the following categories: consciousness, language, neglect, vision, motor strength, ataxia, dysarthria, and sensory loss. Ratings for each item are scored with 3 to 5 grades with 0 as normal (maximum score, signifying severe stroke= 42).

2) Bamford Classification (Bamford et al., 1991), also known as Oxford Community Stroke Project, or Oxford classification: a classification system for stroke that classifies stroke episode as Total anterior circulation stroke (TAC), Partial anterior circulation stroke (PAC), Lacunar stroke (LAC), Posterior circulation stroke (POC), based on the extent of the symptoms.

3) Functional Independence Measure, FIM (Tesio et al., 2002): an assessment tool that aims at evaluating the functional status of patients in performing basic life activities safely and effectively. It comprises 18 items of tasks and assesses patients' need for assistance. Patients are asked to rate on a 7 points ordinal scale their need of assistance in performing a minimum set of skills related to self-care, sphincter control, transfers, locomotion, communication, and social cognition, from complete dependence to complete independence. Scores range from 18 (lowest) to 126 (highest) indicating level of function.

4) Brunnstron approach (Brunnstrom, 1966): an evaluation procedure aimed at assessing muscle tone and voluntary control of movement patterns in stroke patients along a 6 points ordinal scale (1 = flaccidity, 6 = normal motricity).

Table 3 illustrates the patients' scores of the above-mentioned tests.

ID	NIH	BAMFORD	FIM	Brunnstrom
P1	5	1	116	4
P2	6	2	113	3
P3	5	2	102	4
P4	6	2	-	3
P5	4	4	-	4
P6	3	4	-	4
P7	4	4	-	4
P8	6	2	-	3
P9	3	2	111	3
P10	4	4	114	3

Table 3. Neurological and motor data of patients. ID: Participants' Identification Number. NIH: NIH Stroke Scale (Individual total score range: 0 - 34); Bamford [Score range: 1 - 4: 1 = Total anterior circulation stroke (TAC), 2 = Partial anterior circulation stroke (PAC), 3 = Lacunar stroke (LAC), 4 = Posterior circulation stroke (POC)]; FIM: Functional Independent Measure (Score range: 18 -126); Brunnstrom (Score range: 1 - 6).

Motor cortex excitability assessment

The week before the starting of the intervention, patients underwent a TMS assessment, according to the following procedure. Single pulse Transcranial Magnetic Stimulation (sTMS) was used to measure corticospinal excitability by recording motor evoked potentials (MEPs) induced by the stimulation of both the affected (AH) and the unaffected (UH) motor cortices. The evaluation was performed by a trained staff, blinded to group assignment. Focal sTMS to the primary motor cortex (M1) was delivered using a 70-mm figure-of-eight coil, held tangential to the skull and aligned in the parasagittal plane with the handle rotated 45° lateral. Online monitoring of the electromyographic (EMG) activity in response to TMS was performed.

EMG signals were band-pass filtered (50–1000 Hz), digitized, and stored on a computer for offline analysis (Synergy NCS EMG EO IOM System; Viasys Healthcare, Old Working, Surrey UK).

MEPs were recorded from the left and the right First Dorsal Interosseous (FDI) muscles. Pairs of silver/silver chloride surface electrodes were placed over the muscle belly (active electrode) and over the associated joint of the muscle (reference electrode). For each hemisphere, first the optimal scalp position for the TMS induction of an MEP from the contralateral FDI was determined. The resting motor threshold (rMT), which corresponds to the lowest TMS intensity able to induce 3 out of 5 MEPs with peak-to-peak amplitude of at least 50 μV (Wassermann et al., 2008), was determined in both hemispheres. Cortical excitability was tested by delivering 3 TMS pulses to the primary motor cortex of each hemisphere (TMS intensity of 110% above the individual rMT). Muscle activity was monitored by real-time EMG to confirm the relaxed status before the stimulation. The motor area was also quantified, which corresponded to the total number of the excitable sites (i.e., scalp locations over M1 where sTMS induced a MEP in the contralateral muscle).

TMS data were available for 9 patients out of 10. For one participant (P6) motor cortex excitability assessment was not performed due to safety reasons. In fact, this patient had a history of epilepsy, which represents an exclusion criterion to TMS, following international safety guidelines (Rossi et al., 2009). Moreover, in 4 patients, MEPs could not be induced by sTMS of the ipsilesional M1, even at the maximum stimulator output (100% of intensity). As consequence, only in 5 out of 9 patients (see Table 4) the rMT in the affected hemisphere could be determined, and the MEPs recorded in the paretic FDI muscle. Due to this paucity of data, cortical excitability indexes of the damaged M1, even though potentially informative about the functional status of the affected hemisphere, were not further analysed.

ID	rMT		MEPs (mV)		Area	
	AH	UH	AH	UH	AH	UH
P1	45	52	0,2	0,8	9	16
P2	*nr*	55	*nr*	0,7	*nr*	10
P3	57	40	0,4	0,5	15	7
P4	*nr*	39	*nr*	2,4	*nr*	13
P5	70	51	0,5	0,8	12	10
P7	90	41	0,2	0,8	17	3
P8	*nr*	57	nr	0,8	*nr*	10
P9	77	65	0,4	3	7	14
P10	*nr*	44	*nr*	1	*nr*	8

Table 4. Motor cortical excitability data of patients. ID: Participants' Identification Number. rMT: resting Motor Threshold. MEPs: Motor Evoked Potentials measured from First Dorsal Interosseus - FDI, expressed in mV. Area: Sum of the total excitable sites. AH = Affected hemisphere. UH = Unaffected hemisphere. *nr* = Not recorded.

Mental imagery assessment

Participants were asked to complete two mental imagery questionnaires, the Vividness of Visual Imagery Questionnaire (VVIQ, Marks, 1973) and the Vividness of Motor Imagery Questionnaire (VMIQ, Isaac et al., 1986). The VVIQ is comprised by 16 items measuring the ability to form visual mental images; the VMIQ consists of 24 items related to movement (e.g., walking, jumping) and measures motor imagery ability. Both questionnaires require to mentally recreate images and to judge their vividness along a 5-point scale: 1= No image at all; 2= Vague and dim; 3= Moderately clear and vivid; 4= Clear and reasonably vivid; 5= Perfectly clear and vivid as normal vision.

Procedure

Patients underwent a 2-weeks treatment comprising 1 week (Monday to Friday) of the experimental treatment REMIT and 1 week (Monday to Friday) of the standard MBT, for a total of 10 sessions. A crossover design was adopted, with patients assigned to two groups: a group received the MBT in the first week, and the REMIT in the second week (i.e. M-R group), the second group received the REMIT in the first week, and the MBT in the second week (i.e. R-M group). Patients were alternately assigned to one of the two groups, starting with the MBT.

The MBT followed the original protocol by Altschuler and coworkers (1999). Patients sat at a table with their arms on the desk at the two sides of a mirror placed perpendicular to their body midline, with the paretic arm behind (see

Figure 2A). In each treatment session, patients had to practice 19 exercises, each lasting 1 minute, while continuously asked to observe the mirror. Each movement had to be performed moving both hands or arms symmetrically while watching the reflection of the mirror. The instruction emphasised to move the paretic limb as best they could (Altschuler et al., 1999).

The REMIT protocol was similar to that of the MBT, with the only difference that patients kept the intact upper-limb (rather than the paretic limb) behind the mirror, as illustrated in Figure 2B.

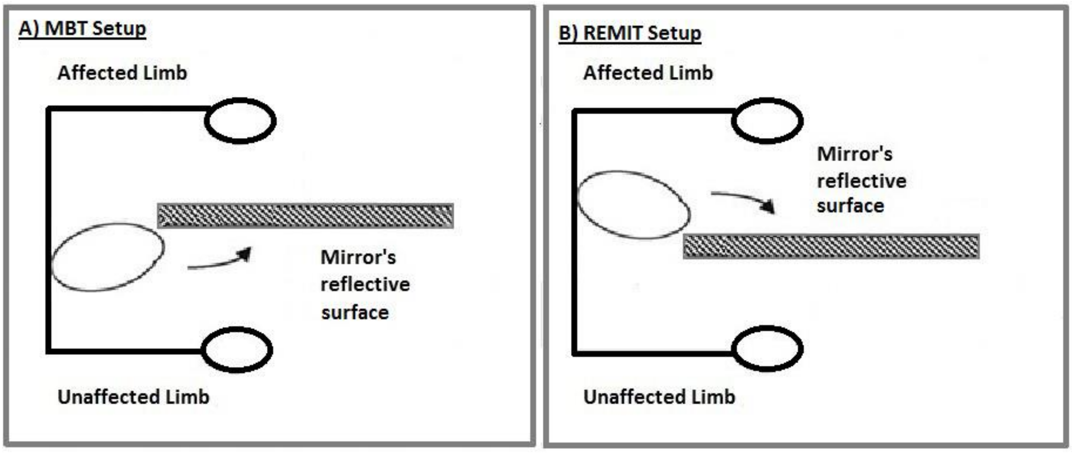


Figure 2. Experimental setup. A) MBT Setup: the paretic arm is placed behind the mirror, out of the sight; the mirror reflects the image of the unaffected arm. B) REMIT Setup: the unaffected limb is placed behind the mirror, out of the sight; the mirror reflects the image of the paretic arm. Modified from (McCabe, 2011).

In particular, patients were required to perform movements with a progressive complexity, following Brunnstrom/Fugl-Meyer model (Brunnstrom, 1966; Fugl-Meyer et al., 1974). Specifically, a shaping procedure was adopted, which started with exercises requiring synergic movements of the upper limbs (e.g., raise and lower the arm of 30°, with the arm flexed to 90° and elbow extended), followed by exercises involving isolated movements of the shoulder (e.g., internal and external rotation of the shoulder, by sliding the forearm toward and outward the mirror, with the arm adducted, the elbow flexed and the forearm on the table on its ulnar side), the forearm (e.g., pronation and supination of the forearm, with elbow and forearm on the table), the wrist (e.g., extension and flexion of the wrist, with elbow and forearm resting on its ulnar side) and, finally, the fingers (e.g., alternate tapping of all the tips on the table, from the index to the little finger and vice-versa, with the forearm and wrist prone on the table). Overall, almost half of the required exercises involved isolated movements of wrist and fingers. Whenever necessary, patients could have a brief rest between consecutive exercises, in order to avoid fatigue.

The entire sequence of movements was shown at the beginning of the first day of each treatment protocol (i.e., MBT and REMIT) through video-clips presented on a computer screen, and patients were asked to try to perform each exercise, in order to familiarize with them. Then, during each daily session, every movement was explained and showed again by the experimenter, who was sat in front of the patients and checked whether they were performing watching through the mirror.

Post-treatment assessment

Illusory sensations

Daily, at the end of each treatment session, participants filled in the Mirror Visual Feedback Questionnaire (MVFQ) [adapted from (Longo et al., 2009)]. With this questionnaire, I aimed to measure differences in the illusory sensations brought about by the two types of treatment, the standard MBT and the experimental version with the intact limb out of view, REMIT.

The MVFQ comprised the following items (translated from Italian): 1) "It felt like I was looking directly at my hand rather than at a mirror image"; 2) "It felt like that both my hands were moving simultaneously"; 3) "It felt like the movements in the mirror were the same I was performing; 4) "It felt like I was moving with less difficulty"; 5) "It felt like that the hand I was looking at was my left/right hand". Participants rated each item using a 7-point Likert scale, with 6 = strongly agree, 3 = neither agree, nor disagree, 0 = strongly disagree. The sum of scores given to each item has been considered indicative of the amount of illusion.

Timeline of the evaluation aimed at assessing treatments' efficacy

Treatments' effects were assessed through clinical tests by certified physiatrist, blinded regarding the group allocation of the participants.

Evaluations were performed at the following time-points:

- t0: the week before the beginning of the experimental program (Baseline).

- t1: at the end of the first week of treatment (Post-treatment 1).

- t2: at the end of the second week of treatment (Post-treatment 2).

