

1 **The neuronal correlates of mirror therapy: an fMRI study on mirror induced visual illusions in**  
2 **stroke patients**

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33 **Key words:** Stroke, Upper-Limbs, Rehabilitation, fMRI, Mirror Therapy

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35 **Word count: 3709**

36

37 **ABSTRACT**

38

39 **Aim**

40 To investigate the neuronal basis for the effects of mirror therapy in stroke patients.

41 **Methods**

42 Twenty-two stroke patients participated in this study. We used functional magnetic resonance imaging  
43 to investigate neuronal activation patterns in two experiments. In the unimanual experiment, patients  
44 moved their unaffected hand, either while observing it directly (no-mirror condition), or while observing  
45 its mirror reflection (mirror condition). In the bimanual experiment, patients moved both hands, either  
46 while observing the affected hand directly (no-mirror condition) or while observing the mirror reflection  
47 of the unaffected hand in place of the affected hand (mirror condition). A two-factorial analysis with  
48 movement (activity versus rest) and mirror (mirror versus no mirror) as main factors was performed to  
49 asses neuronal activity resultant of the mirror illusion.

50 **Results**

51 Data of 18 participants were suitable for analysis. Results showed a significant interaction effect of  
52 movement x mirror during the bimanual experiment. Activated regions were the precuneus and the  
53 posterior cingulate cortex ( $p < .05$  FDR).

54 **Conclusion**

55 In this first study on the neuronal correlates of the mirror illusion in stroke patients we showed that  
56 during bimanual movement the mirror illusion increases activity in the precuneus and the posterior  
57 cingulate cortex, areas associated with awareness of the self and spatial attention. By increasing  
58 awareness of the affected limb the mirror illusion might reduce learned non-use. The fact that we did  
59 not observe mirror-related activity in areas of the motor or mirror neuron system questions popular  
60 theories that attribute the clinical effects of mirror therapy to these systems.

61

62

63 **INTRODUCTION**

64 Mirror therapy was first introduced by Ramachandran and co-workers to alleviate phantom limb pain in  
65 amputees.[1] By showing patients the reflection of their unimpaired arm in a mirror, they retrieved the  
66 sensation of their amputated arm without pain. Since then, the paradigm of mirror therapy has also  
67 been studied in other pain related syndromes (especially chronic regional pain syndrome) [2] and  
68 motor related syndromes (stroke, hand surgery).[3,4] The focus of these studies has been on potential  
69 clinical effects; reduction of pain and improvement of motor function. Relatively little research has  
70 focussed on the mechanisms that underlie the effects of mirror therapy.

71 Presumably, different working mechanisms are behind the effects of mirror therapy on pain  
72 and motor symptoms. For the latter category, the focus of the current study, a number of mechanisms  
73 have been proposed. Ramachandran originally hypothesised that paralysis following stroke might  
74 have a 'learned' component, which could possibly be 'unlearned' by means of the mirror illusion.[5]  
75 Others suggested that mirror therapy might be a form of visually guided motor imagery.[6] Motor  
76 imagery itself has proven to be effective in the rehabilitation of patients with hemiparesis [7] and the  
77 mirror induced visual feedback of the imagined movement might further facilitate this. In addition, it  
78 has been hypothesized that the observation of the mirror illusion might trigger the mirror neuron  
79 system (MNS).[3] Mirror neurons were initially discovered by Di Pellegrino and co-workers in  
80 monkeys[8] and are a particular type of neurons that discharge both with performance of a motor  
81 action and with observation of another individual performing similar motor actions.[9] As single cell  
82 studies are not normally performed in the human brain, there is not yet direct evidence for the  
83 existence of a mirror neuron system in humans. However, brain imaging data do suggest the  
84 existence of a similar system.[10,11] Previous research has indicated the potential of the MNS in  
85 motor recovery by showing that the observation of movements performed by others improves motor  
86 performance in stroke patients.[12] It is conceivable that observing one's own mirrored movement  
87 promotes recovery in a similar way.

88 A number of studies have evaluated the neuronal correlates of mirror therapy by examining  
89 the observation of the mirror reflection of a moving hand in healthy subjects. These studies were  
90 based on the hypothesis that the mirror illusion would increase excitability or activity in primary motor  
91 areas in the hemisphere ipsilateral to the moving hand. Using transcranial magnetic stimulation (TMS)  
92 [13-15], magnetoencephalography (MEG) [16], electroencephalography (EEG) [17] and functional

93 magnetic resonance imaging (fMRI) [18] the authors compared neuronal activity or excitability  
94 ipsilateral to the moving hand with or without observing its mirror reflection. The MEG study reported  
95 the mirror illusion to suppress 20-Hz activity, indicating increased activation of the primary motor  
96 cortex [16], while the EEG study reported that the mirror illusion of movement induced lateralized  
97 readiness potentials, indicating cortical motor preparation for the non-moving hand.[17] On the other  
98 hand, the TMS studies either found no effect of the mirror illusion on motor cortex excitability [13,14] or  
99 indicated that the mirror illusion needs to be combined with motor imagery in order to increase motor  
100 cortex excitability.[15] Finally, an fMRI study from our research group [18] found no increased activity  
101 in sensorimotor areas as a result of the mirror illusion, but did find an increase in activity in the  
102 superior temporal sulcus (STS), presumed to be due to involvement of the MNS.[19]

