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Mirrored, imagined and executed movements differentially activate sensorimotor cortex in amputees with and without phantom limb pain

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ABSTRACT

Extended viewing of movements of the intact hand in a mirror as well as motor imagery has been shown to decrease pain in phantom pain patients. We used functional magnetic resonance imaging to assess the neural correlates of mirrored, imagined and executed hand movements in 14 upper extremity amputees – 7 with phantom limb pain (PLP) and 7 without phantom limb pain (non-PLP) and 9 healthy controls (HC). Executed movement activated the contralateral sensorimotor area in all three groups but ipsilateral cortex was only activated in the non-PLP and HC group. Mirrored movements activated the sensorimotor cortex contralateral to the hand seen in the mirror in the non-PLP and the HC but not in the PLP. Imagined movement activated the supplementary motor area in all groups and the contralateral primary sensorimotor cortex in the non-PLP and HC but not in the PLP. Mirror- and movement-related activation in the bilateral sensorimotor cortex in the mirror movement condition and activation in the sensorimotor cortex ipsilateral to the moved hand in the executed movement condition were significantly negatively correlated with the magnitude of phantom limb pain in the amputee group. Further research must identify the causal mechanisms related to mirror treatment, imagined movements or movements of the other hand and associated changes in pain perception.

1. Introduction

Many amputees feel the continued presence of their amputated limb and 50–80% experience phantom limb pain (PLP) [36]. The magnitude of PLP is related to the changes in the cortical representation of areas adjacent to the amputated limb with more PLP being related to a shift of activation into the deafferented zone in both primary somatosensory and motor cortex [12,25]. It has been suggested that this reorganized cortical map might be related to PLP and that its restitution might relieve it. Ramachandran et al. [37,38] used a mirror box such that movement of the intact arm was perceived as movement of the amputated limb and reported anecdotal evidence of changes in the movement and pain of the phantom. Mirror training is thought to reverse cortical reorganizational changes related to phantom limb pain [13] and might resolve a conflict between motor intention and sensory feedback, which has been found to result in aversive sensation and potentially pain [10,31].

In a randomized controlled trial that used graded motor imagery – a sequential combination of hand laterality training, motor imagery and mirror training patients with complex regional pain syndrome or phantom limb pain showed a decrease in pain as well as an improvement in function post-treatment and at the 6-month follow-up [34] and it was shown that the order of treatment mattered [33]. In lower limb amputees Brodie [3,4] reported a significantly greater number of movements in the phantom when a mirror box was used but also found that executed movements had a similar effect. Hunter et al. [21] showed that a single trial mirror box intervention led to a more vivid awareness and enhanced movement ability of the phantom. Four weeks of mirror training led to significantly more decrease in phantom limb pain than training with a covered mirror or mental visualization [5]. Both Giraux and Sirigu [16] and MacIver et al. [30] showed that imagery alone also affects the cortical map and relieves phantom limb pain in contrast to Chan et al. [5] who did not find such changes. These studies suggest that modification of input into the affected brain region may alter pain sensation. The optimal method to alter pain and brain representation and the brain mechanisms underlying the effects mirror training or motor imagery are still unclear.

In this study mirrored and executed movements of the dominant hand in controls and the intact hand in amputees as well as imagined movements of the non-dominant hand in controls and

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the amputated hand in amputees were examined by self-report and functional magnetic resonance imaging. We tested the hypothesis that mirrored movements of the intact hand might provide input into the cortical representation zone that previously received input from the now amputated limb or, alternatively, resolves the conflict between motor intention and sensory feedback and that this might counteract phantom limb pain. We also assumed that executed and imagined movements might have similar but less pronounced effects.

2. Materials and methods

2.1. Participants

Fourteen unilateral upper limb amputees, 7 with phantom limb pain (PLP, mean age 54.3 ± 8.6 years, range 36–62, 2 female), 7 without phantom limb pain (non-PLP, mean age 50.3 ± 7.2 years, range 41–60, 1 female), and 9 healthy controls (HC, mean age 51.9 ± 6.9 years, range 39–61, 1 female) participated in the study. The average age of the groups did not differ significantly ($F(2,20) = 0.49$; $p = 0.62$). In both patient groups 4 patients had their dominant and 3 patients had their non-dominant hand amputated. All participants gave written informed consent prior to taking part in the study and the local institutional review board approved the protocol, which adhered to the Declaration of Helsinki. The amputations were caused by accident ($N = 13$) or vascular disease ($N = 1$). Non-painful phantom phenomena (e.g. sensations of limb size, movement, and sensation such as tingling or itchy) were reported by 12 of the 14 amputees, telescoping (i.e. the perception of shrinkage of the limb) was perceived in 8 persons (see Table 1 for demographic and clinical characteristics of the participants). In our sample all PLP patients had had long-standing phantom limb pain and the non-PLP had never experienced phantom limb pain. None of the subjects was under pain medication at the time of the study and none of the amputees had phantom limb pain during the assessment.