Motor function assessment

The following tests were used to clinically evaluate motor improvements brought about the REMIT and the MBT.

1) Fugl-Meyer test (Fugl-Meyer et al., 1974): an assessment scale that provides a general sensorimotor impairment index by evaluating motor functioning, balance, sensation and joint functioning. Specifically, it evaluates upper limb, shoulder, elbow, arm, wrist and hand. In addition, it gives information regarding coordination and speed. The severity of each motor function is scored on 3-point ordinal scale (score: 0 - 2, with 2 indicating no deficit). Total score ranges from 0 = plegic, to 66 = normal.

2) Box and Block test (Desrosiers et al., 1994; Mathiowetz et al., 1985b): a task that measures unilateral gross manual dexterity. This test consists of a timed task assessing the ability to manipulate and move small wooden blocks. The number of blocks (dimension = 2.54 cm^3) that can be transported from one compartment of a box to another within 1 min is counted (Mathiowetz et al., 1985b).

3) Hand Grip strength test (Mathiowetz et al., 1985a): an evaluation that tests the grip strength of the paretic hand through a grip strength dynamometer. Patients are instructed to squeeze the dynamometer as hard as possible and hold it for 5 sec. The average of three measurements (kg) is taken as a measure of strength.

4) ABILHAND questionnaire (Penta et al., 2001): an instrument that measures the patient's perceived difficulty in performing everyday manual activities. Specifically, patients are asked to judge on a 3-point scale (0 - 2 score, with 0 = impossible, 1 = difficult, 2 = easy) their ability to perform 23 daily activities that require the use of the upper limbs. The total score is then linear transformed and range from 0 to 100. Higher values correspond to better manual ability.

5) Wrist extension: a test that measures voluntary extension of the wrist, though goniometric assessment (American Academy of Orthopaedic Surgeons, 1965).

Statistical analyses

First, in order to rule out potential differences between the two groups before the intervention, a series of 1-way Analysis of Variance (ANOVA) was run on the baseline scores of each diagnostic test (namely, Fugl-Meyer test, Box and Block test, Hand Grip strength test, ABILHAND questionnaire and wrist extension) with Group (M-R and R-M) as between-subjects factor. No differences were found: FM [$F_{(1,9)} = 0.39$, $p = 0.55$], BB [$F_{(1,9)} = 0.92$, $p = 0.36$], HG [$F_{(1,9)} = 1.62$, p = 0.24], ABILHAND [$F_{(1,9)} = 1.9$, $p = 0.2$], wrist extension [$F_{(1,7)} = 2.2$, $p = 0.19$]. Therefore, the two experimental groups were comparable at baseline.

Then, the effects of the two treatments were assessed with repeated measure (rm)ANOVAs with Group (M-R, R-M) as between-subjects factor, and Time (t0, t1, and t2) as within-subjects factor. Significant effects were further explored with the Newman-Keuls post-hoc test for multiple comparisons. In addition, the influence of the following factors was considered: demographic (age, duration of illness), clinical (NIH), motor cortex excitability (rMT, MEPs, Area), mean lesion volume, imagery abilities (VMIQ and VVIQ questionnaires), illusory effects (MVFQ). These factors were included in a series of Analyses of Covariance (ANCOVAs) as independent variables (standardized mean score used as a linear and interactive covariate).

With respect to the illusory experience brought about by the two Mirror Visual Feedback types, I wanted to investigate whether both conditions were associated with analogous Mirror Illusion sensations. In addition, the stability of such phenomenon during time was of interest. To this aim, first, MVFQ scores obtained at the first day of the MBT and of the REMIT treatments were submitted to a rmANOVA, with Mirror Visual Feedback (Standard, Reversed) as within-subjects factor. To detect any changes due to the cumulative exposure to each treatment, another rmANOVA was run with Mirror Visual Feedback (Standard, Reversed) and Time (Day1, Day2, Day3, Day4 and Day 5) as within-subjects factors.

4.3. Results

Motor assessment

Fugl-Meyer (FM) test. With respect to the FM assessment, results showed only a main effect of Time [$F_{(2, 12)} = 7$, $p < 0.01$)], while the effect of Group [$F_{(1, 6)} = 0.2$, $p = 0.7$)] and the Time by Group interaction [$F_{(2, 12)} = 0.2$, $p = 0.8$)] did not attain the significance level. Newman-Keuls post-hoc comparisons showed an improvement at the two post-treatment assessments (t1 = 29.7 score, t2 = 29.9), as compared to the baseline score (t0 = 26.5). On the other hand, t1 and t2 did not differ each other ($p = 0.6$) (see Table 5).

As shown in Table 6, ANCOVA analyses showed that the main effect of Time was still significant when in the statistical model were included the following covariates: age, duration of illness, NIH score, cortical excitability measures of the unaffected hemishere (rMT, MEPs, Area), lesion volume, the motor imagery score (evaluated through Vividness of Motor Imagery Questionnaire, VVIQ) Mirror Visual Feedback Questionnaire score. In addition, such covariates did not interact with Time and Group.

On the other hand, intriguing results arise when considering the visual imagery ability (indexed by VVIQ scores) as covariate. In this model, an interaction between Time and the Covariate emerges [$F_{(2, 8)} = 6.2$, $p = 0.02$], suggesting that the score obtained at the VVIQ impact on the Fugl-Meyer outcome. In particular, patients with low visual imagery show a worsening at the FM test after the first week of therapy (regardless of whether it was REMIT or MBT), and ameliorate after the second one. On the other hand, those who show higher imagery ability benefit from a greater improvement after the first week, which stabilize after the second one (see Figure 3).

Box and Block (BB) test. Results showed no main effect of: Time [$F_{(2, 16)} = 0.7$, $p = 0.4$], Group [$F_{(1, 8)} = 0.8$, $p = 0.4$], Time by Group interaction [$F_{(2, 16)} = 0.8$, $p = 0.5$]

(see Table 5). However, when considering the role of the unaffected hemisphere area (i.e., the sum of the total excitable sites in the healthy M1, measured through sTMS), a significant interaction Time by Covariate emerges [$F_{(2, 12)} = 7.7$, $p < 0.01$] (see Table 7). Patients who show a greater excitable area in the healthy side of the brain perform better at the BB test on the pre-treatment assessment, worsening during the treatments; on the contrary, those who have a lower number of excitable sites in the unimpaired M1 perform worst at the baseline, but they improve to a large extend during the treatment (see Figure 4a).

Hand Grip (HG) strength test. Results showed no main effect of Time [$F_{(2, 16)} = 0.06$, $p = 0.9$)], Group [$F_{(1, 8)} = 1.9$, $p = 0.2$)], Time by Group interaction [$F_{(2, 16)} = 0.8$, $p = 0.4$)] (see Table 5).

However, when considering the role of the neurological state (NIH score), an interaction Time by Covariate emerges [$F_{(2, 8)} = 4.4$ $p = 0.03$] (see Table 7). While globally it seems that the HG performance is stable over time, this finding is dissimilar across different levels of NIH scale. In particular, patients with high NIH (indicative of worse neurological condition) decline after the first week of treatment and return to baseline levels after the second week; instead, patients with low NIH (indicative of better neurological condition) show an opposite trend: they improve after the first week of treatment, but return to baseline levels after the second (see Figure 4b).

ABILHAND questionnaire. With respect to the ABILHAND, results showed no main effect of Time [$F_{(2, 16)} = 0.3$, $p = 0.7$], Group [$F_{(1, 8)} = 0.6$, $p = 0.5$], Time by

Group interaction [$F_{(2, 16)}$ = 1.6, p = 0.2] (see Table 5). A significant interaction between Time and the covariate MVFQ score was found [$F_{(2, 14)}$ = 4.3, p = 0.03] (see Table 7). Patients more susceptible to the Mirror Illusion report a lower ability of performing bimanual activity in everyday life during the 2 weeks of intervention, while patients with a low illusion score show an opposite trend (see Figure 4c).

Wrist extension. With respect to wrist extension, results showed no main effect of Time [$F_{(2, 12)}$ = 0.8, p = 0.47], Group [$F_{(1, 6)}$ = 2.2, p = 0.2], Time by Group interaction [$F_{(2, 12)}$ = 2, p = 0.2] (see Table 5). No significant effects emerge from covariance analyses.

	TIME			ANOVA			
Group Test	**t0**	**t1**	**t2**	**Time**	**Group**	**Time x Group**	**Post hoc**
FM							
M-T	31.4 (7)	31.7 (9)	33.4 (8)	F=7	F=0.2	F=0.2	B<t1
R-T	26 (5)	27.5 (7)	30.6 (6)	**p<0.01**	p=0.7	p=0.8	B<t2
BB							
M-T	17 (7)	18.2 (7)	16.4 (6)	F=0.7	F=0.8	F=0.8	
R-T	9.4 (4)	10.6 (5)	11 (5)	p=0.4	p=0.4	p=0.5	
HG							
M-T	18.3 (4)	17.2(4)	16 (4)	F=0.06	F=1.9	F=0.8	
R-T	26.6 (4)	26 (4)	25.5 (4)	p=0.9	p=0.2	p=0.4	
ABILHAND							
M-T	68 (6)	62.5 (6)	65.5 (6)	F=0.3	F=0.6	F=1.6	
R-T	57 (6)	59 (5)	60 (7)	p=0.7	p=0.5	p=0.2	
Wrist extension							
M-T	62 (7)	62 (7)	66 (10)	F=0.8	F=2.2	F=2	
R-T	38 (11)	43 (11)	53 (8)	p=0.47	p=0.2	p=0.2	

Table 5. Motor Scores. Baseline (t0), after the first week (t1), and after the second week (t2) assessments, by Group. SEM in Brackets. Group: M - R = MBT - REMIT order; R - M = REMIT-MBT order; FM = Fugl-Meyer assessment; BB = Box and Block test; HG = Hand Grip strength task; ABILHAND = ABILHAND Questionnaire scores.

Covariate	Time	Group	Covariate	Time X Group	Time X Covariate
AGE	**F=5.8 p=0.02**	F=0.09 p=0.8	F=0.1 p=0.9	F=0.19 p=0.83	F=0.15 p=0.86
DUI	**F=5.7 p=0.02**	F=0.00 p=0.98	F=0.11 p=0.75	F=0.59 p=0.57	F=0.47 p=0.64
NIH	**F=5.2 p=0.03**	F=0.1 p=0.73	F=0.06 p=0.8	F=0.2 p=0.8	F=0.2 p=0.86
UH rMT	**F=7 p=0.01**	F=0.11 p=0.75	F=0.92 p=0.38	F=0.3 p=0.77	F=1.1 p=0.36
UH mep	**F=9 p=0.01**	F=1.1 p=0.37	F=0.4 p=0.59	F=1.1 p=0.39	F=0.76 p=0.5
UH area	**F=10.5 p=0.04**	F=0.16 p=0.7	F=0.02 p=0.9	F=0.22 p=0.8	F=3.8 p=0.06
Volume	**F=6.2 p=0.02**	F=0.03 p=0.87	F=0.9 p=0.4	F=0.2 p=0.8	F=0.2 p=0.8
VMIQ	**F=6.4 p=0.02**	F=1.4 p=0.28	F=14.6 p=0.01	F=1.3 p=0.88	F=0.49 p=0.63
VVIQ	**F=9.6 p<0.01**	F=0.0 p=0.99	F=0.02 p=0.88	F=0.15 p=0.86	**F=6.2 p=0.02**
MVFQ	**F=11 p<0.01**	F=0.39 p=0.55	F=0.45 p=0.53	F=0.9 p=0.42	F=2.5 p=0.13

Table 6. Results from the rmANCOVAs in the Fugl-Meyer model. DUI: Duration of Illness; NIH: NIH stroke scale; UH rMT: Unaffected hemisphere resting Motor Threshold; UH mep: Unaffected hemisphere Motor Evoked Potentials; UH area: unaffected hemisphere number of total excitable sites; Volume: Number of lesioned voxels, obtained by MRIcro reconstruction; VMIQ: Vividness of Motor Imagery Questionnaire; VVIQ: Vividness of Visual Imagery Questionnaire; MVFQ: Mirror Visual Feedback Questionnaire.

