Neurorehabilitation &
Neural Repair

Cortical mechanisms of mirror therapy after stroke

Journal:	*Neurorehabilitation & Neural Repair*
Manuscript ID:	NNR-14-0060.R3
Manuscript Type:	Original Research Articles
Date Submitted by the Author:	n/a
Keyword:	mirror therapy, magnetoencephalography, stroke, beta oscillations, motor cortex

Complete List of Authors: Rossiter, Holly; UCL, Institute of Neurology
Borrelli, Mimi; UCL, Institute of Neurology
Borchert, Robin; UCL, Institute of Neurology
Bradbury, David; UCL, Institute of Neurology
Ward, Nick; UCL, Institute of Neurology

Abstract: Background and Objective: Mirror therapy is a new form of stroke rehabilitation that uses the mirror reflection of the unaffected hand in place of the affected hand to augment movement training. The mechanism of mirror therapy is not known but is thought to involve changes in cerebral organisation. We used magnetoencephalography (MEG) to measure changes in cortical activity during mirror training after stroke. In particular, we examined movement-related changes in the power of cortical oscillations in the beta (15-30Hz) frequency range, known to be involved in movement.
Methods: Ten stroke patients with upper limb paresis and thirteen healthy controls were recorded using MEG whilst performing bimanual hand movements in two different conditions. In one, subjects looked directly at their affected hand (or dominant hand in controls) and in the other they looked at a mirror reflection of their unaffected hand in place of their affected hand. The movement-related beta desynchronization was calculated in both primary motor cortices.
Results: Movement-related beta desynchronization was symmetrical during bilateral movement and unaltered by the mirror condition in controls. In the patients, movement-related beta desynchronization was generally smaller than in controls, but greater in contralesional compared to ipsilesional motor cortex. This initial asymmetry in movement-related beta desynchronization between hemispheres was made more symmetrical by the presence of the mirror.
Conclusions: Mirror therapy could potentially aid stroke rehabilitation by normalising an asymmetrical pattern of movement-related beta desynchronization in primary motor cortices during bilateral movement.

Full title: Cortical mechanisms of mirror therapy after stroke

Authors: Holly E Rossiter PhD[1], Mimi R Borrelli MSc[1], Robin Borchert[1], David Bradbury[2], Nick Ward MD[1]

Affiliations: 1. Sobell Department of Motor Neuroscience and Movement Disorders, UCL Institute of Neurology, 33 Queen Square, London, WC1N 3BG, UK. 2. Wellcome Trust Centre for Neuroimaging, UCL Institute of Neurology, 12 Queen Square, London

Corresponding author: Dr Holly Rossiter, address: UCL Institute of Neurology, 33 Queen Square, London, WC1N 3BG, UK, email: h.rossiter@ucl.ac.uk tel: 020334488775

Word count: 3841

Figures: 5

Tables: 2

Abstract:

Background and Objective: Mirror therapy is a new form of stroke rehabilitation that uses the mirror reflection of the unaffected hand in place of the affected hand to augment movement training. The mechanism of mirror therapy is not known but is thought to involve changes in cerebral organisation. We used magnetoencephalography (MEG) to measure changes in cortical activity during mirror training after stroke. In particular, we examined movement-related changes in the power of cortical oscillations in the beta (15-30Hz) frequency range, known to be involved in movement.

Methods: Ten stroke patients with upper limb paresis and thirteen healthy controls were recorded using MEG whilst performing bimanual hand movements in two different conditions. In one, subjects looked directly at their affected hand (or dominant hand in controls) and in the other they looked at a mirror reflection of their unaffected hand in place of their affected hand. The movement-related beta desynchronization was calculated in both primary motor cortices.

Results: Movement-related beta desynchronization was symmetrical during bilateral movement and unaltered by the mirror condition in controls. In the patients, movement-related beta desynchronization was generally smaller than in controls, but greater in contralesional compared to ipsilesional motor cortex. This initial asymmetry in movement-related beta desynchronization between hemispheres was made more symmetrical by the presence of the mirror.

Conclusions: Mirror therapy could potentially aid stroke rehabilitation by normalising an asymmetrical pattern of movement-related beta desynchronization in primary motor cortices during bilateral movement.

Key words: mirror therapy, MEG, beta oscillations, stroke, motor cortex

Introduction:

Mirror Therapy (MT) is emerging as an adjunct to physical therapy in the treatment of upper limb impairment after stroke[1,2]. MT involves asking patients to attempt synchronous bilateral hand movements whilst observing the mirror reflection of their unaffected limb in the position of their affected limb. MT has been found to have a significant positive effect on motor function in stroke patients[3] but there is little understanding as to its mechanism of action.

Studies have been performed using mirror visual feedbackin healthy subjects using both transcranial magnetic stimulation (TMS) and fMRI. TMS studies using MT found that it increased excitability in primary motor cortex (M1) in both hemispheres[4–6], but this has not been tested in stroke patients.

A number of fMRI studies have investigated using mirror visual feedback in healthy subjects using unimanual movements and have found areas that differ in their activation between mirror and no mirror conditions[7–9], Most of these were areas outside the motor network, although Hamzei et al did find changes in premotor areas during MT.

The few fMRI studies to explore MT in stroke patients have used protocols that do not match what is performed in a clinical setting (i.e. bilateral movement with mirror reflection of unaffected hand in place of affected hand)[10,11]. Michielsen et al[12] recorded fMRI whilst performing MT with bilateral movement but found no main effect of mirror. They performed another study exploring the effect of 6 weeks training with MT and observed a shift of brain activation evoked by movement of the affected hand towards the lesioned hemisphere[13].

Based on the studies described above, we hypothesised that MT acts by simultaneously enhancing the potential for neuroplastic change in surviving motor regions and networks, and then taking advantage of this enhancement with concurrent repeated movement. In this study, we therefore asked both healthy subjects and stroke patients to perform movements using mirror visual feedback whilst measuring brain activity using magnetoencephalography (MEG). MEG is a brain imaging technique that directly measures cortical neural activity at different frequencies (termed oscillations) and unlike blood oxygenation level dependent signal used by fMRI, it is unaffected by neurovascular uncoupling. MEG can detect the oscillatory signals generated predominantly by changes in the post-synaptic fields of pyramidal cells[14]. Pyramidal cells are reciprocally connected to GABAergic interneurons and so changes in oscillatory signals are dependent on the balance between inhibition and excitation within these microcircuits[15]. As such, it is possible that oscillatory changes could tell us something about the potential for both local and network plasticity, however there is much more to be understood about this relationship[16]. Oscillations in the beta frequency band (15-30Hz) are known to be important in movement. In M1, they are present at rest and are suppressed during movement (movement-related beta desynchronization - MRBD)[17]. The power of resting beta oscillations and MRBD are both enhanced by benzodiazepines ($GABA_A$-agonists), suggesting they reflect the degree of GABAergic inhibition in M1[18,19]. MEG has been used to examine changes in beta oscillations during mirror therapy and concurrent median nerve stimulation in healthy subjects[20,21] but MT has not been assessed in stroke patients.