2.2. Evaluation of phantom sensations and imagery capability

Duration, intensity, and frequency of phantom limb pain, non-painful phantom sensations, residual limb pain, and residual limb sensations were investigated by a standardized interview [12,46] and the German version of the West Haven–Yale Multidimensional Phantom Limb Pain Inventory (MPI) [14,26] modified to separately evaluate phantom limb pain and residual limb pain [12]. The Questionnaire upon Mental Imagery (QMI) [43] was used to assess imagery ability of the participants. After the measurements in the MR scanner, vividness of the imagination and the sensation of phantom movements were evaluated using the questions adapted from the QMI such as Did you have the feeling that the

Table 1
Demographic and clinical details of the samples.

	Patients with phantom limb pain ($N = 7$)	Patients without phantom limb pain ($N = 7$)	Healthy controls ($N = 9$)	p
N male/female	5/2	6/1	8/1	n.s.
Age in years (M, SD)	54.3 ± 8.6	50.3 ± 7.2	51.9 ± 6.9	n.s.
Time since amputation in years (M, SD)	18.5 ± 14.2	35.6 ± 13.3		0.05
Mean age at amputation in years (M, SD)	35.9 ± 17.7	15.0 ± 10.6		0.05
Handedness (before amputation) N right/left	5/2	6/1	9/0	
Side of amputation N right/left	2/5	5/2		
Amputation of the dominant hand N yes/no	4/3	4/3		
Traumatic amputation N yes/no	6/1	7/0		
Prosthesis: N myoelectric/cosmetic/none	3/1/3	1/2/4		
Subjects with telescoping N yes/no	4/3	4/3		
Subjects with non-painful phantom phenomena N yes/no	7/0	5/2		
Questionaire upon Mental Imagery (QMI)[a] (M, SD, range 1–7)	2.7 ± 0.7	2.9 ± 0.4	2.4 ± 0.5	n.s.
Multidimensional Phantom Limb Pain Inventory – German version (MPI)				
Intensity of phantom limb pain[b] (M, SD, range 0–6)	2.7 ± 1.2	0.0 ± 0.0		0.001
Intensity of residual limb pain[b] (M, SD, range 0–6)	1.4 ± 1.6	0.3 ± 0.4		n.s.
Visual analogue scale of phantom limb sensation[c] (M, SD, range 0–100)	57.3 ± 31.6	34.2 ± 35.3		n.s
Visual analogue scale of residual limb sensation[c] (M, SD, range 0–100)	13.7 ± 2 0.1	17.1 ± 17.5		n.s
Intensity of phantom limb pain before measurement[b] (M, SD, range 0–6)	2.6 ± 1.3	0.0 ± 0.0		0.001
Intensity of residual limb pain before measurement[b] (M, SD, range 0–6)	1.1 ± 1.7	0.0 ± 0.0		n.s.
Phantom limb pain during movements in front of the mirror[b] (M, SD, range 0–6)	0.0 ± 0.0	0.0 ± 0.0		n.s.
Phantom limb sensation during movements in front of the mirror[d] (M, SD, range 0–6)	0.4 ± 0.5	0.7 ± 0.5		n.s.
Phantom limb pain during imagined movements[b] (M, SD, range 0–6)	0.1 ± 0.4	0.0 ± 0		n.s.
Phantom limb sensation during imagined movements[d] (M, SD, range 0–6)	0.29 ± 0.5	0.29 ± 0.5		n.s.
Mirror image belongs to phantom/non-dominant hand[a] (M, SD, range 1–7)	5.1 ± 2.3	5.3 ± 1.7	6.4 ± 1.1	n.s.
Vividness of imagined hand movement at the measurement[a] (M, SD, range 1–7)	3.3 ± 0.9	3.7 ± 1.4	2.8 ± 1.1	n.s.