103         So far, no studies on the neuronal correlates of the mirror illusion have been performed in  
104 patient groups. It seems obvious that caution has to be taken when generalizing results from mirror  
105 studies in healthy participants to stroke patients. Patients have a damaged hemisphere, and alteration  
106 of activity within that hemisphere might not be as easily achieved as in healthy participants.  
107 Furthermore, stroke patients performing mirror therapy are generally instructed to practice bi-  
108 manually, moving affected and un-affected limb together.[3,20,21] As stroke patients move  
109 asymmetrically, placing a mirror between their hands will give them a sudden illusion of normal  
110 movement of the involved hand, creating an incongruence between task performance and visual  
111 feedback. This situation can not be created similarly in healthy controls, and is conceptually different  
112 from the experiments in which healthy controls only move one hand.

113         In summary, while clinical trials have presented promising results of mirror therapy in several  
114 patients groups, the working mechanisms have not yet been investigated in patients. Additionally,  
115 results of the studies on the working mechanisms in healthy participants have not been conclusive and  
116 effects that have been found in these studies cannot be generalized to stroke patients. In the present  
117 study we therefore investigated the neuronal correlates of the mirror illusion in stroke patients. We  
118 used fMRI to compare two different sets of conditions: 1) moving the unaffected hand while observing  
119 it directly versus moving the unaffected hand while observing its mirror reflection and 2) moving both  
120 hands while observing the affected hand directly versus moving both hands while observing the mirror  
121 reflection of the unaffected hand in place of the affected hand. We hypothesised that observing the  
122 mirror reflection would increase neuronal activity in the affected hemisphere.

## 123 **MATERIALS & METHODS**

124

### 125 **Participants**

126 Patients that took part in this experiment were selected participants of a randomized controlled trial  
127 (RCT) investigating the effects of a rehabilitation program of mirror therapy ([www.trialregister.nl](http://www.trialregister.nl)  
128 NTR1052). In this trial, 40 stroke patients were included and randomly assigned to either an  
129 experimental (mirror) group or a control group. Patients from the trial that were eligible to be scanned  
130 in an MRI scanner were asked to take part in the present study. This study took place ahead of the  
131 start of the clinical trial, before patients had been allocated to a treatment group. Inclusion criteria for  
132 the RCT were knowledge of the Dutch language, a Brunnstrom Score for the upper-extremity between  
133 III and V, home dwelling status and at least one year post stroke. Patients with neglect, co-morbidities  
134 that influenced upper-extremity usage or who had suffered multiple strokes were excluded from  
135 participation. For patients to be able to take part in the present study, the following additional inclusion  
136 criteria applied: a Brunnstrom score of IV or V and standard MRI exclusion criteria. Application of  
137 these criteria resulted in a total of 22 eligible patients. The study was approved by the Medical Ethics  
138 Committee of the Erasmus MC Rotterdam and all patients gave written informed consent before  
139 participating in the study. Before the fMRI experiment started the Fugl Meyer [22] assessment of  
140 upper-extremity function was administered to all participants for descriptive purposes.

141

### 142 **fMRI experiment**

143 The fMRI paradigm we used was based on one previously designed in our laboratory.[18] We  
144 performed two separate experiments each involving two conditions within a single scanning session.  
145 In the first experiment patients were instructed to only move their unaffected hand (unimanual), either  
146 while looking directly at it (no mirror condition) or while observing its reflection in a mirror (mirror  
147 condition). In the second experiment patients were instructed to move both their hands (bimanual)  
148 either while looking directly at their affected hand (no mirror condition) or while observing the mirror  
149 reflection of their unaffected hand in place of their affected hand (mirror condition) (see Figure 1). In all  
150 four conditions, patients could see two hands. In the no mirror conditions of both experiments, patients  
151 had a direct view of both their affected and their unaffected hand. In the mirror conditions, patients had

152 a direct view of their unaffected hand and saw the reflection of their unaffected hand in place of their  
153 affected hand, also leading to visual feedback of two hands.

154 During scanning, patients lay on their back in the scanner with their upper arms comfortably  
155 resting on the scanner table alongside their torso and their elbows flexed in such a way that their  
156 hands were 20 centimetres apart above their waists. By means of two mirrors attached to the head  
157 coil above the head, patients were able to look in the direction of their feet and could thus view both  
158 their hands.

159 All four conditions were performed using a block design, consisting of 10 alternating 30s  
160 periods of 5 rest blocks and 5 active blocks. In the active blocks patients had to open and close either  
161 their unaffected hand or both hands, in the rest blocks patients had to hold their hands still. Patients  
162 were instructed to pace the opening of their hands to a metronome with a rhythm of 0.5 Hz. The  
163 onsets of the rest and active conditions were indicated verbally using simple words (start, rest)  
164 generated by a computer program (Matlab 7.1; Mathworks, Sherborn, Mass). Auditory stimuli were  
165 presented to the patients through MRI compatible headphones. The hand movement was practiced  
166 before the scan session started.