In this study, we were interested in the effect of MT on beta oscillations during movement. We were keen to assess MRBD during attempted movement in these patients (i.e. practice) as motor practice is what will drive behavioural improvement.

MEG studies of bilateral hand movement performed by healthy volunteers have observed that beta power in both primary motor cortices decreases during movement[22,23] and here given that our paradigm involves synchronous bilateral hand movements, we expected to see the same. Following stroke however, there is thought to be a disruption in the relationship between motor cortices in each hemisphere[24,25]. Specifically, just prior to movement, there is an increased level of inhibition from contralesional to ipsilesional M1[25]. Although this finding was observed during unilateral movement, we hypothesise that a similar reduction in contralesional to ipsilesional M1 interhemispheric inhibition during bilateral hand movement in our patient group might occur and be reflected in altered MRBD. If this were the case, we would not see such a symmetrical change in MRBD in our patients compared to controls and in particular we would expect MRBD to be enhanced in M1 of the lesioned hemisphere, reflecting increased overall levels of task related inhibition in this cortical region. The key question is then how does adding in MT to bilateral hand movement alter this and it is tempting to speculate that MT may 'normalise' this imbalance. In this study, we therefore hypothesised that i) in healthy controls, during bilateral movement, there would be a symmetrical decrease in beta band power in both primary motor cortices, (ii) following stroke, MRBD during bilateral movement would not be symmetrical, but would be greater (i.e. a larger decrease) in the lesioned hemisphere reflecting increased inhibition, and (iii) MT would normalise this imbalance in the patient group. Given that our protocol is identical to that implemented in clinical practice, we suggest that this differential interaction between

MT and hemisphere in stroke patients and controls might represent its mechanism of action.

Materials and Methods:

Subjects:

Ten stroke patients (mean age 56±12yrs, range 30-71 yrs old, 3 female, 6 dominant-hand affected, 1 left-handed) and thirteen control participants were recruited to this study (mean age 45±15yrs, age range 22-67 yrs old, 9 female, 2 left-handed). All patients suffered from first-ever stroke and weakness of at least wrist and finger extensors and hand interossei. Patients were not suffering from any other neurological disorder. Full written consent was obtained from all subjects in accordance with the Declaration of Helsinki. The study was approved by the Joint Ethics Committee of the Institute of Neurology, UCL and National Hospital for Neurology and Neurosurgery, UCL Hospitals NHS Foundation Trust, London.

Behavioural testing:

All stroke patients were scored on the Nine Hole Peg Test (NHPT), Action Research Arm Test (ARAT), Box and Blocks test and Grip strength. All scores were given in terms of measures for the affected hand as a percentage of the unaffected hand. A principal component analysis (PCA) was performed on all behavioural scores in order to account for ceiling and floor effects in these measures and to create a single motor impairment score (a lower PCA score corresponding to greater impairment).

Motor task:

Subjects made alternating flexion and extension movements synchronously with both hands in response to an auditory cue every 5 seconds. There were 2 cues,

one high pitch followed by one at a lower pitch (this alternated between the two across the recording) and the participant was asked to open their hand (extension) on the high pitch cue and close their hand into a fist (flexion) on the low pitch cue. The participants were instructed to relax their hands after having made each movement. The movements were performed in two different conditions. Patients looked either towards their affected hand (1) directly (*no mirror)*, or (2) as a reflection of their unaffected hand (*mirror*) (Figure 1). Controls looked either towards their dominant hand (1) directly (no mirror), or (2) as a reflection of their non-dominant hand (mirror), but gaze was always in the same direction in all subjects. The other hand was covered up in order not to distract from the observed/mirrored hand. The order of conditions was randomised. There were 60 movements in each block, 30 flexion and 30 extension, each block lasting 5 minutes.

Data analysis:

MEG signals were measured continuously during the task using a whole-head CTF Omega 275 MEG system (CTF, Vancouver, Canada).

Head localization was monitored continuously during the MEG recordings in order to check for excessive movement. The MEG data were pre-processed offline using SPM8 (Wellcome Trust Centre for Neuroimaging, www.fil.ion.ucl.ac.uk/spm)[26]. EMG was recorded from the forearm muscles as part of the MEG dataset. Data were down-sampled to 300Hz and were filtered from 5-100Hz. Data were epoched from -2.5s to +2.5s where time 0 indicated onset of the auditory cue. The different pitch auditory cues had different markers and therefore at this stage, the data were split into flexion and extension movements and analysed separately. Trials with large eye blinks or other artefacts were excluded.

Coregistration to the Montreal Neurological Institute (MNI) coordinates was based on three fiducial points: nasion and left and right preauricular points. We used a single-shell (rather than single sphere) model fit, based on spherical harmonic series[27], to the inner skull surface of the inverse normalized SPM template to more precisely characterize the MEG forward model.

Oscillatory changes in the beta band between rest and grip were localised using the Linearly Constrained Maximal Variance (LCMV) beamformer[28,29] as part of the SPM8 software. The beamforming method is based on the linear projection of sensor data using a spatial filter computed from the lead field of the source of interest and the data covariance[30]. We computed the data covariance matrix using two time windows (passive and active). The passive time window was taken from -2s to -1s with 0 as the onset of the auditory cue. The active time window was from 0.35-1.35s following the auditory cue onset, this was guided by the EMG across participants. The frequency band used for the beamformer was 15-30Hz. We made volumetric t-statistic images per subject using a grid spacing of 10mm. At each location, the source orientation was taken to be in the direction yielding maximal signal variance[31]. From these t-statistic images, we extracted the source signal from the location of peak change in beta power (15-30Hz) within the primary motor cortices both contralateral (CM1) and ipsilateral (IM1) to the observed hand. Morlet-wavelet time-frequency analysis was used to explore the changes in beta across a trial from these locations. The spectrograms were rescaled in order to show percentage change from baseline (-2.5s to 0s) and averaged across trials. The mean movement-related percentage beta desynchronization (MRBD) (15-30Hz) was then extracted from the 2s movement period for primary motor cortex contralateral to the

hand being observed (CM1) and ipsilateral to the hand being observed (IM1) for each participant.