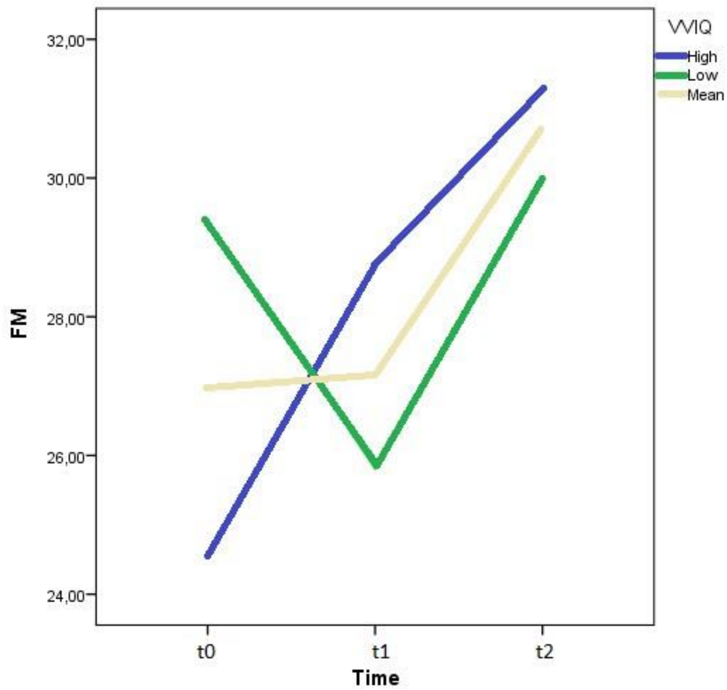
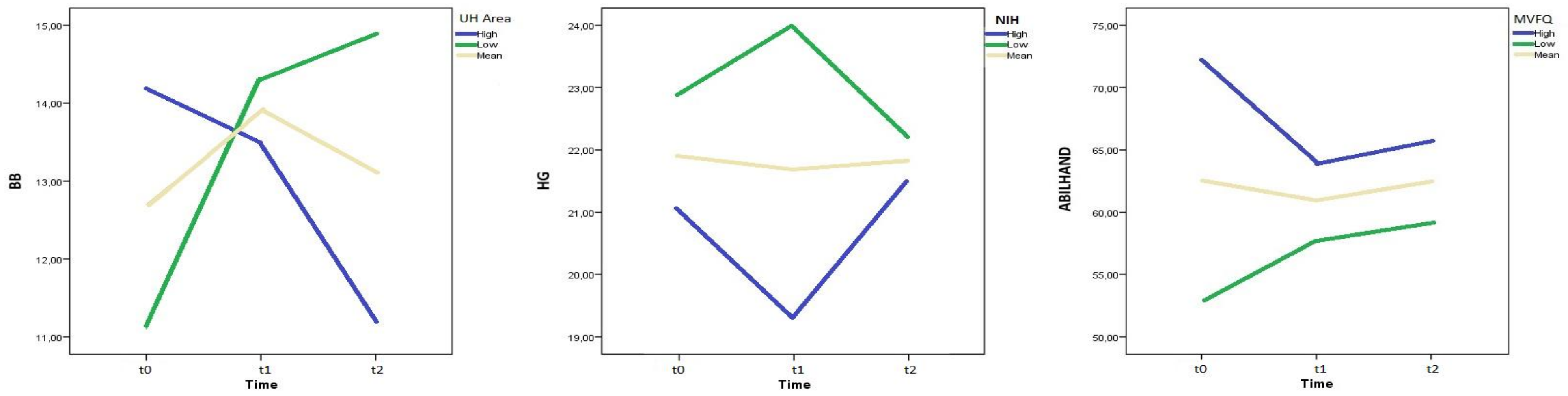


Figure 3. ANCOVA: Interaction between Time and VVIQ scores for the Fugl-Meyer (FM) test. Patients with High VVIQ (blue line) and with low VVIQ (green line). VVIQ: Vividness of Visual Imagery Ability Questionnaire.

Test	Covariate	Time	Group	Covariate	Time X Group	Time X Covariate
BB	**UH area**	F=1.2	F=0.93	F=0.00	F=1.2	**F=7.7**
		p=0.36	p=0.37	p=0.94	p=0.33	**p<0.01**
HG	**NIH**	F=0.08	F=1.5	F=0.02	F=0.54	**F=4.4**
		p=0.9	p=0.26	p=0.88	p=0.59	**p=0.03**
ABILHAND	**MVFQ**	F=0.5	F=0.04	F=1	F=0.15	**F=4.3**
		p=0.6	p=0.84	p=0.34	p=0.87	**p=0.03**

Table 7. Results from the rmANCOVAs in the secondary outcomes. Models with a within-subjects factor, Time (t0, t1 and t2), and a between-subjects factor, Group (M - R and R - M), with the standardized variables mean score as a linear and interactive covariate. BB: Box and Blocks test; HG: Hand Grip strength test; UH area: Unaffected hemisphere number of total excitable sites; NIH: NIH stroke scale; MVFQ: Mirror Visual Feedback Questionnaire.



4a) Box and Block (BB) test

4b) Hand Grip (HG) strength test

4c) ABILHAND questionnaire

Figure 4. Significant interactions in the ANCOVA models.

4a) ANCOVA**:** Interaction between Time and unaffected M1 area in the Box and Block test. Patients with a high number of excitable sites in the healthy M1 (Blue line) and patients with a low number of excitable sites in the healthy M1 (Green line). UH: Unaffected hemisphere.

4b) ANCOVA: Interaction between Time and NIH in the Hand Grip strength test. Patients with high NIH (Blue line) and patients with low NIH scores (Green line). NIH: NIH Stroke Scale.

4c) ANCOVA: Interaction between Time and MVFQ in the ABILHAND Questionnaire. Patients with high illusion (Blue line) and patients with low illusion (Green line). MVFQ: Mirror Visual Feedback Questionnaire.

Mirror Illusion

Mirror Visual Feedback Questionnaire. The rmANOVA did not show a main effect of Mirror Visual Feedback (Standard, Reversed) [$F_{(1, 9)} = 4$, $p = 0.07$], indicating that overall stroke patients showed a reliable illusion during both conditions, regardless the type of feedback. Therefore, the sensations brought about by Reversed Mirror Visual Feedback (mean of MVFQ: 17.6) are comparable to that experienced during Standard Mirror Visual Feedback (mean of MVFQ: 22). Even when changes in the illusory sensations were assessed over time, no significant effects were found: Mirror Visual Feedback [$F_{(1, 9)} = 1.9$, $p = 0.19$)], Time [$F_{(4, 36)} = 1.9$, $p = 0 .13$], Mirror Visual Feedback by Time interaction [$F_{(4, 36)} = 0.58$, $p = 0.67$)] (see Figure 5).

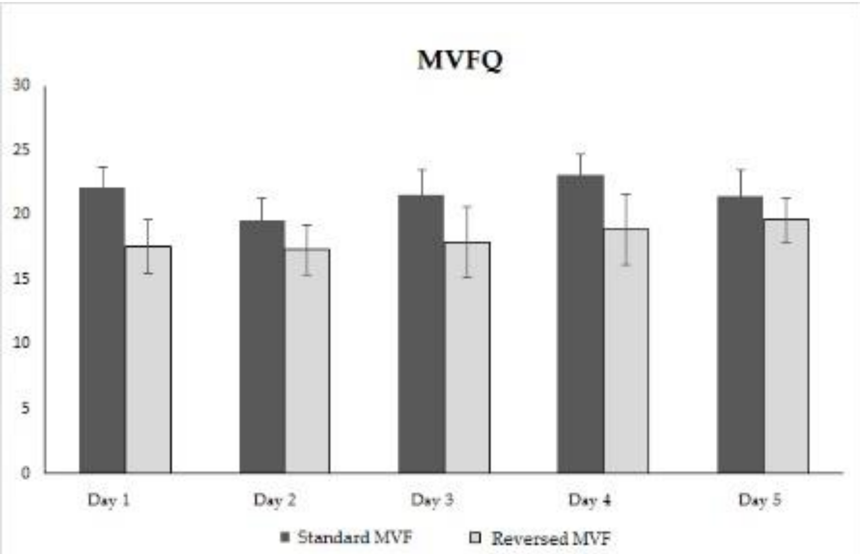


Figure 5. MVFQ. Mean total MVFQ score of stroke patients, with Standard Mirror Visual Feedback (namely, under MBT condition; dark grey bar) and with Reversed Mirror Visual Feedback (namely, under REMIT condition; light grey bar). MVFQ: Mirror Visual Feedback Questionnaire. Error bars = SEM.

4.4. Discussion

The present study yields two main results. Firstly, I demonstrated that a novel treatment based on reversed Mirror Visual Feedback has comparable effects to those of standard Mirror Visual Feedback on different motor outcomes. Hence, the REMIT is equally effective as the classic MBT, at least with respect to the improvement of motor performance measured through the Fugl-Meyer test (Michielsen et al., 2011; Dohle et al., 2009). However, this new intervention, as well as the MBT, was not associated to significant recovery on secondary outcomes such as Box and Block (BB) test, Hand Grip (HG) test, ABILHAND questionnaire and wrist extension. Moreover, further analyses shed light on a set of factors that affects Mirror Visual Feedback-related consequences on different motor outcomes. In particular: 1) the effects on sensorimotor recovery (FM) seem mediated by mental imagery ability; 2) the extension of the contralesional motor area (expressed as the sum of total excitable sites) shapes the Mirror Visual Feedback effects on unilateral gross manual dexterity (BB); 3) overall neurological state of the participants (e.g., NIH score) influences the effects on HG test; 4) subjective susceptibility to the Mirror Illusion plays a role in determining manual ability of the paretic hand (ABILHAND questionnaire).

The second relevant finding is that the pattern of illusory sensations associated with reversed Mirror Visual Feedback is similar to that elicited by standard Mirror Visual Feedback, indicating that both interventions are equally effective in fooling the brain into thinking that the hand out of the sight is the same as the one in the mirror. Therefore, both Mirror therapies (namely, REMIT and MBT) are associated with comparable illusory sensations.

The effects on motor recovery of reversed Mirror Visual Feedback and underpinning mechanisms

The pattern of results that emerges from the present study strongly evidences that a modified version of the standard Mirror Visual Feedback, where the feedback provided is reversed and patients are fooled into thinking that the good arm is moving badly, leads to significant motor improvements. Remarkably, the related gains correspond to those elicited by classic MBT conditions, where the mirror provides the illusory image of the affected hand that works properly. This evidence opens up new interpretations of Mirror therapies, whose mechanisms are still under investigation (see Chapter 1).

Crucially, given the overlap between REMIT and MBT effects found in the present study, the two interventions likely share similar mechanisms. Here, it is important to remark that participants were required to move both hands symmetrically, following the original protocol (Altschuler et al., 1999). Namely, also during the MBT sessions, the paretic arm out of the sight had to be moved as best as patients could. Consequently, the output sent by the brain to both limbs (and thus also to the one behind the mirror) plays a crucial role in characterizing the Mirror Visual Feedback of both the standard and the experimental therapy intervention. Therefore, the mechanisms at the basis of the present Mirror therapies may act on both aspects: not only the visual feedback provided by the mirror is relevant, but also the motor output sent by the brain. The underpinning process should be able to take advantage of the illusory image of the bad arm finally performing well (MBT condition), as well as to overcome the illusory image of the good arm working badly (REMIT condition). In other words, such mechanism should cope with the conflicting incoming input inherent in Mirror Visual Feedback conditions between visual and motor information as well as between expected and actual feedback and ground on those mismatches its reparative function.

Crucially, focusing on the REMIT condition and its peculiarities, that represents the novelty of the present study, helps in disentangling which of the hypotheses put forward to interpret MBT (Chapter 1) results could apply also for REMIT effects.