167 The four conditions were presented to the patients in random order. During the mirror  
168 conditions, a large mirror was placed between the subjects' hands in such a way that the mirror image  
169 of the unaffected hand was superimposed on the position of the affected hand. The large mirror was  
170 made of MRI-compatible material (plexiglass) and was shaped in such a way that it fitted inside the  
171 scanner bore and fully obstructed the view of the hand behind the mirror (see Figure 2). In this way, a  
172 visual illusion of two normal hands was created. Before the scanning session, patients practiced  
173 outside the scanner with a regular mirror as used during mirror therapy in order to make sure they  
174 experienced the visual illusion. While it is hard to objectively quantify the presence or strength of the  
175 illusion, all subjects reported that the illusion of seeing the affected hand moving in an unimpaired  
176 fashion was similar to their experience during the mirror exercises outside the MRI scanner.

177 Imaging was performed on a 3T MR system (HD platform, GE Healthcare, Milwaukee, Wis).  
178 For anatomical reference, a high-resolution, 3-dimensional, inversion recovery, fast spoiled gradient  
179 echo, T1-weighted image was acquired (TR/TE/TI 10.7/2.2/300 ms, 18° flip angle, matrix 416×256,  
180 and field of view 250×175 mm<sup>2</sup>). For functional imaging, a single-shot, T2\*-weighted, gradient echo  
181 echo-planar imaging (EPI) sequence was used (TR/TE 3000/30 ms, 75° flip angle, matrix 64×96, field

182 of view  $220 \times 220 \text{ mm}^2$ ). An fMRI acquisition lasted for 5m15s, including 15 seconds of dummy scans  
183 that were discarded. For each of the four conditions 100 volumes were collected. The imaging volume  
184 covered the entire brain including the cerebellum.

185

## 186 **Statistical analyses**

187 The imaging data were analyzed using statistical parametric mapping software (SPM5, Wellcome  
188 Department of Cognitive Neurology, University College London, UK) implemented in MATLAB version  
189 7.1 (Mathworks, Sherborn, MAS).

190 All functional images for each participant were realigned to the first scan of each condition and  
191 then coregistered to the T1-weighted anatomical scan. Subsequently, images were transformed to  
192 standard Montreal Neurologic Institute space. To prevent warping around the lesions, we used a  
193 segmentation-based normalization approach.[23] Finally, normalized images were spatially smoothed  
194 by using a Gaussian filter of 8-mm FWHM.

195 Preliminary analyses showed that the realignment parameters estimated during spatial  
196 preprocessing were sometimes correlated with the task design. Therefore, we decided not to model  
197 the realignment parameters in the design matrix as regressors of no interest, as this would have  
198 resulted in cancelling out task-related activation. Instead, we used the ArtRepair Toolbox [24], which  
199 evaluates all volumes, detects the ones most affected by movement, and deweights these in the  
200 general linear model estimation. The experimental block design was convolved with the canonical  
201 hemodynamic response function, and the resulting model was estimated using a high-pass filter at  
202 128 s in order to remove low-frequency artifacts.

203 In the first-level analysis, statistical maps were calculated for each of the four task blocks (i.e.  
204 movement with mirror, movement without mirror, rest with mirror, rest without mirror) for each patient  
205 and each experiment (bimanual and unimanual) separately. Statistical maps of patients with left-sided  
206 lesions were flipped about the midsagittal plane, so that the affected hemisphere corresponded to the  
207 right side of the brain for all patients. The statistical maps were used for second level analyses.

208

209

210 **Second level analysis**

211 We performed a two factorial analysis with movement (activity versus rest) and mirror (mirror versus  
212 no mirror) as main factors for both the unimanual and the bimanual experiments separately. Main  
213 effects of movement and mirror as well as the interaction between movement and mirror were  
214 investigated. Significance was set at  $p < .05$  (FDR corrected) with a minimum cluster size of 20.

215

216 **RESULTS**

217 Four patients were discarded from further analysis, one due to scanner failure, and three due to  
218 scanner artefacts in their data sets. The remaining analyses were thus conducted using data sets of  
219 18 patients. The characteristics of these patients are presented in Table 1.