A mixed-effects ANOVA was performed on MRBD comprising 2 within-subjects factors (mirror, hemisphere) and 1 between-subjects factor (group). This was done for both flexion and extension movements separately. A correlation was performed on MRBD and motor impairment.

An asymmetry index was also calculated from the MRBD values by taking (CM1_MRBD-IM1_MRBD)/(CM1_MRBD+IM1_MRBD). A mixed-effects ANOVA was performed on this index with the within-subject factor of mirror (no mirror vs mirror conditions) and the between subjects factor of group (patients vs controls).

T-tests were then performed as post-hoc analysis to interpret further the results of the ANOVA.

Results:

Behavioural scores were as follows (all scores given with affected hand as percentage of unaffected hand); NHPT mean=25±23%, box and block mean=42±29%, grip strength mean=51±30%, ARAT mean=70±31%. The raw behavioural scores are shown in Table 1.

Figure 2 shows group average time-frequency spectrograms for each condition, beta MRBD is clear in all conditions as a blue patch following the auditory cue to move at 0s. An ANOVA of MRBD focussing on flexion movements revealed a significant effect of group ($F=4.43$, $p=0.048$) and of hemisphere ($F=5.56$, $p=0.03$), a significant interaction between hemisphere and mirror condition ($F=8.93$, $p=0.007$) and also a significant 3-way interaction between hemisphere*mirror*group ($F=7.02$, $p=0.015$). Table II shows all the results of this ANOVA. Performing post-hoc t-tests

on these data, we find that in the no mirror condition of the contralateral M1, there is a significantly smaller MRBD in patients than controls (p=0.01). There is also a significantly smaller MRBD in CM1 as compared to IM1 in the no mirror condition in the patient group (p=0.005).

An ANOVA of the asymmetry index for flexion movements indicated that there was a very significant effect of condition (F=9.09, p=0.007). The effect of group did not quite reach significance (F=3.89, p=0.063), however there was a significant interaction between condition and group (F=7.20, p=0.014). Performing post-hoc t-tests on these data, we find that there is a significant difference in the asymmetry index between patients and controls in the no mirror condition (p=0.009) and that there is a significant difference between the mirror vs no mirror condition in patients (p=0.02). So the asymmetry index was different between patients and controls in the control no mirror condition, and in the patient group, the asymmetry index was altered by the addition of the mirror. This can be seen in the box plot in Figure 4.

Figure 3 shows a summary of MRBD across all these conditions. MRBD was smaller in patients compared to controls, especially in CM1 (M1 of the lesioned hemisphere in patients). In the control group, there was no effect of mirror condition on MRBD in either hemisphere. However in the patient group, MRBD was greater in IM1 (M1 of the intact hemisphere) than CM1 (M1 of the lesioned hemisphere). Furthermore, the presence of the mirror reduced MRBD in IM1 but increased it in CM1. In other words, MRBD became less asymmetric when the mirror was introduced.

Baseline beta values were extracted and no significant differences were found in beta values at rest between patients and controls. We also explored mu oscillations (7-14Hz) and saw a desynchronization in this frequency during

movement. When an ANOVA was performed on these data using the same factors as in the beta band, a significant difference was found between patients and controls but no differences were found due to hemisphere or mirror condition. MRBD in both CM1 and IM1 was not found to correlate with motor impairment in any of the conditions. With regards to extension movements, there were no significant differences in any of the comparisons including the ANOVAs.

Discussion:

Our results show that the effect of MT on motor cortex function in each hemisphere during bilateral hand movements was different in stroke patients compared to healthy controls. In controls, MRBD was the same in each hemisphere and unaltered by MT. In the patient group however, an imbalance in MRBD between hemispheres during bilateral hand movement was made more symmetrical by MT (Figure 3). It is interesting to consider what this tells us about how MT might work. One possibility is that relative change in MRBD in each hemisphere represents a 'normalisation' of the balance in motor cortex activity in ipsilesional and contralesional hemispheres.

The balance of activity between ipsilesional and contralesional motor cortices after stroke has been investigated in previous work. For example, excessive task-related inhibition from contralesional to ipsilesional motor cortex has been observed following stroke[25]. Indeed, a number of non-invasive brain stimulation studies have attempted to reverse this imbalance and so facilitate recovery after stroke by reducing excitability in contralesional motor cortex. This finding was obtained during unimanual affected hand movement, and so may not be relevant for interpreting our findings during bilateral movement. In our study, MRBD was equal in both hemispheres during bilateral movements in healthy controls but not in patients.

MRBD in the ipsilateral (contralesional) M1 was larger than in contralateral (ipsilesional) M1 pointing to a significant imbalance of activity between hemispheres before MT was introduced. With the mirror in place, this asymmetry of MRBD became less marked in patients, but was unaltered in healthy controls. This 'normalisation' of MRBD balance between the hemispheres could represent a readjustment of a stroke-related excitatory-inhibitory imbalance between the two motor cortices, creating a pattern of activity more similar to healthy controls.

It is tempting to speculate whether the effect of MT is related to differential effects on movement-related intracortical GABAergic inhibition in each hemisphere. From what we know about the relationship between beta power and intracortical GABAergic inhibition[18,19], our results suggest that in stroke patients, MT reduces GABAergic inhibition in contralesional rather than M1 of the lesioned hemisphere. A reduction in inhibitory mechanisms is thought to be an important factor in enhancing long-term potentiation and therefore experience-dependent plasticity[32,33]. This might suggest that in the patient group, we see an increase in the potential for experience-dependent plasticity during the mirror condition in the ipsilateral (contralesional) M1 rather than the contralateral (ipsilesional) M1. Although this might seem surprising at first, there is a body of literature indicating that the activations seen in contralesional motor cortex following stroke can aid functional recovery[34]. Nevertheless, it seems more likely that MT involving bilateral movement works through altering the balance of activity between hemispheres, rather than on one hemisphere or the other. Although our results don't specifically measure interhemispheric influences, they do point towards alterations in hemispheric balance.

Generally, the studies that have looked at the mirror illusion in fMRI have not performed mirror therapy as it would be done clinically (i.e. bilateral movement with a

mirror in place of the affected hand), they have mainly used unilateral movements[11,12] and some have used virtual reality videos of hand movements in place of a mirror setup[10]. In the present study, we were careful to use a protocol that reflected a clinical MT protocol and so we feel more able to relate our results to a mechanism of the clinically applied MT.