Under the MBT, the recall of the Mirror Neuron System has been tempting: the information provided through the mirror would give the visual input necessary for the activation of mirror motor areas. The present findings related to the efficacy of the REMIT are difficult to reconcile with this proposal. In fact, under REMIT condition, the mirror gives the image of an arm that is not working properly; it follows that the putative mirror neurons-based imitation mechanism should lead to a worsening of the performance. As seen, this is not the case, since our data provide evidence of comparable beneficial effects after both Mirror therapies.

On the other hand, the apparent role of motor imagery should not be evoked for two main reasons. Firstly, mentally recreating the movement of the arm out of the sight was not directly asked during the therapy as part of the instruction, differently from other studies (Stevens and Stoykov, 2003). Even supposing that some patients have used this strategy, it was not explicitly required by the present experimental design, and thus it may not applied by all participants. Second, and more relevant, an interesting link was found between Mirror Visual Feedback and visual, but not motor, imagery (see also Chapter 2). The association between visual imagery abilities and motor improvement brought about the REMIT and the MBT emphasizes a key role of crossmodal - visual - influences in driving Mirror Visual Feedback-induced motor effects.

Finally, also the Ramachandran's originally proposal that the beneficial effect of Mirror Visual Feedback for reversing the 'learnt' component of the motor impairment, namely a putative learned non-use of the paretic limb (Ramachandran and Altschuler, 2009) cannot explain the REMIT's effects. In this view, the mirror reflection of the paretic limb should eventually lead to a reinforcement, rather than a reversal, of learned paralysis, hence worsening motor functions. As seen, this was not the case.

In this regard, of importance are the results from the analysis of the subjective sensations brought about by the reversed and standard mirror reflection (Mirror

Visual Feedback Questionnaire, MVFQ): the observation through the mirror of the impaired limb (i.e., during REMIT) leads to similar sensations as those elicited when the unimpaired limb is offered as a reflection (i.e., during MBT). The comparable illusory effects under both mirror conditions suggest that the standard and reversed mirror feedbacks are capable of creating comparable, efficient, multisensory and sensorimotor mismatches, both giving rise to the Mirror Illusion. The repetitive exposure to the Mirror Visual Feedback does not influence the magnitude of its illusory effects, which appear resistants to repetition and stable after consecutive inductions.

To sum up, the present data support the hypothesis that depicts the conflict connatural to Mirror Visual Feedback conditions as playing a vital role in boosting the effects of Mirror therapies (Michielsen et al., 2010), in line with the previous findings (Chapter 2 and 3).

The REMIT and MBT effects on motor recovery

Exploring in details the effects of the standard and experimental Mirror therapies (considering both MBT and REMIT) found in the present work, they are in line with previous evidence. In particular, the improvement on FM appears in accordance with previous reports on MBT (Michielsen et al., 2011; Dohle et al., 2009). Interestingly, in the above-mentioned studies, the intervention lasted longer, namely 6 weeks (Michielsen et al., 2011), and was often provided in combination with standard therapy (see, for example Dohle et al., 2009). Here I found that a shorter period (2 weeks) of training with the Mirror Visual Feedback is sufficient to elicit motor gains, even if not coupled with additional physical therapies. In addition, the improvement on the FM scale is unrelated to the clinical features of participants, further underscoring the efficacy of the intervention regardless the different clinical profiles of stroke patients. Indeed, factors such as age, duration of disease, neurological state (i.e., NIH scale), motor cortex excitability parameters of the unaffected motor cortex did not affect the Mirror therapies outcomes. The finding that the improvement brought about the MBT is independent from the illness duration is of great interest, underlying that MBT may represent a valuable treatment option even for patients in a chronic stage (mean of 33 months in the present study) (see also, Michielsen et al., 2011).

A novel, intriguingly, finding of the present study is the demonstration that MBT-induced improvements are shaped by the visual imagery ability of stroke patients. This effect, toghether with evidence obtained in the preceding experimental works in stroke patients and healthy adults (Chapters 2), further supports the view that the crossmodal sensory component of the mirror plays a major role in shaping the influence of the mirror feedback on the motor outcome. This may be more relevant in older adults, such as our brain-damaged patients, who are known to rely greatly on visual control and feedback during motor activities (Voelcker-Rehage, 2008) and to take advantage of visual strategies when learning and performing motor tasks (Swinnen et al., 2011).

The present Mirror therapies did not improve every tested functions, expected to show a recovery, at variance with the results of Cho and Cha (2015) and Amasyal and Yaliman (2016) who found significant improvements after the MBT on a broader range of clinical tests. However, it should be noted the methodological differences with the present study. In first study (Cho and Cha, 2015), MBT was coupled with transcranial Direct Current Stimulation (daily therapy duration = 45 min, 3 times a week for 6 weeks), while in the second study (Amasyali and Yaliman, 2016), the MBT was given in combination with conventional physiotherapy for a long duration (e.g., 3 weeks, 5 days/week, 30 min/day). Thus, both the duration of the single session of MBT and of the whole rehabilitation protocol, as well as the use of adjuvant physical/brain stimulation therapies, may explain the more restricted effects in our interventions.

Notwithstanding, more in depth exploratory analyses showed intriguing associations of the Mirror therapies effects with some clinical characteristics of the patients, which warrant further investigations. First, the effects of Mirror Visual Feedback on unilateral gross manual dexterity (BB test) appeared associated to the cortical excitability of the unaffected motor cortex. In particular, patients with a smaller area of excitable sites in the healthy hemisphere seem to benefit more of the Mirror therapies, regardless the type of feedback (reversed vs. standard). Results from the study described in Chapter 3 showed that observing a slowed down movement (simulating the reversed Mirror Visual Feedback) is more effective for increasing cortical excitability of the motor cortex controlling the hand behind the mirror. Noteworthy, in the REMIT, the hemisphere ipsilateral to the hand in front of the mirror is the one that controls for the healthy hand. The finding that also the REMIT improves motor function, as the MBT does, suggests that cortical excitability changes (if any) in the healthy motor cortex under reversed Mirror Visual Feedback conditions may support motor recovery, likely by taking over the motor control of both the healthy and paretic hand. However, given the lack of such data in this study (see methods section), this hypothesis remains speculator.

Finally, ABILHAND questionnaire scores seem to predict the subjective illusory feelings (assessed through MVFQ). Indeed, the susceptibility to the Mirror Illusion appears linked to the self-perceived motor ability in everyday-life: patients who gave low scores on this scale appearing less susceptible to the Mirror Illusion. This finding nicely complements the results from the investigation of the Mirror Illusion in stroke patients with hemiparesis (Chapter 2). In fact, in the previous study, I showed that stroke patients who experienced low illusory effects are those who report little to no use of the paretic limb in everyday-life activity (MAL scale). Again, it seems that the illusory sensations brought about by the mirror are linked to the perception of the contribution of the affected side of the body in their real, everyday life. In other words, the more patients have positive experience of functioning of the paretic limb, the more the retrieval of illusory sensations under Mirror Visual Feedback conditions. However, such subjective feelings are not able to influence the effects of the therapy (see also below). Crucially, are patients who are less persuaded that their affected hand could move as well as the intact hand under Mirror Visual Feedback condition, which reported greater generalizability of the therapeutic effects in everyday-life (measured through ABILHAND questionnaire scores) after the Mirror Therapies. This counterintuitive result suggests that the mechanisms underlying Mirror Visual Feedback and its effects are not prompt by voluntary control, namely there is little space in this therapy for a placebo effect.

Chapter 5

General discussion

The series of experiments described in the present doctoral thesis illustrates the role of conflicting incoming information inherent in MVF conditions and their effects on the motor system, providing new interpretations of the underpinning mechanisms.

Brief overview of findings

In Chapter 2, through a neuropsychological investigation of the Mirror Illusion, I characterized the MVF experience (indexed by an ad-hoc questionnaire), in a group of brain-damaged participants with motor deficit, demonstrating inter-individual differences in the related illusory feelings. Through this study, I could delineate the subjective sensations brought about by the observation of an illusory arm in patients with acquired difficulties in moving their own affected limb. The analyses pointed out how this illusion is differently experienced by participants, following the aftermaths of the cerebrovascular accident they suffered. This diversification has been found to be linked to clinical and motor features of the patients, unrevealing the role of certain abilities and brain areas in determining the susceptibility to the illusion. In particular, I have demonstrated how the view of reflected self-movements can differentially modulate the Mirror Illusion, depending on the damaged sensorimotor function. Indeed, whether the tactile sense is affected, the MVF of the bad arm moving goodly is associated with increased Mirror Illusion. On the other hand, in case of a severe motor impairment, the (illusionary) sight of the movements is not perceived as concerning the impaired limb.

These visuo-motor effects appear further shaped by the location of the cortical lesions: damages to low- and high-level areas of the motor cortical system enhance the Mirror Illusion, while lesions to parietal regions may differentially impact it, disrupting or facilitating specific processes involved in multisensory, body- and self-related, processing. Specifically, lesions affecting the precentral gyrus, middle and superior frontal regions and the inferior parietal cortex are positively associated with the illusion, whereas damages to the postcentral gyrus and to superior parietal regions are negatively related to it. Together, those pieces of evidence strongly supports the pivotal role of conflicting information in MVF conditions, suggesting how selective lesions and impairments may differentially characterize it.

In Chapter 3, through a Mirror Box-like paradigm in healthy participants, I showed that the observation of an altered visual feedback concomitant the execution of unilateral movements induces corticospinal changes that are related to the speed of the watched movement. Results point out that the greater the mismatch between observed and performed action, the greater the modulation of cortical excitability. In particular, the utmost modulatory effect is registered during the observation of a slower pace. Crucially, this effect appears not constrained to the muscle involved in the observed movement, rather it extends bilaterally to both hands, suggesting a widespread motor cortical activation. Together, these pieces of evidence have represented encouraging premises for the potential applicability of a modified version of the classic Mirror Box Therapy in stroke patients with motor deficit, where the conflict inherent in this arrangement has been further exacerbated. Consequently, this has opened up the hypothesis that an altered visual feedback might represent a promising strategy for improving motor recovery after stroke, as compared to the classic Mirror Box Therapy.

This hypothesis is explored in Chapter 4, where I directly verify the efficacy of a modified version of Mirror Box Therapy, the REMIT therapy, during which the MVF is reversed and the conflict is further exaggerated, for promoting the amelioration of post-stroke upper-limb motor impairments. Results showed that not only the novel intervention is associated with beneficial effects, but also these precisely reproduce those obtained through standard MVF and classic Mirror Box Therapy. The perfect overlap of findings, despite the intrinsic dissimilarities between the two techniques, calls for the potential involvement of similar mechanisms. Nonetheless, the peculiarities of the novel intervention help in disambiguating the available hypotheses. Indeed, the fact that the illusory image provided during REMIT is the one of the good arm working badly rules out the potential beneficial role of the Mirror Neurons Systems being the underpinning mechanism of the motor gains, as resonance mechanisms would have led to the imitation of a bad performance. The efficacy of the REMIT also rules out the reversal of the learned non-use phenomenon: if this mechanism would be involved, a further worsening of motor function should have been expected, but this was not the case. Nor the motor imagery is relevant for the efficacy of the MVF, rather it is the visual imagery that shapes its benefits. The more plausible interpretation of the mechanisms activated by the illusory mirror feedback (of the good arm behaving as it would be affected by paresis under REMIT and of the paretic arm finally working well under classic MBT) is that they are intrinsically linked to conflicts between incoming information from different modalities and sensorimotor processes. My proposal is that the sensorimotor mismatch itself, behaving as a strong reparative mechanism, triggers the motor system, leading to the observed clinical improvements.

Together, the results of these experiments demonstrate that the Mirror Illusion, in stroke patients, appears as a pervasive and stable phenomenon characterized by individual peculiarities and shaped by clinical and lesion factors. The multi-componentiality of MVF conditions calls for the mediation of many factors that, combined, contribute to the multifaceted MVF experiences. Collectively, the pieces of evidence provided depict the conflicts inherent in those conditions as pivotal in influencing motor behaviour, both at cortical and clinical levels.