220

221 **Table 1**

222 **Patient characteristics of the study participants**

<b>Characteristic</b>	<b>Total*</b>	
No. of patients	18	223 224
Age (years)	54.7 ± 9.9	226
Time since stroke (years)	5.2 ± 3.6	227
Sex (male/female)	10/8	228
Affected side dominant/nondominant)	6/12	229
Type of stroke (infarct/haemorrhage)	16/2	230
Location of stroke lesion		231
- Cortical (with or without subcortical)	13	232
- Subcortical	3	233
- Brainstem <sup>†</sup>	2	234
FM score	41.9 ± 11.3	235

237 \*Values are mean ± standard deviation

238 <sup>†</sup> Brainstem lesions are located in the pons, above the crossing of the cortico-spinal tract

239

240 In a two-factorial design, we examined main effects of movement (task condition versus rest),  
241 mirror (mirror condition versus no mirror condition), and the interaction effect between movement and  
242 mirror for both experiments. Observed activation patterns for the main effect of movement were in  
243 accordance with the expected activation for uni- and bimanual hand motor tasks. Activity was  
244 observed in the pre- and postcentral gyrus (primary motor and sensory cortex), the medial superior



245 frontal gyrus (SMA), at the junction of the superior frontal sulcus and the precentral sulcus (premotor  
246 cortex) and in the cerebellum.

247

248 **Table 2**

249 **Areas of activation (unimanual experiment: main effect of movement)**

Anatomical location	Side	Cluster size	Z-score	MNI		
				x	y	z
Pre- and Post central Gyrus	Unaffected	2103	7.57	-34	-26	54
Cerebellum	Unaffected	1287	7.28	16	-56	-20
Middle Occipital/Temporal gyrus	Affected	612	6.18	42	-64	4
Medial Frontal Gyrus, Superior Frontal Gyrus, Cingulate Gyrus	Affected, Unaffected	448	6.17	-6	-6	56
Middle Occipital/Temporal gyrus	Unaffected	491	5.98	-48	-70	-2
Precentral Gyrus	Unaffected	36	5.72	-52	0	8

250

251

252 **Areas of activation (bimanual experiment: main effect of movement)**

Anatomical location	Side	Cluster size	Z-score	MNI		
				x	y	z
Pre- and Post central Gyrus	Unaffected	853	7.11	-42	-20	52
Medial Frontal Gyrus, Superior Frontal Gyrus, Cingulate Gyrus	Unaffected	331	6.43	-6	-4	58
Pre- and Post Central Gyrus, Medial and Superior Frontal Gyrus, Cingulate Gyrus	Affected	665	6.36	36	-16	56
Middle Occipital gyrus, Inferior Temporal gyrus	Affected	33	5.63	44	-72	0
Middle Occipital/Temporal gyrus, Inferior Temporal gyrus	Unaffected	55	5.35	-46	-68	0

253

254 Areas are thresholded at  $p < .05$  (FDR corrected) with a minimum cluster size of 20 voxels. MNI =  
 255 Montreal Neurological Institute.

256

257 Analysis of the main effect of mirror (mirror versus no mirror) showed no significant areas of  
 258 activation in either of the two experiments. The interaction of mirror x movement showed no significant  
 259 activation for the unimanual experiment but it did show significant activation in the bimanual  
 260 experiment; in the precuneus and the posterior cingulate cortex. Post-hoc analysis revealed that this  
 261 was caused by increased activity in the movement with mirror condition versus the movement without  
 262 mirror condition. Table 3 and Figure 3 show the activated clusters for the interaction effect of  
 263 movement x mirror in the bimanual experiment.

264

265 **Table 3**

266 **Areas of activation (bimanual experiment: interaction movement x mirror)**

Anatomical location	Side	Cluster size	Z-score	MNI		
				x	y	z
Precuneus	Affected,	78	5.28	2	-58	58
	Unaffected,					
	Central					
Posterior Cingulate Cortex	Unaffected	117	4.68	-6	-36	8

267

268 Areas are thresholded at  $p < .05$  (FDR corrected) with a minimum cluster size of 20 voxels. MNI =

269 Montreal Neurological Institute.

270

## 271 **DISCUSSION**

272 In an attempt to unravel the working mechanism of mirror therapy, this study investigated the neuronal  
273 correlates of the mirror-induced visual illusion in stroke patients. In this first study, to our knowledge, in  
274 which stroke patients participated instead of healthy volunteers, we showed increased activity as a  
275 result of the mirror illusion during bimanual movement in two areas: the precuneus and the posterior  
276 cingulate cortex. We found no differential effect on neuronal activity of the mirror illusion during  
277 unimanual movement, nor did we find evidence for the mirror illusion to increase activity in motor  
278 areas or the mirror neuron system.

279 A network including both the precuneus and posterior cingulate cortex is reported to be  
280 associated with mental representation of the self.[25] More specifically, research showed that the  
281 precuneus is activated when actions are being interpreted as being controlled by the self as well as  
282 during self centred mental imagery strategies [26] whereas the cingulate cortex becomes activated  
283 during spatial navigation [27] and has been found to process information about the spatial positions of  
284 the limbs in monkeys.[28] The mirror illusion of a normal moving affected hand thus seems to increase  
285 alertness and spatial attention towards this hand. The fact that we did not find this activation during the  
286 unimanual condition, suggests that it is not so much the illusion of a virtual moving hand that causes  
287 this activation, but the mismatch between the movement one performs and the movement that is

288 observed. Research showing that the cingulate cortex becomes activated during conflict monitoring,  
289 specifically during action conflicts, supports this.[29]