In this study, we found no correlation between MRBD and motor impairment. This is in keeping with the findings of Saleh et al who found no correlation between degree of fMRI activation and hand function in stroke patients during the mirror condition[10]. This suggests that if our findings reflect the mechanism of action of MT, then it would work as effectively in patients with differing levels of impairment. We studied patients with a wide range of impairment and also a varying amount of time after stroke. This wide range of time after stroke may have led to greater variability in our results as the excitatory-inhibitory balance may vary at different stages following stroke. Having said that, all but one of our patients were at a chronic stage (see Table 1) and so are likely to have plateaued in terms of their recovery. We also performed a correlation between months after stroke and MRBD amplitude in the patient group and this was not found to be significant ($p>0.24$). It would be of interest to look longitudinally at these changes within patients and see how these results changed over time. There was also a mixture of dominant and non-dominant hemisphere affected which may have added to the variability across patients. The fact that we see a change in the MRBD despite these sources of variability might point to the general applicability of MT, but the effects of these sources of variability need to be explored further in larger groups to be certain.

Anecdotally, most patients were very engaged in the therapy and were often fascinated by the illusion of seeing the reflection of their unaffected hand. It would be

interesting to investigate the effect of the belief in the illusion on the results we describe here. It is possible that stronger effects are seen in those with a more convincing illusion.

No significant differences were found between MT and non-MT conditions during extension movements. Finger extension was generally more difficult than flexion for our stroke patients. It might be that some degree of illusion is required for the effect and this is more difficult when the discrepancy between real and mirrored movement is too great. This could obviously have a negative effect on the applicability of MT clinically.

There was some difference in the average age between patients and controls but having correlated all MRBD measures with age in the control group, there was no significant correlation and so we do not believe that this would have unduly affected our results.

Structural information on the site of stroke was not available for all patients and therefore it is not possible to make any statement about how this may have affected MRBD in our study. It would be interesting to investigate how MRBD results may be affected by lesion location/volume in a future study.

In this study, we chose to use observation of the dominant hand in our healthy subjects as a control comparison to observation of the affected hand. In a previous dataset, we have directly compared non-dominant and dominant hand measures of MRBD in a control group whilst performing unimanual movements and found no significant differences (under review) so we were comfortable using the dominant hand as a comparison. Also, in this study participants were performing bilateral movement and it was only the focus of their gaze that was towards the dominant

hand. We would not expect this to make a significant difference as the controls were receiving the same visual feedback in the mirror and no-mirror condition as they could complete the movement correctly. The control group provided a baseline level of MRBD as a comparison for the patients and the direction of their gaze is unlikely to have influenced the results in this case.

As the patients were impaired, the movement amplitude and accuracy is likely to have differed between patients and controls. Whilst this is a possible limitation, it is unlikely to explain the difference due to the addition of the mirror in the patient group.

In summary, this study is the first to measure cortical activity directly during MT using MEG and to examine the differential effects in controls and stroke patients. Our results suggest a rebalancing of MRBD between hemispheres in stroke patients. Alterations in beta oscillations have been linked to changes in intracortical GABA-ergic inhibitory function and it is interesting to speculate on whether our results reflect a MT enhanced potential for experience dependent plasticity within motor networks. In future studies, it would be of interest to determine whether the effects of MT on cortical physiology that we have observed here are necessary (i.e. biomarkers) for a beneficial effect on motor function.

Acknowledgements:

This research was supported by the European Commission under the 7th Framework Program-HEALTH-Collaborative Project Plasticise (Contract no. 223524) www.plasticise.eu (Dr Rossiter), and The Wellcome Trust (Mr Robin Borchert, Dr Nick Ward).

References:

1. Ezendam D, Bongers RM, Jannink MJA. Systematic review of the effectiveness of mirror therapy in upper extremity function. *Disabil Rehabil*. 2009;31(26):2135-2149. doi:10.3109/09638280902887768.

2. Rothgangel AS, Braun SM, Beurskens AJ, Seitz RJ, Wade DT. The clinical aspects of mirror therapy in rehabilitation: a systematic review of the literature. *Int J Rehabil Res Int Z Für Rehabil Rev Int Rech Réadapt*. 2011;34(1):1-13. doi:10.1097/MRR.0b013e3283441e98.

3. Thieme H, Mehrholz J, Pohl M, Behrens J, Dohle C. Mirror therapy for improving motor function after stroke. *Stroke J Cereb Circ*. 2013;44(1):e1-2.

4. Garry MI, Loftus A, Summers JJ. Mirror, mirror on the wall: viewing a mirror reflection of unilateral hand movements facilitates ipsilateral M1 excitability. *Exp Brain Res Exp Hirnforsch Expérimentation Cérébrale*. 2005;163(1):118-122. doi:10.1007/s00221-005-2226-9.

5. Fukumura K, Sugawara K, Tanabe S, Ushiba J, Tomita Y. Influence of mirror therapy on human motor cortex. *Int J Neurosci*. 2007;117(7):1039-1048. doi:10.1080/00207450600936841.

6. Nojima I, Mima T, Koganemaru S, Thabit MN, Fukuyama H, Kawamata T. Human motor plasticity induced by mirror visual feedback. *J Neurosci Off J Soc Neurosci*. 2012;32(4):1293-1300. doi:10.1523/JNEUROSCI.5364-11.2012.

7. Matthys K, Smits M, Van der Geest JN, et al. Mirror-induced visual illusion of hand movements: a functional magnetic resonance imaging study. *Arch Phys Med Rehabil*. 2009;90(4):675-681. doi:10.1016/j.apmr.2008.09.571.

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

9. Fritzsch C, Wang J, Dos Santos LF, Mauritz K-H, Brunetti M, Dohle C. Different effects of the mirror illusion on motor and somatosensory processing. *Restor Neurol Neurosci*. 2013. doi:10.3233/RNN-130343.

10. Saleh S, Adamovich SV, Tunik E. Mirrored Feedback in Chronic Stroke: Recruitment and Effective Connectivity of Ipsilesional Sensorimotor Networks. *Neurorehabil Neural Repair*. 2013. doi:10.1177/1545968313513074.

11. Wang J, Fritzsch C, Bernarding J, et al. Cerebral activation evoked by the mirror illusion of the hand in stroke patients compared to normal subjects. *NeuroRehabilitation*. 2013. doi:10.3233/NRE-130999.