5.1. Mirror Visual Feedback experience

As discussed in Chapter 1, the experience of performing movements facing a mirror placed perpendicular to the body midline is associated with subjective sensations of watching directly the limb out of the sight, facilitating the retrieval of illusory feelings (Ramachandran and Altschuler, 2009). It has been proposed that this arrangement fools the brain into thinking that both arms are moving symmetrically (Franz and Packman, 2004). In doing so, and by taking advantage of the universal human tendency of performing bimanual activity in synchrony (Swinnen, 2002), this condition is associated with improvements in motor performance.

What clearly emerges from the present set of experiments is that MVF experience is a pervasive phenomenon that occurs easily in stroke patients affected by acquired motor impairment, as well as in healthy participants (Chapter 2). This finding is further corroborated by the lack of the association between the illusion and the overall size of the lesion, underlying that even brain-damaged systems can benefit from the MVF. This is in line with previous evidence that shows how the brain preserves its natural capability to integrate input from different sensory modalities in many pathological conditions featured by a brain damage (Bolognini et al., 2016).

In addition, the illusion is not influenced by age, schooling, or by duration of illness or general condition of the patients, further demonstrating its pervasiveness. Moreover, findings from Chapter 4 clearly show that the repetitive exposure over consecutive days does not alter the stability of the illusory sensations. This evidence, in turn, fully supports the application of MVF rehabilitative programs that are intrinsically features by repeated sessions over weeks.

Crucially, results from Chapter 4 strongly evidenced that also the clinical effects of the Mirror therapies are not affected by demographic (age and gender), clinical (duration of illness and lesion extension) nor motor (measured through a set of clinical indexes of impairment) factors. While this finding recalls the pervasiveness of the illusionary effects found in Chapter 2, it provides new indications about the suitability of clinical interventions based on MVF. Specifically, the fact that the induced effects are not dependent upon the duration of illness is promising, confirming how Mirror Therapies may represent a valuable treatment option even for more chronic patients. This evidence is in line with previous reports that show clinical improvements of the Mirror Box Therapy also years after the injury (Michielsen et al., 2011). To note, the opposite is not always true; rather, Mirror Box Therapy seems detrimental when applied soon after stroke. Specifically, a recent pilot study on acute patients (< 8 days) shows no significant improvement after Mirror Box Therapy, as compared to a control group (Yeldan et al., 2015).

Globally, those pieces of evidence further underline the wide applicability of MVF interventions among a broad range of post stroke motor profiles.

5.2. Individual differences in the MVF experience in stroke

Whereas globally the MVF experience appears as a pervasive phenomenon that occurs easily, the counterpart - which represents the novelty of the present set of findings - is that many factors contribute to it, especially considering its multi-componential nature. Indeed, by directly analysing the subjective sensations brought about by the MVF conditions in stroke patients with motor impairment, I demonstrated inter-individual differences in experiencing the so-called Mirror Illusion. Within the framework of promising but not conclusive results due to the huge variability of the effects (for review, see Thieme et al., 2013), and underpinning mechanisms still under investigation (for a review, see Deckoninck et al., 2014), the present results clearly pointed out individual differences in the related experience. In fact, more in depth analyses presented in Chapter 2 showed that the degree of motor and tactile impairments, as well as the lesional profile of participants, differentially affects the experience. Those findings underline how different elements are combined together to determine the phenomena at the basis of Mirror Box Therapy. The role of those different factors could be acknowledged within a view that depicts the twofold conflict (between sensory information and among sensorimotor processes) inherent in this arrangement as the potential driving mechanism under MVF conditions (Michielsen et al., 2010). In fact, when facing conflicting information coming from different sensory systems, the brain has to combine them into a single percept. What seems to be happening is that, following Bayesian rules (Ernst and Bülthoff, 2004), the weight of all the information is different, being influenced from both the intact senses that may try to predominate and the missing or unreliable input from the impaired sensory modalities. What clearly emerges here is that stroke patients with acquired motor deficits following a brain damage are differentially vulnerable to crossmodal interferences (Bolognini et al., 2016). In particular, I have demonstrated how the view of self-reflected movements can differentially modulate the Mirror Illusion, depending on the underlying balance between damaged and unimpaired modalities. In particular, the greater the tactile deficit, the greater the illusion; instead, the greater the motor deficit, the less the illusory effects, underlying how a spared motor output is necessary to generate the illusion. In addition, the effects are further shaped by cortical lesions, with interesting dissociations between cerebral areas (lesions to superior parietal and postcentral regions disrupt the illusion; instead, lesions to middle and superior frontal cortices, inferior parietal cortex the precentral gyrus increase it), (see Chapter 2).

This supports the view of a mutual interplay between sensory and motor consequences of one own actions, further underlying the role of sensorimotor integration mechanisms.

A remarkable evidence of the role of individual factors can also be observed in Experiment 4, where many components appear able to shape the effects of Mirror therapies. Here, the investigation of factors that could mediate such effects indicates that visual imagery is relevant for promoting clinical gains in motor domain and also unravels the role of the unaffected hemisphere and general clinical conditions in mediating those improvements. While the effect related to the visual imagery represents a nice complementary result of that emerged in Chapter 2, and further strengthens that evidence, the latter two findings are intriguing and deserve further in depth consideration in future studies.

What clearly emerges is that it is not just the experience of performing movements in front of a mirror that induces the well-known and widely investigated effects, rather many other factors play a crucial role in influencing the outcomes. Therefore, it is reasonable to think that the driven mechanisms are not exclusively based on the mere observation of movements, as every participant behaves differently following its peculiarities. Rather, the putative working mechanisms exploit more complex motor and cognitive processes.

5.3. Beyond visual feedback: multisensory integration and sensorimotor processes

Results from the present series of experiments strongly evidence how MVF conditions, albeit their simplicity, appear as complex multi-componential phenomena. As seen, it is not only the view of the movement, or the passive exposure to a visual feedback superimposed over the hidden body part, that prompts the illusory sensations, the cortical changes and the motor improvements. Rather, those effects are mediated by a complex interplay between information from different modalities as well as sensorimotor processes.

Specifically, any effect with visual feedback of an action could be due to intricate interactions between vision, motor commands, and proprioceptive signals (Tsakiris et al., 2010). Under MVF conditions, these three different kinds of information need to be unified into a single percept: while subjects are required to perform movements watching through the mirror, the visual information from the reflection has to be compared not only with the motor output sent by the brain, but also with the proprioceptive signals from the unseen hand. Results of the present set of experiment strongly indicate that different integrations of the three, following individual spared and impaired capabilities, lead to a various range of illusionary experiences.

Regarding the first two sources of information, namely vision and motor commands, present results suggest that visual information is sufficient to elicit the illusion when coupled with motor information, i.e., the motor efferent copy of the output sent by the brain is compared with the visual feedback from the mirror, giving rise to an illusion of movement; as the negative association between the motor component and the illusion suggests, the more the damage (i.e., the less the motor output), the less the illusion (Chapter 2). In addition, the explicit instruction of moving the paretic hand as better as possible (Altschuler et al., 1999), requiring an active role of the more impaired limb, prompts the key participation of motor information and related sensorimotor processes of the affected hemisphere. The affected motor system, far from being a mere receptor of the visual feedback, becomes the promotor of effects. Therefore, following its spared ability, the damaged motor system itself is able to actively contribute to the observed effects. As seen, some residual motor function of the affected limb are necessary to generate the illusory sensations (Chapter 2). Noteworthy, by sustaining the residual function of the affected hemisphere through tDCS stimulation, it is possible to further enhance MVF driven beneficial effects on motor outcomes (Cho and Cha, 2015).

Regarding proprioceptive signals, the study did not evaluate the integrity or the impairment of the proprioceptive function in stroke participants, therefore the role of proprioception in the illusion can not be fully delineate. Future research should deeply assess the role of proprioceptive capability in influencing multisensory the illusory sensations in post stroke patients with upper limb motor impairment to further elucidate the factors that could influence the MVF experience.

On the other hand, data from Experiment 2 provide intriguing suggestions with respect to somatosensory signals, showing that the presence of tactile deficit in the hidden limb further enhances the illusion. In particular, this finding shows that, in presence of unreliable tactile sensations, stroke patients rely more on the visual feedback. Therefore, the mirror-induced illusory sensations need to overcome and dominate the unreliable, real, tactile sensations in order to be able to alter them, in turn promoting the feeling of moving the paretic arm. Instead, reliable, tactile sensations reduce the Mirror Illusion because in this case the visual feedback cannot overcome and dominate over the real touch. Brain diseases, by altering the acuity of one sense, may render such weakened sense more vulnerable to crossmodal interferences from the intact senses (Bolognini et al., 2016).

Intriguingly, the relation between mirror feedback and somatosensation is bidirectional: not only spared tactile capability influences the illusion (Chapter 2), but also, in a complementary fashion, visual information ameliorates somatosensory deficit. Some pilot findings, in fact, pointed out how Mirror Box Therapy may improve tactile sensitivity (Colomer et al., 2016) and surface sensibility (Dohle et al., 2009). These findings are of relevance in rehabilitation settings, given the key role of sensation in driving post stroke motor recovery (Bolognini et al., 2016).

In addition, results on kinaesthesia under MVF conditions in healthy subjects reveal how also information from the hand in front of the mirror are important in determining the illusory sensations (Chancel et al., 2016). Specifically, it emerges how those phenomena (there indexed through the illusory displacement of the feel position of the hidden limb) result from the combination of congruent signals from the two arms: the visual afferents related to the virtually moving arm and the somaesthetic afferents of the contralateral limb. Taken as a whole, those findings further confirm how mirror-related effects are not purely visual (Chancel et al., 2016).

5.4. Multisensory and sensorimotor conflicts

The evidence discussed so far points at the integration between various modalities and sensorimotor processes sustained by different cerebral networks in characterizing MVF settings. This combination do not convey the mere merging of incoming information, where all the input are put together into a single percept, but it rather actively influences the extent of multisensory and sensorimotor binding, depending on their level of functioning. Consequently, different balances between all those components seem lead to differences in the related phenomena (Chapter 2 and 4).

Again, the mirror effects due to bimanual conditions are not simple visual phenomena, i.e., it is not just the visual input prompting the motor outcomes. Otherwise, the mere observation of a movement should evoke the same beneficial consequences. This is not the case, since studies directly comparing MVF conditions and the direct view of the limbs, showed the superiority of the first intervention at behavioral, cortical and clinical levels (Chapter 1).

It is important to underline that the present findings pertain MVF conditions in which some kind of motor output is given also to the hand behind the mirror. In fact, efforts toward the hidden limb are always explicitly required, in accordance with original protocol (Altschuler et al., 1999); it follows the importance of the motor input sent by the brain, namely the commands to the muscles to move, regardless the smoothness of the resulting output. As seen in Chapter 1, every motor command is accompanied by a copy with the expected consequences (Frith and Wolpert, 2000). Whether the consequences deviate from what it is expected, a conflict arises. Specifically, the greater the mismatch between expected and actual motor consequences, the greater the conflict (Chapter 3 and 4). Not only the conflict is important and should be further exploited (Chapter 3), but also, it could be the key booster of the changes. In fact, under standard MVF conditions, the mirror creates a great conflict between the actual sensory feedback and the expected motor output. This mismatch is beneficial as, by exploiting the natural tendency of bimanual activity, it provides the image of an (illusory) working limb (Ramachandran and Altschuler, 2009). In this case, the output sent by the brain to the impaired limb is finally coupled with a feedback of successful performance. Similar beneficial effects could be obtained by further exploiting the key role of the conflict, providing a feedback that is greatly different from that expected, when the mismatch between incoming input and sensory modalities is farther exaggerated (Chapter 3 and 4). In fact, the actual image of unsuccessful result, while a successful movement is expected, leads to a great widespread motor activation in order to compensate for the erroneous feedback (see Chapter 3). Finally, the impaired performance of a motor act expected to be correct, as the one that comes along with the exaggerated conflict under REMIT conditions, drives to more powerful motor efforts, which result in appreciable improvements (Chapter 4).