290 Two previous studies in healthy participants have reported increased neuronal activity in  
291 similar areas as a result of an incongruence between movement observation and action.[30-32] Dohle  
292 et al.[32] found that such incongruence increased activation in occipital and posterior parietal areas,  
293 amongst them the precuneus, which they suggest to play a decisive role during mirror therapy.[21] In  
294 an earlier experiment Fink et al.[30] also reported on the neural correlates of a conflict between visual  
295 and proprioceptive information. Using positron emission tomography (PET) they investigated the effect  
296 of the mirror illusion during either in-phase movements or out-of-phase movements that are perceived  
297 as in-phase movements due to the mirror. Their results showed an effect of the mirror in the  
298 dorsolateral prefrontal cortex (DLPCF) and in the superior posterior parietal cortex (Brodmann's area  
299 (BA) 7). BA 7 is a point of convergence between vision and proprioception and plays a role in visuo-  
300 motor coordination. The precuneus, which in our study became activated during the bimanual mirror  
301 condition, is part of BA 7 [26] but is located more medial than the area of activation reported by Fink et  
302 al. The main difference between our study and Fink et al.'s is that in the latter participants actively had  
303 to create the motor-sensory conflict (by moving out-of-phase), while in our patient group this resulted  
304 from the involved arm not being able to perform similar movements as the non-involved arm. The  
305 situation created by Fink et al. likely induces a larger cognitive burden for the participants, which may  
306 explain increased DLPCF activation, while the main effect of the mirror they observed in BA 7 is in line  
307 with our findings of precuneus activation in the presence of a motor-sensory conflict, albeit under  
308 different experimental settings.

309 The question is how the involvement of the areas we found activated by the mirror illusion  
310 relates to the reported improvements in motor function following mirror therapy.[3,21] Possibly, by  
311 increasing the spatial attention towards the affected limb the mirror illusion might help in overcoming  
312 the learned non-use phenomenon [21,33], and as a result of the ensuing increased use of the limb  
313 improve motor performance. An alternative hypothesis, supported by the fact that we only observed  
314 increased cortical activation during the bimanual experiment, might be that the effects of the mirror lie  
315 in an enhancement of spatial coupling between limbs. It is well known that when two arms move  
316 simultaneously, movements become more temporally and spatially stable.[34] In stroke rehabilitation,  
317 this phenomenon has been exploited in the form of bimanual training programs. Several studies have

318 shown that bimanual training strategies have a favourable effect over unimanual training [35],  
319 demonstrating that spatial coupling can cause the affected limb to take on the properties of the non-  
320 effected limb, thereby improving motor performance. The hypothesis that the mirror illusion enhances  
321 this spatial coupling is supported by studies on healthy volunteers, showing that the mirror illusion  
322 increased the tendency of one limb to take on the spatial properties of the other limb.[36,37]

323         So far, several reviews and clinical studies have attributed the effects of mirror therapy to  
324 activation of motor areas or the mirror neuron system (MNS).[3,38-40] As mentioned, in the present  
325 study we did not observe activation resulting from the mirror illusion in these areas (e.g. M1, PMC,  
326 SMA, Broca's area), neither in the unimanual experiment nor in the bimanual experiment. Research  
327 performed in healthy subjects has so far also been unable to provide convincing evidence for the  
328 activation of these areas by the mirror illusion, only the fMRI study of Matthys et al. showed some  
329 evidence for MNS activation by reporting increased activation within STS.[18] However, although STS  
330 is reported to be related to the MNS [19], this area has been associated with many different  
331 behaviours and its exact function remains poorly understood.[41] Matthys et al. also reported  
332 activation in the superior occipital gyrus, an area connected with the PPC through the dorsal stream.  
333 Activation of this area may reflect increased attentional demands for the integration of vision and  
334 proprioception induced by the mirror, which is in line with our present results. It has to be noted that  
335 the analysis strategy of Matthys et al. differed from the strategy employed in the present paper.  
336 Contrary to our approach, Matthys et al. did not apply an ANOVA design and thus did not examine the  
337 effect of the mirror, the hand motor performance and its interaction separately. As a final note on the  
338 proposed activation of the MNS or motor system by the mirror illusion, a previous study showed that  
339 whereas observation of actions attributed to another individual activated the motor system,  
340 observation of identical actions linked to the self did not.[42] The MNS thus seems to distinguish  
341 between observing actions linked to the self and actions linked to others. This finding further  
342 undermines the notion that the mirror illusion of self-performed movements might trigger the motor or  
343 mirror neuron system.