12. Michielsen ME, Smits M, Ribbers GM, et al. The neuronal correlates of mirror therapy: an fMRI study on mirror induced visual illusions in patients with stroke. *J Neurol Neurosurg Psychiatry*. 2011;82(4):393-398. doi:10.1136/jnnp.2009.194134.

13. Michielsen ME, Selles RW, van der Geest JN, et al. Motor recovery and cortical reorganization after mirror therapy in chronic stroke patients: a phase II randomized controlled trial. *Neurorehabil Neural Repair*. 2011;25(3):223-233. doi:10.1177/1545968310385127.

14. Murakami S, Okada Y. Contributions of principal neocortical neurons to magnetoencephalography and electroencephalography signals. *J Physiol*. 2006;575(Pt 3):925-936. doi:10.1113/jphysiol.2006.105379.

15. Yamawaki N, Stanford IM, Hall SD, Woodhall GL. Pharmacologically induced and stimulus evoked rhythmic neuronal oscillatory activity in the primary motor cortex in vitro. *Neuroscience*. 2008;151(2):386-395. doi:10.1016/j.neuroscience.2007.10.021.

16. Traub RD, Bibbig A, LeBeau FEN, Buhl EH, Whittington MA. Cellular mechanisms of neuronal population oscillations in the hippocampus in vitro. *Annu Rev Neurosci*. 2004;27:247-278. doi:10.1146/annurev.neuro.27.070203.144303.

17. Pfurtscheller G, Lopes da Silva FH. Event-related EEG/MEG synchronization and desynchronization: basic principles. *Clin Neurophysiol*. 1999;110:1842-57.

18. Hall SD, Stanford IM, Yamawaki N, et al. The role of GABAergic modulation in motor function related neuronal network activity. *NeuroImage*. 2011;56(3):1506-1510. doi:10.1016/j.neuroimage.2011.02.025.

19. Muthukumaraswamy SD, Myers JFM, Wilson SJ, et al. The effects of elevated endogenous GABA levels on movement-related network oscillations. *NeuroImage*. 2012;66C:36-41. doi:10.1016/j.neuroimage.2012.10.054.

20. Tominaga W, Matsubayashi J, Deguchi Y, et al. A mirror reflection of a hand modulates stimulus-induced 20-Hz activity. *NeuroImage*. 2009;46(2):500-504. doi:10.1016/j.neuroimage.2009.02.021.

21. Tominaga W, Matsubayashi J, Furuya M, et al. Asymmetric Activation of the Primary Motor Cortex during Observation of a Mirror Reflection of a Hand. *PLoS ONE*. 2011;6(11):e28226. doi:10.1371/journal.pone.0028226.

22. Gross J, Pollok B, Dirks M, Timmermann L, Butz M, Schnitzler A. Task-dependent oscillations during unimanual and bimanual movements in the human primary motor cortex and SMA studied with magnetoencephalography. *NeuroImage*. 2005;26(1):91-98. doi:10.1016/j.neuroimage.2005.01.025.

23. Formaggio E, Storti SF, Boscolo Galazzo I, et al. Modulation of event-related desynchronization in robot-assisted hand performance: brain oscillatory changes in active, passive and imagined movements. *J Neuroengineering Rehabil*. 2013;10:24. doi:10.1186/1743-0003-10-24.

24. Ward NS, Cohen LG. Mechanisms underlying recovery of motor function after stroke. *Arch Neurol*. 2004;61(12):1844-1848. doi:10.1001/archneur.61.12.1844.

25. Murase N, Duque J, Mazzocchio R, Cohen LG. Influence of interhemispheric interactions on motor function in chronic stroke. *Ann Neurol*. 2004;55(3):400-409. doi:10.1002/ana.10848.

26. Litvak V, Mattout J, Kiebel S, et al. EEG and MEG data analysis in SPM8. *Comput Intell Neurosci*. 2011;2011:852961. doi:10.1155/2011/852961.

27. Nolte G. The magnetic lead field theorem in the quasi-static approximation and its use for magnetoencephalography forward calculation in realistic volume conductors. *Phys Med Biol*. 2003;48(22):3637-3652.

28. Hillebrand A, Barnes GR. Beamformer analysis of MEG data. *Int Rev Neurobiol*. 2005;68:149-171. doi:10.1016/S0074-7742(05)68006-3.

29. Vrba J, Robinson SE. Signal processing in magnetoencephalography. *Methods*. 2001;25:249-71.

30. Van Veen BD, van Drongelen W, Yuchtman M, Suzuki A. Localization of brain electrical activity via linearly constrained minimum variance spatial filtering. *IEEE Trans Biomed Eng*. 1997;44(9):867-880. doi:10.1109/10.623056.

31. Sekihara K, Nagarajan SS, Poeppel D, Marantz A. Asymptotic SNR of scalar and vector minimum-variance beamformers for neuromagnetic source reconstruction. *IEEE Trans Biomed Eng*. 2004;51(10):1726-1734. doi:10.1109/TBME.2004.827926.

32. Hensch TK. Critical period plasticity in local cortical circuits. *Nat Rev Neurosci*. 2005;6(11):877-888. doi:10.1038/nrn1787.

33. Benali A, Weiler E, Benali Y, Dinse HR, Eysel UT. Excitation and inhibition jointly regulate cortical reorganization in adult rats. *J Neurosci Off J Soc Neurosci*. 2008;28(47):12284-12293. doi:10.1523/JNEUROSCI.1952-08.2008.

34. Rossiter HE, Eaves C, Davis E, et al. Changes in the location of cortico-muscular coherence following stroke. *NeuroImage Clin*. 2013;2:50-55. doi:10.1016/j.nicl.2012.11.002.

Table I: Demographic information and raw behavioural scores for each patient's affected (Aff) and unaffected (Un) hand on the 4 motor tasks performed.