Results of the present set of studies show how some residual motor abilities are necessary to give rise to the Mirror Illusion (Chapter 2). Notwithstanding, general indications about which level of motor impairment (i.e., mild, moderate or severe) should patients present to benefit more from MVF intervention could not be given. In fact, the evidence from Chapter 4 points out that Mirror Therapies are equally effective regardless the degree of motor deficit. The latter result, although referring to a small sample of patients, seems to reassure that even more impaired participants may profit from Mirror Therapies. Indeed, motor exercises with the MVF have been widely adopted as a valuable tool also for patients that are not able to actually move their paretic limb (Dohle et al., 2009). Interestingly, recent evidence pointed out how Mirror Box Therapy is likewise adequate as passive mobilization in improving motor function in severely impaired chronic patients, and more effective in ameliorating light touch sensitivity deficit (Colomer et al., 2016).

Regarding severe stroke cases, it has been reported what follows: *"There is some evidence that the clinical effect of MT* [namely, Mirror (Box) Therapy] *is most prominent when the difference between the real and the mirrored visual feedback is greatest, i.e. in patients with no motor function at all (Dohle et al., 2011)"* [in Wang et al., 2013, page 594]. Hence, the postulated link between the efficacy of the therapy and the motor functionality of the patients seems to assign a key role to the *lack* of motor functionality itself.

This point should be questioned.

Firstly, not only severely impaired stroke patients benefit from the therapy (see Chapter 1, but see Thieme et al., 2013). Additionally, the pieces of evidence from the present thesis point out how the MVF effects are not driven (only) by the (lack of) motor functionality, rather by an intricate balance between sensorimotor processes and sensory modalities, in which the conflict between different incoming information is pivotal. The following reflections support this claim.

As seen, the present findings do not call for a relation between the efficacy of the therapy and the degree of motor impairment of the patients (see also Chapter 4). From the other hand, it could be possible that participants with no motor function at all may encounter difficulties in experiencing the illusion, given the found link between spared motor function and susceptibility to the illusory feelings (Chapter 2). In addition, results of both Chapter 2 and 4 suggest that the more the patients have positive experiences regarding the functioning of their impaired limb (as assessed through questionnaires such as MAL and ABILHAND), the more the retrieve of illusory sensations under MVF conditions. Therefore, the absence of movement of the paretic hand may play a detrimental role when considering the potential function of the perception patients have with respect to the ability of their affected limb.

Considering the key function of motor output under bimanual conditions, also the role of the embodiment put forward to explain MVF phenomena (Romano et al., 2013) could be expanded. Following this intriguing hypothesis, the mirror reflection that shows an accurate motor behavior might automatically activate the correct residual motor patterns in the impaired hand, thanks to the attribution of some properties of an external object to one's own body (De Vignemont, 2011). Given the present results, as already discussed, the embodiment of the hand in the mirror is not a passive automatic effect, rather it depends upon a wide range of other components.

The notion of embodiment should also take into consideration the illusory movements that stem from the reflected hand, as well as the comparison with the real movements of the limb out of the sight. Whether the illusory movements are perceived as too different from patients' abilities, they could prevent the establishing of the illusion (see Chapter 2). Present evidence from healthy subjects suggests that when the visual feedback is too fast, as compared to participants' possibilities, it acts against motor cortex modulation (see Chapter 3). Therefore, patients with no motor function at all may have difficulties in embodying the mirrored image, given the huge difference between the two in terms of movements. In case of no movement, the hand in the mirror could be too different from the real movements of the affected limb. In addition, present results clearly showed that similar clinical effects are elicited under REMIT condition, where the feedback provided is of incorrect behavior (see Chapter 4).

Thus, calling back the above-mentioned claim that the clinical effect of Mirror Box Therapy is more prominent when the difference between the real and the mirrored visual feedback is greatest, and taking as a whole the present evidence, another interpretation should be put forward. Specifically, it is not only the motor component that drives those effects, rather it seems more relevant the incongruence between expected and actual feedback, motor output and sensory input, as well as the conflict between incoming multisensory information. The greater is the mismatch between all those components, the greater the conflicting condition created by the MVF that the brain has to face. In this way, the beneficial effects driven by Mirror Box Therapy, which are grounded on those conflicts, are maximized.

Therefore, a theoretical perspective that summarizes all the evidence from the PhD thesis, taking into account all the inconstancies found in the literature, needs to emphasize the role of the sensorimotor and multisensory conflicts; only this component seems able to combine and account for the multifaceted application of the MVF in clinical settings.

Concluding remarks

Two decades after the first introduction of MVF in clinical settings (Ramachandran et al., 1995), unfortunately, little has changed, as this practice is still being passed on only anecdotally among operators. On the one hand, it should be acknowledged that the effects of the MVF arrangements have been largely studied and exploited in healthy subjects with the aim of assessing the consequences of manipulating the limb out of the sight (Chapter 1). On the other, regrettably, it is a matter of fact that the mechanisms thanks to which MVF is effective in post-stroke motor rehabilitation are still under investigation. Given the plethora of various uses of MVF, the high variability of behavioural gains obtained –which prevent to determine conclusive inferences about its efficacy- could be partly explained by differences in the setup. Regrettably, a recent systematic review on the clinical aspects of MVF interventions in rehabilitation clearly pointed out how published studies do not provide sufficient information on the clinical protocols used (Rothgangel et al., 2011). It is important to keep this in mind when attempting to draw conclusions from the great amount of different clinical trials performed and studies published. With this respect, it is essential to identify which instructions are given to patients and interpret the findings accordingly. For instance, uni- or bi-manual movements, the adoption of concomitant strategies (e.g., motor imagery) and the type of exercises proposed are factors often neglected. Notwithstanding, they represent important confounders that add variability in the studies and mystify both the underpinning mechanisms and the related findings.

Notably, many efforts have been done in recent years to unravel the neural correlates of the Mirror Illusion and the related clinical improvements (Chapter 1). However, those results are not conclusive, since there are no shared guidelines for performing MVF interventions. For instance, in a recent overview of findings related to cerebral activation under MVF conditions (Deckonink et al., 2014), Authors made the effort to distinguish between uni- and bi-manual exercises during MVF conditions. With respect to studies in stroke patients with motor impairment, evidence showed that bimanual conditions are associated with ipsilateral precentral gyrus activation (Kang et al., 2011; 2012) and contralateral posterior cingulate cortex activation (Michielsen et al., 2011). On the other hand, both uni- and bi-manual conditions are associated with and increased activities in both contra- (Michielsen et al., 2011) and ipsi-lateral (Michielsen et al., 2011; Wang et al., 2013) precunei. However, on a broader perspective, it should be note that the majority of published studies did not specified which kind of motor exercise was required to perform during the mirror therapy. We still need to uncover the more effective exercises; subsequently, it would be possible to modify them in order to build an individual oriented therapy.

With this respect, in a seminal study in healthy subjects, in which the mirror was used as a tool to investigate the effects of the visual feedback on reaching movements, interesting dissociations between different types of exercises were found (Snijders et al., 2007). Specifically, these results support the direction-dependent weighting of visual and proprioceptive information, with vision relatively more dominant in the azimuthal direction, and proprioception relatively stronger in the radial direction. Consequently, it would be possible that exercises in the azimuthal direction (left – right) direction are more susceptible of MVF influence. In other words, an arm that is moving toward the midline could fool the brain into thinking that both arms are moving toward each other in the mirror condition, and thus may exploit to a greater extent the above mentioned tendency of performing bimanual movements. This may be associated with greater functional recovery. On the other hand, radial (near-far) exercises could be less indicated for upper limb rehabilitation. Conversely, it could be speculated that those exercises are more useful for the lower limb, as the vast majority of legs movements that sustain locomotion are carried out in the radial direction. Additionally, given the findings of Chapter 2, it could be possible that different types of exercises are more effective in case of concomitant tactile impairment. Although intriguing, those suggestions remain purely speculative and warrant future ad hoc investigations.

Another important aspect is the role of imagery. In some studies, participants were instructed to imagine the movement of the arm out of the sight (e.g., Stevens and Stoykov, 2003). With this respect, a different scenario arises when participant are overtly required to imagine the movement of the paretic side, namely to mentally rehearse the exercises without any overt effort. In this case, motor mental imagery abilities may play a crucial role and thus influence the effects of the therapy; however, when not explicitly required, motor imagery plays a minor role as compared to visual imagery, as shown in Chapters 2 and 4.

To sum up, notwithstanding the broad use of the MVF as an easy way to facilitate motor recovery with promising results, the lack of a shared knowledge represents a big flaw and many factors that are often neglected may confound its efficacy. Only by gaining a common use of the MVF it is possible to deeply understand its mechanisms and develop useful rehabilitation strategies. On the other hand, findings and suggestions that stem for the present dissertation could be intriguing since they depict MVF related phenomena as reliable, easy to elicit and stable to maintain, despite the repetitive exposure typical of multiple sessions of the rehabilitative interventions.

Regarding the underpinning processes, the results here presented identify in the twofold conflicts under MVF conditions the key factor to interpret and predict the related consequences in the motor domain, both at a behavioural and a clinical level. Specifically, the multisensory mismatch regarding visual, motor and somatosensory information, together with the sensorimotor conflict between expected and actual feedback, influences the consequences of performing actions while observing the mirror placed perpendicular to the body midline.

What is crucial to remark is that those results pertain to situations in which patients are required to carry out bimanual exercises, and thus the Mirror Visual Feedback could exploit the universal tendency of performing symmetric movements (Swinnen 2002). In fact, both in experiment 2 and 4 participants were required to move the paretic hand as better as possible, according to the original protocol (Altschuler et al., 1999), and similarly to previous reports (Dohle et al., 2009; Wu et al., 2013). To note, in this case, the objective motor performance of the paretic hand is of no interest, namely, regardless the correctness and the smoothness of the movement performed by the paretic side, what is essential is the attempt from the brain. Accordingly, the reflection given by the mirror could be seen as the illusory visual feedback to the motor input sent (Ramachandran and Altschuler, 2009). In addition, in this condition, the tendency to carry out movements bilaterally could represent the counterpart of the motor input from the brain. On contrary, when only uni-manual movements are performed, other mechanisms may arise. In this case, the visual information may fully overcome the (absent) information from the hand out of the sight, following the well known visual capture effect (Holmes et al., 2004) where the visual input, is weighted more than the kinesthetic signals coming from that hidden hand (van Beers et al., 1999). However, this point remains speculative and further studies directly comparing uni- and bimanual exercises under Mirror Visual Feedback conditions are warranted.

References

Albert ML. A simple test of visual neglect. Neurology, 23: 658–664, 1973.

Altschuler, E. L., & Hu, J. (2008). Mirror therapy in a patient with a fractured wrist and no active wrist extension. *Scandinavian Journal of Plastic and Reconstructive Surgery and Hand Surgery*, *42*(2), 110-111.

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

Amasyali, S. Y., & Yaliman, A. (2016). Comparison of the effects of mirror therapy and electromyography-triggered neuromuscular stimulation on hand functions in stroke patients: a pilot study. International Journal of Rehabilitation Research, 39(4), 302-307.

Amedi, A., Malach, R., & Pascual-Leone, A. (2005). Negative BOLD differentiates visual imagery and perception. Neuron, 48(5), 859-872.

American Academy of Orthopaedic Surgeons. (2011). Joint motion: method of measuring and recording.

Avanzino, L., Raffo, A., Pelosin, E., Ogliastro, C., Marchese, R., Ruggeri, P., & Abbruzzese, G. (2014). Training based on mirror visual feedback influences transcallosal communication. European Journal of Neuroscience, 40(3), 2581-2588.

Avenanti, A., Bolognini, N., Maravita, A., & Aglioti, S. M. (2007). Somatic and motor components of action simulation. Current Biology, 17(24), 2129-2135.