344         We acknowledge that our study has some limitations. In general, detecting study-related  
345 effects in fMRI experiments in stroke patients is difficult as the neuronal circuitry may be distorted and  
346 heterogeneous between subjects. Some authors try to get around this issue by including only patients  
347 with minor lesions, which is a major source of selection bias. In the current study, in order to enlarge

348 the contrast between mirror and no mirror conditions in the bimanual experiment, we explicitly enrolled  
349 patients with larger motor deficits. Consequently, the within-group variability was considerable, which  
350 may have decreased the power to detect differences between conditions. Another issue, related to the  
351 severity of their motor deficit, is that some patients had problems keeping their head still during the  
352 experimental task. As these movements were task-related, we could not simply regress out head  
353 motion-related activation as this would have cancelled out task-related activation as well. However, we  
354 used an alternative, sensitive method to deal with this issue (see Methods section), and activation  
355 patterns we observed are in accordance with the expected activation for uni- and bimanual hand  
356 motor tasks, implicating the validity of our analysis. A general limitation of this and similar studies is  
357 that it is difficult to objectify the strength of the illusion patients experience when inside the scanner. To  
358 maximize the mirror illusion during scanning, all patients practiced with a standard mirror used for  
359 mirror therapy outside the scanner, and they all reported similar illusion strength during the  
360 measurements inside the scanner as outside.

361 In conclusion, the present study showed that during bimanual movement, the mirror illusion  
362 alters neuronal activation in the precuneus and posterior cingulate cortex, areas related to alertness  
363 and spatial-awareness. We found no differential effect on neuronal activity of the mirror illusion during  
364 unimanual movements, nor did we find evidence for the mirror illusion to increase activity in motor  
365 areas. By increasing awareness of the affected limb, possibly due the mismatch between action and  
366 observation, the mirror illusion might reduce learned non-use. The fact that we did not observe any  
367 activation in areas belonging to the motor or mirror neuron system questions popular theories that  
368 contribute the clinical effects of mirror therapy to these systems. As research into the working  
369 mechanism of mirror therapy has so far mainly focussed on these systems, we suggest that future  
370 research should adopt a broader perspective, amongst other things taking the ideas as proposed in  
371 this paper into account. Since a better understanding of why and how mirror therapy works may lead  
372 to a more effective application and might help in selecting patients for which mirror therapy will be  
373 most effective, it is important that efforts to unravel the neuronal correlates of mirror therapy continue.

374

375 **ACKNOWLEDGMENTS**

376 We want to thank Martine Eckhardt for her help with the measurements and organization of the study  
377 and Maarten Bobbert, Daphne Heemskerk and David Warmerdam for their help with the analysis of  
378 the data and interpretation of results. Also, we would like to acknowledge the two anonymous  
379 reviewers for their valuable suggestions on the data analysis and the manuscript. This study was  
380 supported by Hersenstichting Nederland, Fonds NutsOhra, Innovatiefonds Zorgverzekeraars, Prinses  
381 Beatrix Fonds and Wetenschappelijk College Fysiotherapie.

382

383 Competing Interest: None declared

384

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392 **REFERENCES**

- 393 [1] Ramachandran VS, Rogers-Ramachandran D, Cobb S. Touching the phantom limb. *Nature*.  
394 1995 Oct 12;377(6549):489-90.
- 395 [2] McCabe CS, Haigh RC, Ring EF, Halligan PW, Wall PD, Blake DR. A controlled pilot study of  
396 the utility of mirror visual feedback in the treatment of complex regional pain syndrome (type 1).  
397 *Rheumatology (Oxford)*. 2003 Jan;42(1):97-101.
- 398 [3] Yavuzer G, Selles R, Sezer N, Sutbeyaz S, Bussmann JB, Koseoglu F, et al. Mirror therapy  
399 improves hand function in subacute stroke: a randomized controlled trial. *Arch Phys Med Rehabil*.  
400 2008 Mar;89(3):393-8.
- 401 [4] Rosen B, Lundborg G. Training with a mirror in rehabilitation of the hand. *Scandinavian journal*  
402 *of plastic and reconstructive surgery and hand surgery / Nordisk plastikkirurgisk forening [and] Nordisk*  
403 *klubb for handkirurgi*. 2005;39(2):104-8.
- 404 [5] Ramachandran VS. Phantom limbs, neglect syndromes, repressed memories, and Freudian  
405 psychology. *International review of neurobiology*. 1994;37:291-333; discussion 69-72.
- 406 [6] Stevens JA, Stoykov ME. Using motor imagery in the rehabilitation of hemiparesis. *Arch Phys*  
407 *Med Rehabil*. 2003 Jul;84(7):1090-2.
- 408 [7] Sharma N, Pomeroy VM, Baron JC. Motor imagery: a backdoor to the motor system after  
409 stroke? *Stroke*. 2006 Jul;37(7):1941-52.
- 410 [8] di Pellegrino G, Fadiga L, Fogassi L, Gallese V, Rizzolatti G. Understanding motor events: a  
411 neurophysiological study. *Exp Brain Res*. 1992;91(1):176-80.
- 412 [9] Rizzolatti G, Craighero L. The mirror-neuron system. *Annual review of neuroscience*.  
413 2004;27:169-92.
- 414 [10] Iacoboni M, Woods RP, Brass M, Bekkering H, Mazziotta JC, Rizzolatti G. Cortical  
415 mechanisms of human imitation. *Science*. 1999 Dec 24;286(5449):2526-8.
- 416 [11] Rizzolatti G, Fogassi L, Gallese V. Neurophysiological mechanisms underlying the  
417 understanding and imitation of action. *Nature reviews*. 2001 Sep;2(9):661-70.
- 418 [12] Ertelt D, Small S, Solodkin A, Dettmers C, McNamara A, Binkofski F, et al. Action observation  
419 has a positive impact on rehabilitation of motor deficits after stroke. *Neuroimage*. 2007;36 Suppl  
420 2:T164-73.
- 421 [13] Garry MI, Loftus A, Summers JJ. Mirror, mirror on the wall: viewing a mirror reflection of  
422 unilateral hand movements facilitates ipsilateral M1 excitability. *Exp Brain Res*. 2005 May;163(1):118-  
423 22.
- 424 [14] Funase K, Tabira T, Higashi T, Liang N, Kasai T. Increased corticospinal excitability during  
425 direct observation of self-movement and indirect observation with a mirror box. *Neuroscience letters*.  
426 2007 May 29;419(2):108-12.
- 427 [15] Fukumura K, Sugawara K, Tanabe S, Ushiba J, Tomita Y. Influence of mirror therapy on  
428 human motor cortex. *The International journal of neuroscience*. 2007 Jul;117(7):1039-48.
- 429 [16] Tominaga W, Matsubayashi J, Deguchi Y, Minami C, Kinai T, Nakamura M, et al. A mirror  
430 reflection of a hand modulates stimulus-induced 20-Hz activity. *Neuroimage*. 2009 Jun;46(2):500-4.