ID	Age	Affected side	Location of lesion	Time after stroke (in months)	NHPT (pegs per sec)	Box and block (boxes per min)	Grip strength (lb)	ARAT

					Aff	Un	Aff	Un	Aff	Un	Aff	Un
1	45	Dominant	anterior MCA	52	0	0.67	10	56	19	88	27	57
2	54	Non-dominant	posterior MCA	10	0	0.59	0	52	8	63	0	57
3	60	Non-dominant	inferior MCA	35	0	0.75	0	69	7	68	2	57
4	71	Dominant	lacunar/subcortical	91	0.01	0.68	7	61	9	49	30	57
5	64	Dominant	anterior MCA	79	0.04	0.52	17	52	54	88	54	57
6	67	Non-dominant	inferior MCA	92	0.09	0.59	22	34	45	66	50	57
7	65	Non-dominant	lacunar/subcortical	1	0.18	0.69	34	54	14	53	55	57
8	52	Dominant	inferior MCA	43	0.19	0.57	35	51	64	88	57	57
9	54	Dominant	lacunar/subcortical	114	0.29	0.58	40	52	51	54	56	57
10	30	Dominant	anterior MCA	35	0.5	0.78	30	45	42	55	57	57

Table II: Results of mixed-effects ANOVA on flexion movements

Factors	F	P
Hemisphere	5.562	0.029
Hemisphere*Group	4.171	0.055

Mirror	0.065	0.802
Mirror*Group	0.630	0.437
Hemisphere*Mirror	8.925	0.007
Hemisphere*Mirror*Group	7.024	0.015
Group	4.425	0.048

Figure legends:

Figure 1: Illustration of experimental conditions. The arrow shows the direction of gaze towards the observed/mirrored hand.

Figure 2: Group averaged spectrograms of each condition with controls on the top row and stroke patients on the bottom row. 0s is the time at which participants heard the auditory cue to move. Whilst movements were made bimanually, participants focussed on one hand (affected hand in patients, dominant hand in controls). Spectrograms are taken from primary motor cortex both contralateral (CM1) and ipsilateral (IM1) to the hand being observed. The same bimanual movements are made in all conditions. The direction of gaze is the same in all conditions. The difference between the two experimental conditions is the presence or absence of the mirror. In other words, in one condition, participants looked directly at their hand (no mirror) and in the other condition, the participants looked at a mirror reflection of the opposite hand (non-dominant in controls, unaffected in patients) (mirror). The colour axis represents percentage change in power compared to baseline.

Figure 3: Movement related beta desynchronization (MRBD) during bilateral hand movement. This figure shows percentage MRBD on the y axis and hemisphere on the x axis during flexion movements. CM1 and IM1 are the primary motor cortices

contralateral and ipsilateral to the hand being observed respectively. As the patient group are observing their affected hand this means that CM1 is in fact M1 of the lesioned hemisphere and IM1 is M1 of the intact hemisphere. The control group are represented by squares and the patient group by circles. The no mirror condition is labelled in grey and the mirror condition is labelled in black. Error bars indicate standard error of the mean.

Figure 4: Box-plot of asymmetry index for patients and controls in both no mirror and mirror conditions.

Figure 5: Location of each patient's lesion shown as axial sections on their structural MRIs.

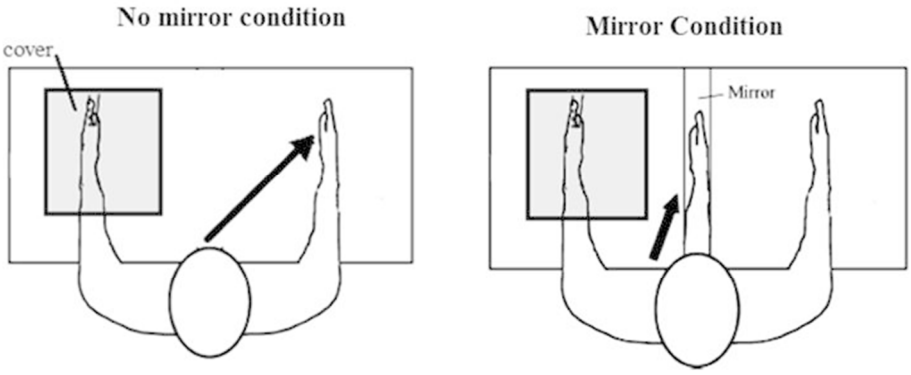
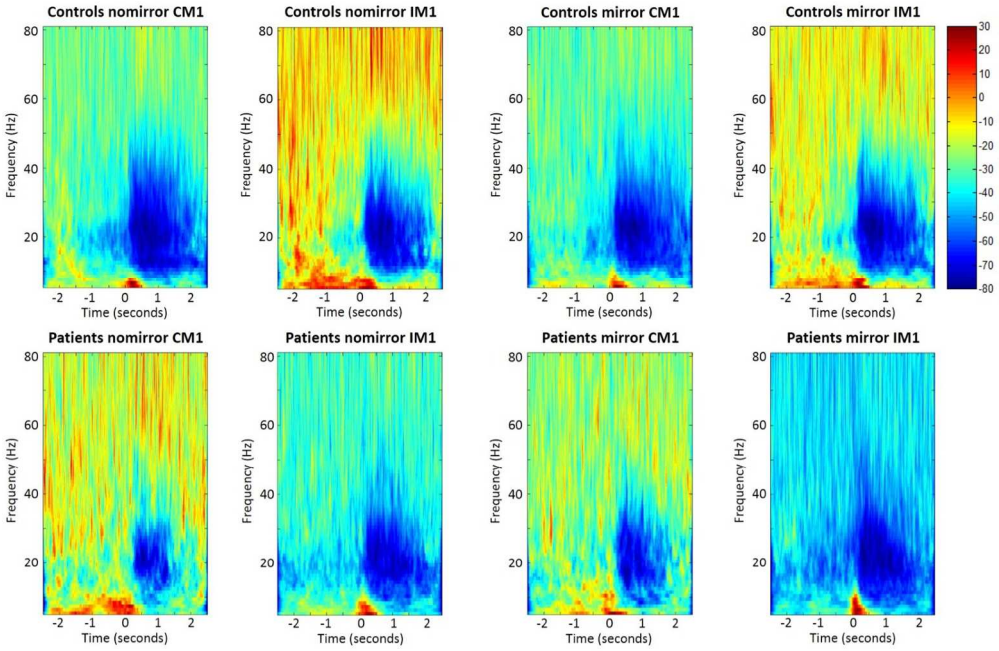
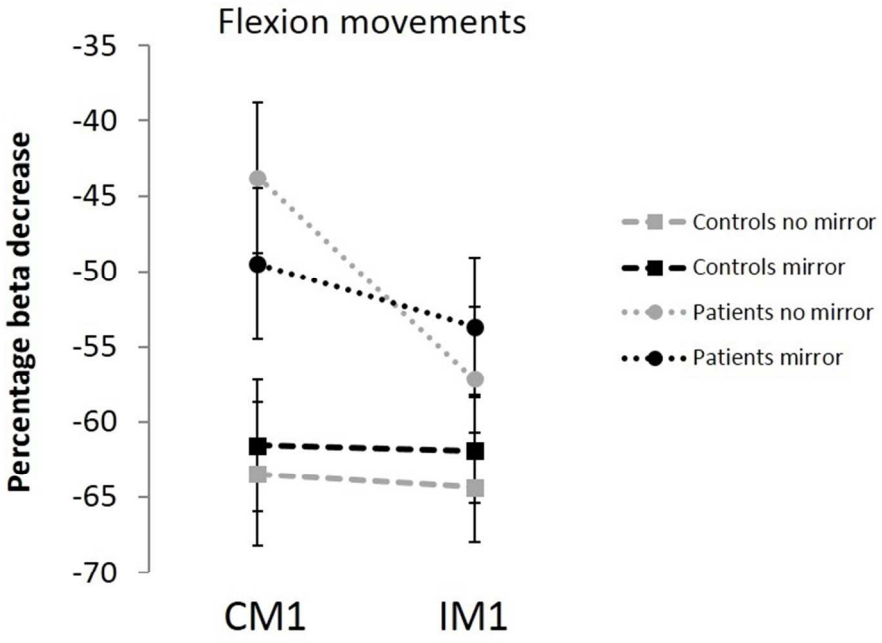