Bamford, J., Sandercock, P., Dennis, M., Warlow, C., & Burn, J. (1991). Classification and natural history of clinically identifiable subtypes of cerebral infarction. The Lancet, 337(8756), 1521-1526.

Barbin, J., Seetha, V., Casillas, J. M., Paysant, J., & Pérennou, D. (2016). The effects of mirror therapy on pain and motor control of phantom limb in amputees: A systematic review. Annals of physical and rehabilitation medicine.

Barth, J. M., Stezar, G. L., Acierno, G. C., Kim, T. J., & Reilly, M. J. (2014). Mirror book therapy for the treatment of idiopathic facial palsy. Ear, nose, & throat journal, 93(9), E11-4.

Bayon-Calatayud, M., Benavente-Valdepeñas, A. M., & Vazquez-Muñoz, M. D. P. (2016). Mirror therapy for distal radial fractures: A pilot randomized controlled study. Journal of Rehabilitation Medicine, 48(9), 829-832.

Bisiach, E., Cappa, S., & Vallar, G. (1983). Guida all'esame neuropsicologico. Cortina.

Blakemore, S. J., & Frith, C. (2003). Self-awareness and action. Current opinion in neurobiology, 13(2), 219-224.

Bolognini, N., & Maravita, A. (2007). Proprioceptive alignment of visual and somatosensory maps in the posterior parietal cortex. Current Biology, 17(21), 1890-1895.

Bolognini, N., Russo, C., & Edwards, D. J. (2016). The sensory side of post-stroke motor rehabilitation. Restorative neurology and neuroscience, (Preprint), 1-16.

Bolognini, N., Russo, C., & Vallar, G. (2015). Crossmodal illusions in neurorehabilitation. Frontiers in behavioral neuroscience, 9.

Braun, S. M., Beurskens, A. J., Borm, P. J., Schack, T., & Wade, D. T. (2006). The effects of mental practice in stroke rehabilitation: a systematic review. Archives of physical medicine and rehabilitation, 87(6), 842-852.

Brott, T., Adams, H. P., Olinger, C. P., Marler, J. R., Barsan, W. G., Biller, J., ... & Hertzberg, V. (1989). Measurements of acute cerebral infarction: a clinical examination scale. Stroke, 20(7), 864-870.

Brunetti, M., Morkisch, N., Fritzsch, C., Mehnert, J., Steinbrink, J., Niedeggen, M., & Dohle, C. (2015). Potential determinants of efficacy of mirror therapy in stroke patients–A pilot study. Restorative neurology and neuroscience, 33(4), 421-434.

Brunnstrom, S. (1966). Motor testing procedures in hemiplegia: based on sequential recovery stages. Physical therapy, 46(4), 357.

Buccino, G., Solodkin, A., & Small, S. L. (2006). Functions of the mirror neuron system: implications for neurorehabilitation. Cognitive and behavioral neurology, 19(1), 55-63.

Cacchio, A., De Blasis, E., De Blasis, V., Santilli, V., & Spacca, G. (2009). Mirror therapy in complex regional pain syndrome type 1 of the upper limb in stroke patients. Neurorehabilitation and neural repair.

Carson, R. G., & Ruddy, K. L. (2012). Vision modulates corticospinal suppression in a functionally specific manner during movement of the opposite limb. The Journal of Neuroscience, 32(2), 646-652.

Cavanna, A. E., & Trimble, M. R. (2006). The precuneus: a review of its functional anatomy and behavioural correlates. Brain, 129(3), 564-583.

Chancel, M., Brun, C., Kavounoudias, A., & Guerraz, M. (2016). The kinaesthetic mirror illusion: How much does the mirror matter?. Experimental brain research, 234(6), 1459-1468.

Cho, H. S., & Cha, H. G. (2015). Effect of mirror therapy with tDCS on functional recovery of the upper extremity of stroke patients. Journal of physical therapy science, 27(4), 1045-1047.

Colomer, C., NOé, E., & Llorens, R. (2016). Mirror therapy in chronic stroke survivors with severely impaired upper limb function: a randomized controlled trial. European journal of physical and rehabilitation medicine.

Crawford, J. R., & Garthwaite, P. H. (2002). Investigation of the single case in neuropsychology: Confidence limits on the abnormality of test scores and test score differences. Neuropsychologia, 40(8), 1196-1208.

De Renzi, E., & Faglioni, P. (1978). Normative data and screening power of a shortened version of the Token Test. Cortex, 14(1), 41-49.

De Renzi, E., Motti, F., & Nichelli, P. (1980). Imitating gestures: a quantitative approach to ideomotor apraxia. Archives of Neurology, 37(1), 6-10.

De Vignemont, F. (2011). Embodiment, ownership and disownership. Consciousness and cognition, 20(1), 82-93.

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. Neurorehabilitation and neural repair, 1545968314546134.

Demeurisse, G., Demol, O., & Robaye, E. (1980). Motor evaluation in vascular hemiplegia. European neurology, 19(6), 382-389.

Desrosiers, J., Bravo, G., Hébert, R., Dutil, E., & Mercier, L. (1994). Validation of the Box and Block Test as a measure of dexterity of elderly people: reliability, validity, and norms studies. Archives of physical medicine and rehabilitation, 75(7), 751-755.

Diller, L., & Weinberg, J. (1976). Hemi-inattention in rehabilitation: the evolution of a rational remediation program. Advances in neurology, 18, 63-82.

Dohle, C., Püllen, J., Nakaten, A., Küst, J., Rietz, C., & Karbe, H. (2009). Mirror therapy promotes recovery from severe hemiparesis: a randomized controlled trial. Neurorehabilitation and 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. Experimental brain research, 208(4), 543-555.

Ernst, M. O., & Bülthoff, H. H. (2004). Merging the senses into a robust percept. Trends in cognitive sciences, 8(4), 162-169.

Fadiga, L., Craighero, L., & Olivier, E. (2005). Human motor cortex excitability during the perception of others' action. Current opinion in neurobiology, 15(2), 213-218.

Fadiga, L., Fogassi, L., Pavesi, G., & Rizzolatti, G. (1995). Motor facilitation during action observation: a magnetic stimulation study. Journal of neurophysiology, 73(6), 2608-2611.

Fink, G. R., Marshall, J. C., Halligan, P. W., Frith, C. D., Driver, J., Frackowiak, R. S., & Dolan, R. J. (1999). The neural consequences of conflict between intention and the senses. Brain, 122(3), 497-512.

Fiorio, M., & Haggard, P. (2005). Viewing the body prepares the brain for touch: effects of TMS over somatosensory cortex. European Journal of Neuroscience, 22(3), 773-777.

Foell, J., Bekrater-Bodmann, R., Diers, M., & Flor, H. (2014). Mirror therapy for phantom limb pain: brain changes and the role of body representation. European Journal of Pain, 18(5), 729-739.

Fortis, P., Maravita, A., Gallucci, M., Ronchi, R., Grassi, E., Senna, I., ... & Tesio, L. (2010). Rehabilitating patients with left spatial neglect by prism exposure during a visuomotor activity. Neuropsychology, 24(6), 681.

Franz, E. A., & Packman, T. (2004). Fooling the brain into thinking it sees both hands moving enhances bimanual spatial coupling. Experimental Brain Research, 157(2), 174-180.

Frith, C. D., & Wolpert, D. M. (2000). Abnormalities in the awareness and control of action. Philosophical Transactions of the Royal Society of London B: Biological Sciences, 355(1404), 1771-1788.

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. Restorative neurology and neuroscience, 32(2), 269-280.

Fugl-Meyer, A. R., Jääskö, L., Leyman, I., Olsson, S., & Steglind, S. (1974). The post-stroke hemiplegic patient. 1. a method for evaluation of physical performance. Scandinavian journal of rehabilitation medicine, 7(1), 13-31.

Fukumura, K., Sugawara, K., Tanabe, S., Ushiba, J., & Tomita, Y. (2007). Influence of mirror therapy on human motor cortex. International Journal of Neuroscience, 117(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. Neuroscience letters, 419(2), 108-112.

Gallivan, J. P., McLean, D. A., Smith, F. W., & Culham, J. C. (2011). Decoding effector-dependent and effector-independent movement intentions from human parieto-frontal brain activity. The Journal of Neuroscience, 31(47), 17149-17168.

Ganis, G., Thompson, W. L., & Kosslyn, S. M. (2004). Brain areas underlying visual mental imagery and visual perception: an fMRI study. Cognitive Brain Research, 20(2), 226-241.

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. Experimental Brain Research, 163(1), 118-122.

Ghazanfar, A. A., & Schroeder, C. E. (2006). Is neocortex essentially multisensory?. Trends in cognitive sciences, 10(6), 278-285.

Goldberg, I. I., Harel, M., & Malach, R. (2006). When the brain loses its self: prefrontal inactivation during sensorimotor processing. Neuron, 50(2), 329-339.

Hamzei, F., Läppchen, 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. Neurorehabilitation and neural repair, 26(5), 484-496.

Harris, A. J. (1999). Cortical origin of pathological pain. The Lancet, 354(9188), 1464-1466.

Harris, C. M., & Wolpert, D. M. (1998). Signal-dependent noise determines motor planning. Nature, 394(6695), 780-784.

Hoff, M., Kaminski, E., Rjosk, V., Sehm, B., Steele, C. J., Villringer, A., & Ragert, P. (2015). Augmenting mirror visual feedback-induced performance improvements in older adults. European Journal of Neuroscience, 41(11), 1475-1483.

Holmes, N. P., & Spence, C. (2005). Visual bias of unseen hand position with a mirror: spatial and temporal factors. Experimental Brain Research, 166(3-4), 489-497.

Holmes, N. P., Crozier, G., & Spence, C. (2004). When mirrors lie:"Visual capture" of arm position impairs reaching performance. Cognitive, Affective, & Behavioral Neuroscience, 4(2), 193-200.

Hubbard, E. M. (2007). Neurophysiology of synesthesia. Current psychiatry reports, 9(3), 193-199.

Isaac, A., Marks, D. F., & Russell, D. G. (1986). An instrument for assessing imagery of movement: The Vividness of Movement Imagery Questionnaire (VMIQ). Journal of Mental Imagery.

Jax, S. A., Rosa-Leyra, D. L., & Coslett, H. B. (2015). Enhancing the mirror illusion with transcranial direct current stimulation. Neuropsychologia, 71, 46-51.

Jeannerod, M. (1994). The representing brain: Neural correlates of motor intention and imagery. Behavioral and Brain sciences, 17(02), 187-202.

Jeannerod, M. (2001). Neural simulation of action: a unifying mechanism for motor cognition. Neuroimage, 14(1), S103-S109.

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. Annals of rehabilitation medicine, 35(6), 747-758.

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. Journal of neuroengineering and rehabilitation, 9(1), 1.

Läppchen, 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. Journal of neurophysiology, 108(10), 2857-2861.

Liepert, J., Dettmers, C., Terborg, C., & Weiller, C. (2001). Inhibition of ipsilateral motor cortex during phasic generation of low force. Clinical Neurophysiology, 112(1), 114-121.

Longo, M. R., & Sadibolova, R. (2013). Seeing the body distorts tactile size perception. Cognition, 126(3), 475-481.

Longo, M. R., Betti, V., Aglioti, S. M., & Haggard, P. (2009). Visually induced analgesia: seeing the body reduces pain. The Journal of Neuroscience, 29(39), 12125-12130.

Marks, D. F. (1973). Visual imagery differences in the recall of pictures. British journal of Psychology, 64(1), 17-24.

Mathiowetz, V., Kashman, N., Volland, G., Weber, K., Dowe, M., & Rogers, S. (1985a). Grip and pinch strength: normative data for adults. Arch Phys Med Rehabil, 66(2), 69-74.

Mathiowetz, V., Volland, G., Kashman, N., & Weber, K. (1985b). Adult norms for the Box and Block Test of manual dexterity. American Journal of Occupational Therapy, 39(6), 386-391.