431 [17] Touzalin-Chretien P, Dufour A. Motor cortex activation induced by a mirror: evidence from  
432 lateralized readiness potentials. *J Neurophysiol*. 2008 Jul;100(1):19-23.

433 [18] Matthys K, Smits M, Van der Geest JN, Van der Lugt A, Seurinck R, Stam HJ, et al. Mirror-  
434 induced visual illusion of hand movements: a functional magnetic resonance imaging study. *Arch Phys*  
435 *Med Rehabil*. 2009 Apr;90(4):675-81.

436 [19] Iacoboni M, Dapretto M. The mirror neuron system and the consequences of its dysfunction.  
437 *Nature reviews*. 2006 Dec;7(12):942-51.

438 [20] Altschuler EL, Wisdom SB, Stone L, Foster C, Galasko D, Llewellyn DM, et al. Rehabilitation  
439 of hemiparesis after stroke with a mirror. *Lancet*. 1999 Jun 12;353(9169):2035-6.

440 [21] Dohle C, Pullen J, Nakaten A, Kust J, Rietz C, Karbe H. Mirror Therapy Promotes Recovery  
441 From Severe Hemiparesis: A Randomized Controlled Trial. *Neurorehabil Neural Repair*. 2008 Dec 12.

442 [22] Fugl-Meyer AR, Jaasko L, Leyman I, Olsson S, Steglind S. The post-stroke hemiplegic patient.  
443 1. a method for evaluation of physical performance. *Scandinavian journal of rehabilitation medicine*.  
444 1975;7(1):13-31.

445 [23] Crinion J, Ashburner J, Leff A, Brett M, Price C, Friston K. Spatial normalization of lesioned  
446 brains: performance evaluation and impact on fMRI analyses. *Neuroimage*. 2007 Sep 1;37(3):866-75.

447 [24] Mazaika P, Whitfield-Gabrieli S, Reiss A. Artifact Repair for fMRI Data from High Motion  
448 Clinical Subjects. *Human Brain Mapping* 2007; Chigago; 2007.

449 [25] Fransson P, Marrelec G. The precuneus/posterior cingulate cortex plays a pivotal role in the  
450 default mode network: Evidence from a partial correlation network analysis. *Neuroimage*. 2008 Sep  
451 1;42(3):1178-84.

452 [26] Cavanna AE, Trimble MR. The precuneus: a review of its functional anatomy and behavioural  
453 correlates. *Brain*. 2006 Mar;129(Pt 3):564-83.

454 [27] Moffat SD. Aging and spatial navigation: what do we know and where do we go?  
455 *Neuropsychology review*. 2009 Dec;19(4):478-89.

456 [28] Bakola S, Gamberini M, Passarelli L, Fattori P, Galletti C. Cortical Connections of Parietal  
457 Field PEc in the Macaque: Linking Vision and Somatic Sensation for the Control of Limb Action. *Cereb*  
458 *Cortex*. Feb 22.

459 [29] Botvinick MM, Braver TS, Barch DM, Carter CS, Cohen JD. Conflict monitoring and cognitive  
460 control. *Psychological review*. 2001 Jul;108(3):624-52.

461 [30] Fink GR, Marshall JC, Halligan PW, Frith CD, Driver J, Frackowiak RS, et al. The neural  
462 consequences of conflict between intention and the senses. *Brain*. 1999 Mar;122 ( Pt 3):497-512.

463 [31] Leube DT, Knoblich G, Erb M, Kircher TT. Observing one's hand become anarchic: an fMRI  
464 study of action identification. *Consciousness and cognition*. 2003 Dec;12(4):597-608.