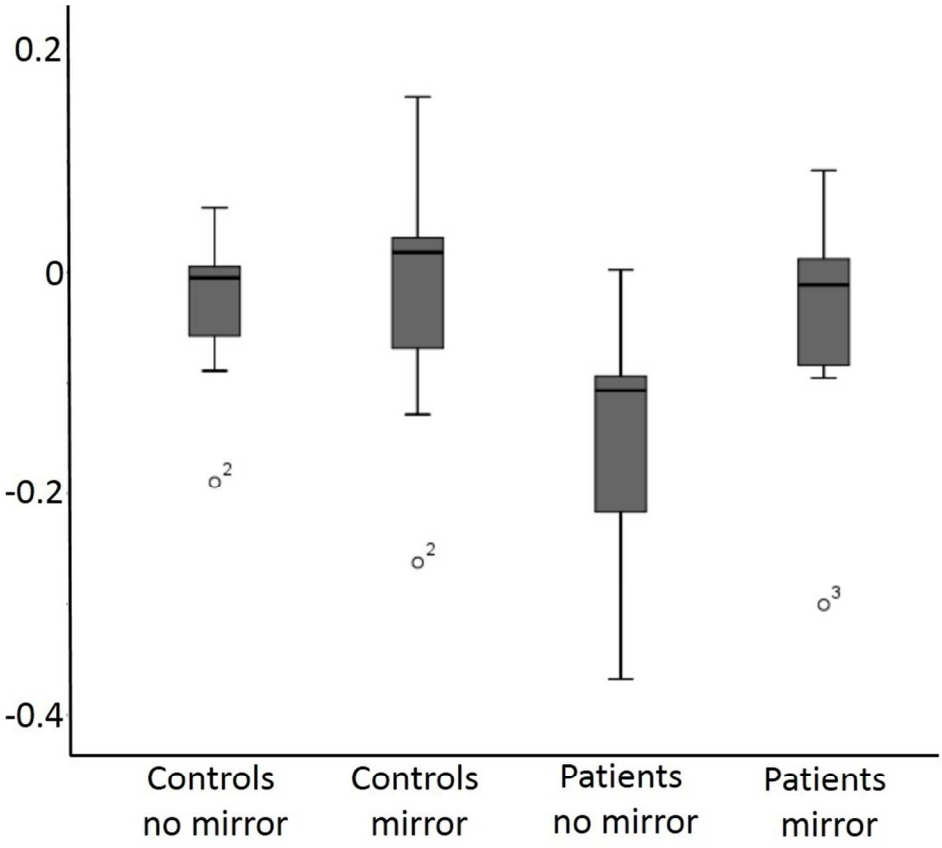
Illustration of experimental conditions. The arrow shows the direction of gaze towards the observed/mirrored hand.
134x55mm (150 x 150 DPI)



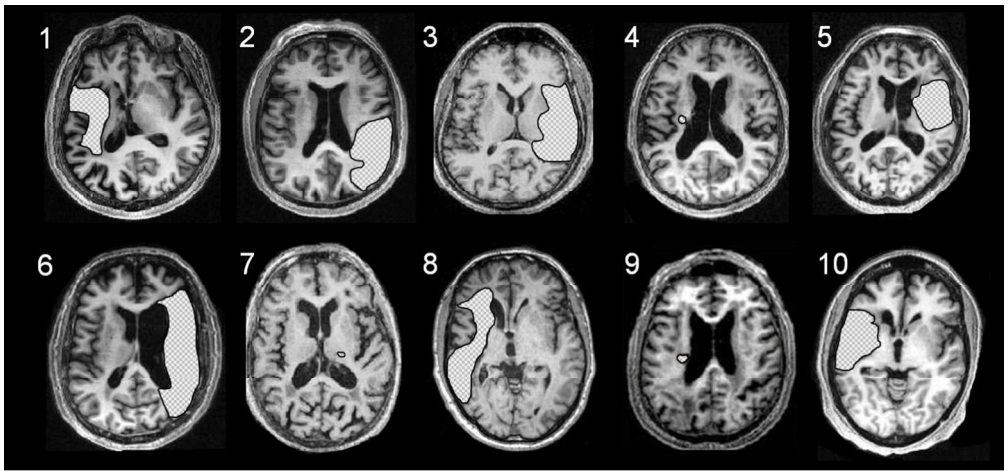
284x187mm (150 x 150 DPI)



144x98mm (150 x 150 DPI)



167x145mm (150 x 150 DPI)



254x117mm (149 x 149 DPI)

Response to Reviewer 2's comments:

Comments to the Author
In this second revision, the authors have made progress in addressing the prior comments, but the revision still falls short in some places. In what I hope will be the final review I write on this manuscript, I try to be as explicit as possible about the remaining changes that are desirable. It is hoped that the authors receive these comments in the constructive spirit with which they are offered.

I appreciate the clear delineation of a priori hypotheses in the introduction. However, if I understand correctly, the results actually indicate a pattern opposite to that of hypothesis (ii), with a reduction rather than the hypothesized enhancement of MRBD in ipsilesional M1. This is interesting, and certainly bears discussion – currently this point has been ignored.