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. Archives of physical medicine and rehabilitation, 90(4), 675-681.

McCabe, C. (2011). Mirror visual feedback therapy. A practical approach. Journal of hand therapy, 24(2), 170-179.

McCabe, C. S., & Blake, D. R. (2007). Evidence for a mismatch between the brain's movement control system and sensory system as an explanation for some pain-related disorders. Current pain and headache reports, 11(2), 104-108.

McCabe, C. S., Haigh, R. C., Halligan, P. W., & Blake, D. R. (2005). Simulating sensory–motor incongruence in healthy volunteers: implications for a cortical model of pain. Rheumatology, 44(4), 509-516.

Medina, J., Khurana, P., & Coslett, H. B. (2015). The influence of embodiment on multisensory integration using the mirror box illusion. Consciousness and cognition, 37, 71-82.

Meige H. (1905) Les amnesies motrices fonctionnelles et le traitement des hemiplegiques. Rev Neurol (Paris); 13: 183–184.

Metral, M., Chancel, M., Brun, C., Luyat, M., Kavounoudias, A., & Guerraz, M. (2015). Kinaesthetic mirror illusion and spatial congruence. Experimental brain research, 233(5), 1463-1470.

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. Neurorehabilitation and Neural Repair, 25(3), 223-233.

Michielsen, M. E., Smits, M., Ribbers, G. M., Stam, H. J., Van Der Geest, J. N., Bussmann, J. B., & Selles, R. W. (2010). The neuronal correlates of mirror therapy: an fMRI study on mirror induced visual illusions in patients with stroke. Journal of Neurology, Neurosurgery & Psychiatry, jnnp-2009.

Mills, K. R., Boniface, S. J., & Schubert, M. (1992). Magnetic brain stimulation with a double coil: the importance of coil orientation. Electroencephalography and Clinical Neurophysiology/Evoked Potentials Section, 85(1), 17-21.

Nojima, I., Mima, T., Koganemaru, S., Thabit, M. N., Fukuyama, H., & Kawamata, T. (2012). Human motor plasticity induced by mirror visual feedback. The Journal of Neuroscience, 32(4), 1293-1300.

Novembre, G., Ticini, L. F., Schütz-Bosbach, S., & Keller, P. E. (2012). Distinguishing self and other in joint action. Evidence from a musical paradigm. Cerebral Cortex, 22(12), 2894-2903.

Oldfield, R. C. (1971). The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia, 9(1), 97-113.

Park, E. J., Baek, S. H., & Park, S. (2016). Systematic review of the effects of mirror therapy in children with cerebral palsy. Journal of Physical Therapy Science, 28(11), 3227-3231.

Park, S. D., Kim, J. Y., & Song, H. S. (2015). Effect of application of transcranial direct current stimulation during task-related training on gait ability of patients with stroke. Journal of physical therapy science, 27(3), 623.

Penta, M., Tesio, L., Arnould, C., Zancan, A., & Thonnard, J. L. (2001). The abilhand questionnaire as a measure of manual ability in chronic stroke patients rasch-based validation and relationship to upper limb impairment. Stroke, 32(7), 1627-1634.

Radajewska, A., Opara, J. A., Kucio, C., Blaszczyszyn, M., Mehlich, K., & Szczygiel, J. (2013). The effects of mirror therapy on arm and hand function in subacute stroke in patients. International Journal of Rehabilitation Research, 36(3), 268-274.

Ramachandran, V. S., & Altschuler, E. L. (2009). The use of visual feedback, in particular mirror visual feedback, in restoring brain function. Brain, awp135.

Ramachandran, V. S., Rogers-Ramachandran, D., & Cobb, S. (1995). Touching the phantom limb. Nature, 377(6549), 489-490.

Reissig, P., Garry, M. I., Summers, J. J., & Hinder, M. R. (2014). Visual feedback-related changes in ipsilateral cortical excitability during unimanual movement: implications for mirror therapy. Neuropsychological rehabilitation, 24(6), 936-957.

Rizzolatti, G., & Craighero, L. (2004). The mirror-neuron system. Annual Review of Neuroscience, 27, 169-192.

Rode, G., Michel, C., Rossetti, Y., Boisson, D., & Vallar, G. (2006). Left size distortion (hyperschematia) after right brain damage. Neurology, 67(10), 1801-1808.

Romano, D., Bottini, G., & Maravita, A. (2013). Perceptual effects of the mirror box training in normal subjects. Restorative neurology and neuroscience, 31(4), 373-386.

Romano, D., Sedda, A., Dell'Aquila, R., Dalla Costa, D., Beretta, G., Maravita, A., & Bottini, G. (2014). Controlling the alien hand through the mirror box. A single case study of alien hand syndrome. Neurocase, 20(3), 307-316.

Rorden, C., & Brett, M. (2000). Stereotaxic display of brain lesions. Behavioural neurology, 12(4), 191-200.

Rosén, B., & Lundborg, G. (2005). Training with a mirror in rehabilitation of the hand. Scandinavian Journal of Plastic and Reconstructive Surgery and Hand Surgery, 39(2), 104-108.

Rossi, S., Hallett, M., Rossini, P. M., Pascual-Leone, A., & Safety of TMS Consensus Group. (2009). Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. Clinical neurophysiology, 120(12), 2008-2039.

Rossini, P. M., Barker, A. T., Berardelli, A., Caramia, M. D., Caruso, G., Cracco, R. Q., ... & De Noordhout, A. M. (1994). Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. Electroencephalography and clinical neurophysiology, 91(2), 79-92.

Rossiter, H. E., Borrelli, M. R., Borchert, R. J., Bradbury, D., & Ward, N. S. (2015). Cortical mechanisms of mirror therapy after stroke. Neurorehabilitation and neural repair, 1545968314554622.

Rothgangel, A. S., Braun, S. M., Beurskens, A. J., Seitz, R. J., & Wade, D. T. (2011). The clinical aspects of mirror therapy in rehabilitation: a systematic review of the literature. International Journal of Rehabilitation Research, 34(1), 1-13.

Sadibolova, R., & Longo, M. R. (2014). Seeing the body produces limb-specific modulation of skin temperature. Biology letters, 10(4), 20140157.

Saleh, S., Adamovich, S. V., & Tunik, E. (2014). Mirrored Feedback in Chronic Stroke Recruitment and Effective Connectivity of Ipsilesional Sensorimotor Networks. Neurorehabilitation and neural repair, 28(4), 344-354.

Saleh, S., Yarossi, M., Manuweera, T., Adamovich, S., & Tunik, E. (2017). Network interactions underlying mirror feedback in stroke: A dynamic causal modeling study. NeuroImage: Clinical, 13, 46-54.

Schenkenberg, T., Bradford, D. C., & Ajax, E. T. (1980). Line bisection and unilateral visual neglect in patients with neurologic impairment. Neurology, 30(5), 509-509.

Schultz, J., Imamizu, H., Kawato, M., & Frith, C. D. (2004). Activation of the human superior temporal gyrus during observation of goal attribution by intentional objects. Journal of cognitive neuroscience, 16(10), 1695-1705.

Selles, R. W., Schreuders, T. A., & Stam, H. J. (2008). Mirror therapy in patients with causalgia (complex regional pain syndrome type II) following peripheral nerve injury: two cases. Journal of rehabilitation medicine, 40(4), 312-314.

Snijders, H. J., Holmes, N. P., & Spence, C. (2007). Direction-dependent integration of vision and proprioception in reaching under the influence of the mirror illusion. Neuropsychologia, 45(3), 496-505.

Soliman, T. M., Buxbaum, L. J., & Jax, S. A. (2016). The mirror illusion's effects on body state estimation. Cognitive Neuropsychology, 33(1-2), 102-111.

Stein, B. E., & Stanford, T. R. (2008). Multisensory integration: current issues from the perspective of the single neuron. Nature Reviews Neuroscience, 9(4), 255-266.

Stevens, J. A., & Stoykov, M. E. P. (2003). Using motor imagery in the rehabilitation of hemiparesis. Archives of physical medicine and rehabilitation, 84(7), 1090-1092.

Sütbeyaz, S., Yavuzer, G., Sezer, N., & Koseoglu, B. F. (2007). Mirror therapy enhances lower-extremity motor recovery and motor functioning after stroke: a randomized controlled trial. Archives of physical medicine and rehabilitation, 88(5), 555-559.

Swinnen, S. P. (2002). Intermanual coordination: from behavioural principles to neural-network interactions. Nature Reviews Neuroscience, 3(5), 348-359.

Swinnen, S., Heuninckx, S., Van Impe, A., Goble, D., Coxon, J., & Wenderoth, N. (2011). Aging and movement control: the neural basis of age-related compensatory recruitment.

Taub, E., & Berman, A. J. (1968). Movement and learning in the absence of sensory feedback. The neuropsychology of spatially oriented behavior, 173-192.

Tesio, L., Granger, C. V., Perucca, L., Franchignoni, F. P., Battaglia, M. A., & Russell, C. F. (2002). The FIM™ instrument in the United States and Italy: a comparative study. American journal of physical medicine & rehabilitation, 81(3), 168-176.

Thieme, H., Mehrholz, J., Pohl, M., Behrens, J., & Dohle, C. (2013). Mirror therapy for improving motor function after stroke. Stroke, 44(1), e1-e2.

Tsakiris, M., Hesse, M. D., Boy, C., Haggard, P., & Fink, G. R. (2007). Neural signatures of body ownership: a sensory network for bodily self-consciousness. Cerebral cortex, 17(10), 2235-2244.

Tsakiris, M., Longo, M. R., & Haggard, P. (2010). Having a body versus moving your body: neural signatures of agency and body-ownership. Neuropsychologia, 48(9), 2740-2749.

Uswatte, G., Taub, E., Morris, D. P. P. T., Light, K. P. P. T., & Thompson, P. A. (2006). The Motor Activity Log-28 assessing daily use of the hemiparetic arm after stroke. Neurology, 67(7), 1189-1194.

van Beers, R. J., Sittig, A. C., & van Der Gon, J. J. D. (1999). Integration of proprioceptive and visual position-information: An experimentally supported model. Journal of neurophysiology, 81(3), 1355-1364.

Voelcker-Rehage, C. (2008). Motor-skill learning in older adults—a review of studies on age-related differences. European Review of Aging and Physical Activity, 5(1), 5.

von Rein, E., Hoff, M., Kaminski, E., Sehm, B., Steele, C. J., Villringer, A., & Ragert, P. (2015). Improving motor performance without training: the effect of combining mirror visual feedback with transcranial direct current stimulation. Journal of neurophysiology, 113(7), 2383-2389.

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, 33(4), 593-603.

Wassermann, E., Epstein, C., & Ziemann, U. (2008). Oxford handbook of transcranial stimulation. Oxford University Press.

Wolpert, D. M., & Ghahramani, Z. (2000). Computational principles of movement neuroscience. nature neuroscience, 3, 1212-1217.

World Medical Association. (1991). Declaration of Helsinki. Law, medicine & health care: a publication of the American Society of Law & Medicine, 19(3-4), 264.

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. Archives of physical medicine and rehabilitation, 94(6), 1023-1030.

Yavuzer, G., Selles, R., Sezer, N., Sütbeyaz, S., Bussmann, J. B., Köseoğlu, F., ... & Stam, H. J. (2008). Mirror therapy improves hand function in subacute stroke: a randomized controlled trial. Archives of physical medicine and rehabilitation, 89(3), 393-398.

Yeldan, I., Huseyınsınoglu, B. E., Akıncı, B., Tarakcı, E., Baybas, S., & Ozdıncler, A. R. (2015). The effects of very early mirror therapy on functional improvement of the upper extremity in acute stroke patients. Journal of physical therapy science, 27(11), 3519.

Yomogida, Y., Sugiura, M., Sassa, Y., Wakusawa, K., Sekiguchi, A., Fukushima, A., ... & Kawashima, R. (2010). The neural basis of agency: an fMRI study. Neuroimage, 50(1), 198-207.