465 [32] Dohle C, Kleiser R, Seitz RJ, Freund HJ. Body scheme gates visual processing. *J*  
466 *Neurophysiol*. 2004 May;91(5):2376-9.

467 [33] Taub E. Somatosensory deafferentation research with monkeys: implications for rehabilitation  
468 medicine. In: Wilkins W, ed. *Behavioral Psychology in Rehabilitation Medicine: Clinical Applications*.  
469 Baltimore 1980:371-401.

- 470 [34] Kelso JA, Holt KG, Rubin P, Kugler PN. Patterns of human interlimb coordination emerge from  
471 the properties of non-linear, limit cycle oscillatory processes: theory and data. *Journal of motor*  
472 *behavior*. 1981 Dec;13(4):226-61.
- 473 [35] Stoykov ME, Corcos DM. A review of bilateral training for upper extremity hemiparesis.  
474 *Occupational therapy international*. 2009;16(3-4):190-203.
- 475 [36] Altschuler EL. Interaction of vision and movement via a mirror. *Perception*. 2005;34(9):1153-5.
- 476 [37] Franz EA, Packman T. Fooling the brain into thinking it sees both hands moving enhances  
477 bimanual spatial coupling. *Exp Brain Res*. 2004 Jul;157(2):174-80.
- 478 [38] Ramachandran VS, Altschuler EL. The use of visual feedback, in particular mirror visual  
479 feedback, in restoring brain function. *Brain*. 2009 Jul;132(Pt 7):1693-710.
- 480 [39] Ezendam D, Bongers RM, Jannink MJ. Systematic review of the effectiveness of mirror  
481 therapy in upper extremity function. *Disability and rehabilitation*. 2009 May 20:1-15.
- 482 [40] Rizzolatti G, Fabbri-Destro M, Cattaneo L. Mirror neurons and their clinical relevance. *Nature*  
483 *clinical practice*. 2009 Jan;5(1):24-34.
- 484 [41] Hein G, Knight RT. Superior temporal sulcus--It's my area: or is it? *J Cogn Neurosci*. 2008  
485 Dec;20(12):2125-36.
- 486 [42] Schutz-Bosbach S, Mancini B, Aglioti SM, Haggard P. Self and other in the human motor  
487 system. *Curr Biol*. 2006 Sep 19;16(18):1830-4.
- 488
- 489

490 **Figure 1**

491 Schematic representation of the four conditions. FE indicates which hands perform the flexion-

492 extension movements, and the arrow is used to indicate the direction of gaze in each condition.

493

494 **Figure 2**

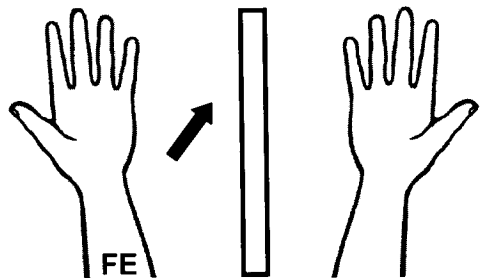
495 Participant lying in the scanner during the mirror condition. The unaffected hand is not visible, as it is  
496 positioned in front of the mirror.

497 **Figure 3**

498 Activation map of the interaction effect of movement x mirror for the bimanual experiment ( $p < .05$ , FDR  
499 corrected, minimum cluster size 20). Label A: posterior cingulate cortex; Label B: precuneus.

## Unimanual experiment

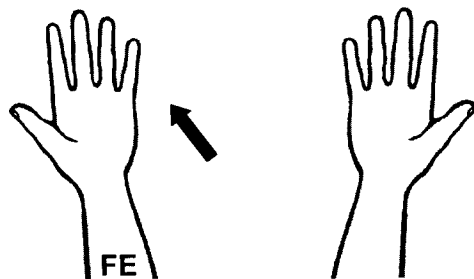
Mirror condition



Unaffected side

Affected side

No mirror condition

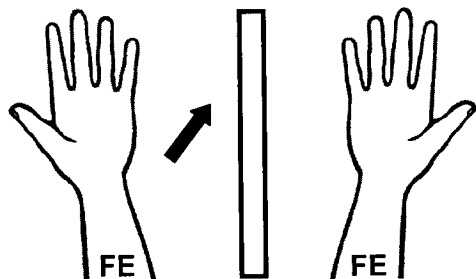


Unaffected side

Affected side

## Bimanual experiment

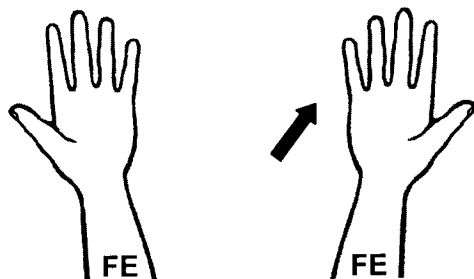
Mirror condition



Unaffected side

Affected side

No mirror condition



Unaffected side

Affected side