This is a misunderstanding. MRBD is a decrease in beta power (a negative number). An 'enhancement' of MRBD is in fact a larger negative number. I have altered this sentence in order to make it clearer: "but would be greater (i.e. a larger decrease) in the lesioned hemisphere"

The presentation of some raw data in the response to reviews is also appreciated, as are the additional analyses. Some of these data should be incorporated into the main paper. Supplemental Fig 1 (p29) showing the group averages of each condition should definitely be included – presumably the first two figures in each row refer to pre-treatment and the latter two to post-treatment: this should be clarified.

Figure 1 of our response has been included as Figure 2 in the manuscript.

The last comment is a repeated misunderstanding – We scanned subjects during bimanual movement with and without a mirror reflection in order to understand what is happening in the brain during this visual mirror feedback. We were not looking for a 'before and after' effect as we are interested in the 'conditioning' effect of mirror therapy. Subjects therefore performed exactly the same auditory cued bimanual task throughout. In half the conditions they looked at the actual hand (dominant in controls or affected in patients) and in half they looked at the mirror reflection of the opposite hand. In this way, the movement and direction of gaze are exactly the same throughout. The only difference is the presence of the mirror. The figure is clearly labelled with the first two as no mirror condition and the last 2 as mirror condition.

I have written a figure legend explaining this further: "Figure 2: Group averaged spectrograms of each condition with controls on the top row and stroke patients on the bottom row. 0s is the time at which participants heard the auditory cue to move. Whilst movements were made bimanually, participants focussed on one hand (affected hand in patients, dominant hand in controls). Spectrograms are taken from primary motor cortex both contralateral (CM1) and ipsilateral (IM1) to the hand being observed. The same bimanual movements are made in all conditions. The direction of gaze is the same in all conditions. The difference between the two experimental conditions is the presence or absence of the mirror. In other words, in one condition, participants looked directly at their hand (no mirror) and in the other condition, the participants looked at a mirror reflection of the opposite hand (non-dominant in controls, unaffected in patients) (mirror). The colour axis represents percentage change in power compared to baseline."

Spectrograms from individual subjects should also be included, but rather than showing the data from each subject for one condition (as in Supplemental Figs 2&3), it would be more useful to show the data from a representative control subject and a representative stroke patient for each of the

four conditions shown in Supplemental Fig 1. The Results section should start with these figures and include brief descriptions of the findings illustrated.

We don't believe it is necessary to show both group averaged spectrograms of all 8 conditions as well as a representative individual spectrogram of each condition as this will look very similar and demonstrate the same information. We do not think the individual spectrogram would add anything extra to the manuscript. I have added in some text to the beginning of the results section explaining the group average spectrogram figure as suggested above: "Figure 2 shows group average time-frequency spectrograms for each condition, beta MRBD is clear in all conditions as a blue patch following the auditory cue to move at 0s." If the editors wish us to include all the figures, then we would do so, but we don't think it enhances the manuscript.

Supplemental Fig 8 (p34), showing the box plot of the asymmetry indices, should also be included at the appropriate juncture.

I have added the boxplot of the asymmetry index in as Figure 4 although it displays very similar information to that of Figure 3. We think that having slightly different representations of the same data may be confusing to readers.

None of the other supplemental figures are necessary, but the Results section should include the data on baseline beta and movement-related mu oscillations (a brief presentation in the text should suffice) – inclusion of these data strengthens the paper as it demonstrates the specificity of the observed findings. It would have substantially eased the pain (on both sides) if such data had been included originally, or at least in the first revision.

Text has now been included in the results section on baseline beta and mu oscillations: "Baseline beta values were extracted and no significant differences were found in beta values at rest between patients and controls. We also explored mu oscillations (7-14Hz) and saw a desynchronization in this frequency during movement. When an ANOVA was performed on these data using the same factors as in the beta band, a significant difference was found between patients and controls but no differences were found due to hemisphere or mirror condition."

I see that the authors have made some attempts to clarify the confusing abbreviations CM1 and IM1, but these abbreviations (or their expanded forms) remain in many places and continue to engender confusion. The confusion stems from expressing contra/ipsi-laterality with respect to the hand used, which of course are exactly the reverse with respect to the lesioned hemisphere. Here the authors should appreciate that these terms and abbreviations are probably second nature to them, but are likely to be quite confusing to readers. There are a number of possible solutions to this. Perhaps the simplest would be to drop the abbreviations altogether, and use "ipsilesional M1" and "contralesional M1" rather than "ipsilateral M1" and "contralateral M1".

We agree that the abbreviations are confusing and it is a common problem with this type of data. As we also include a control group, it is not possible to solely use contralesional and ipsilesional M1 as we need to compare to the healthy controls who do not have a lesioned hemisphere.

We believe that we have clearly explained what each abbreviation means the first time they are mentioned: "we extracted the source signal from the location of peak change in beta power (15-30Hz) within the primary motor cortices both contralateral (CM1) and ipsilateral (IM1) to the observed hand." "primary motor cortex contralateral to the hand being observed (CM1) and ipsilateral to the hand being observed (IM1) for each participant." and reiterated this in the discussion: "MRBD in the ipsilateral (contralesional) M1 was larger than in contralateral (ipsilesional)

M1 pointing to a significant imbalance of activity between hemispheres before MT was introduced." This is the most we can do to make it as transparent as possible.

Using the abbreviations is also helpful for the figures and the meaning of the abbreviations is once more explained in the figure legends: "Whilst movements were made bimanually, participants focussed on one hand (affected hand in patients, dominant hand in controls). Spectrograms are taken from primary motor cortex both contralateral (CM1) and ipsilateral (IM1) to the hand being observed." "CM1 and IM1 are the primary motor cortices contralateral and ipsilateral to the hand being observed respectively. As the patient group are observing their affected hand this means that CM1 is in fact M1 of the lesioned hemisphere and IM1 is M1 of the intact hemisphere."

A number of awkward sentence constructions remain and should be fixed (annotated by page/line):
Multiple places: "MT in healthy subjects" seems odd since a therapeutic effect is irrelevant for healthy subjects. Similarly, "perform MT" (5/12) seems strange.
4/52: rewrite thus: "shift of brain activation evoked by movement of the affected hand towards the lesioned hemisphere".
7/31: rewrite thus: "given in terms of measures for the affected …."
8/33: should be "muscles"?
13/57: replace comma after "stroke" with a period and start new sentence.
15/7: similarly, replace comma after "study" with a period and start new sentence.

These alterations have been made as recommended. We have altered "MT in healthy subjects" to "using mirror visual feedback in healthy subjects" as we feel this is clearer in terms of it not being used as a therapy in these situations. We have also changed "perform MT" to "perform movements using mirror visual feedback".