[a] 1 = perfectly clear and vivid, 7 = no image present at all.
[b] 0 = no pain, 6 = very intense pain.
[c] VAS = 0–100.
[d] 0 = No sensation, 6 = very intense sensation.

mirror image belongs to the phantom/non-dominant hand? How vivid was the imagination of the hand movement? All answers were rated on a scale ranging from 1 = perfectly clear and vivid to 7 = no image present at all.

2.3. Training

To avoid muscle activity during imagery, the subjects were trained to imagine movements while electromyographic (EMG) recordings of the musculus extensor digitorum of the intact hand or the most proximal muscle of the residual limb were taken prior to functional magnetic resonance imaging (fMRI) in the laboratory. The training consisted of 10 blocks of ∼90 s each and 4 min rest between the blocks and lasted for 1 h. EMG levels during imagined movements were fed back and the training continued until the EMGs did no longer exceed the baseline level.

2.4. Experimental procedure

The experiment consisted of three parts in randomized order. In the 'executed movement' condition the participants were instructed to make a fist with the intact (amputees) or dominant (HC) hand with a frequency of 0.5 Hz. They watched their intact or dominant hand in this condition. In the condition 'mirrored movements' the subjects moved the intact (amputees) or dominant hand (HC) in the same manner and watched the mirror image in the mirror box placed on the belly of the subject (see Fig. 1) through a mirror fixed on the fMRI head coil. In the condition 'imagined movements' the amputees imagined making a fist with the phantom and the HC with the non-dominant hand. For all conditions the subjects had their eyes open. All movements or imagined movements were paced externally by a metronome at the rate of 0.5 Hz, whose sound was delivered by earphones. All conditions were separate blocks of fMRI measurements with durations of about 3 min each and were separated by breaks of about 5 min.

2.5. fMRI measurement

The fMRI scans were conducted with a Siemens 1.5 T scanner using echoplanar imaging (EPI, matrix $64 * 64$, TE = 60 ms, TR = 3.3 s) and 24 slices of 4 mm thickness (1 mm gap, in-plane resolution = $3.44 * 3.44$ mm) angulated in parallel to the AC–PC line and adjusted to include all frontal, central, parietal and occipital cortical areas as well as upper parts of the temporal cortex and the cerebellum. Fifty-seven whole-brain scans including 4 blocks of executed or imagined movements with 6 scans each interspersed with 5 blocks of 6 scans of rest were gathered per condition and the first three volumes were excluded from the analysis to allow for signal stability following onset transients. This sums up to 80 s of executed or imagined movements per condition. For anatomical reference a T1-weighted anatomical data set (MPRAGE; slice thickness 1 mm, no gap, TR 11.4 ms, TE 4.4 ms, flip angle 12°) was obtained.

Fig. 1. The person's view of the moving hand and its mirror image.

2.6. Statistical analysis

Functional MRI data were evaluated with SPM2 (Wellcome Institute of Imaging Neuroscience, London, UK) implemented in Matlab 6.1 (Mathworks Inc., Natick, MA). To test for possible hemisphere-specific effects the same calculations were performed with reduced patient samples that included only patients with right- or left-sided amputations. These results did not reveal hemisphere-specific activations and therefore all the patients were included in one calculation with a virtual left side amputation. Thus, for patients with an amputation on the right side the data were flipped sagitally to obtain a "homogeneous" sample of patients with a left side amputation. Then the data were realigned, corrected for slice-timing effects, normalized to a template (Montreal Neurological Institute, MNI) and smoothed with Gaussian kernel of 9 mm^3 (full-width at half-maximum). Separate random effect models were used for each group (PLP, non-PLP and HC) and condition (executed, mirrored and imagined movements). In the second order analysis the groups (PLP, non-PLP and HC) were compared by single t-tests. We report significant voxels with $p < 0.05$ (false discovery rate (FDR) corrected and an extent threshold of 15 voxels ($45 \times 45 \times 45$ mm) [45]. For group comparisons in the group map regions of interest (ROIs) that included primary somatosensory cortex, secondary somatosensory cortex, primary motor cortex, and the supplementary motor area were defined with MARINA 0.6.1 (Bender Institute of Neuroimaging, Giessen, Germany) by using the anatomical structures in the programme (pre-, post-central gyrus, paracentral lobule, supplementary motor area, and rolandic operculum) and were used for small volume correction. Pearson correlation analyses were performed by correlating the individual maximum b values of a sphere with 10 mm radius around the maximum peak in the activation cluster of the one-sample t-test of both patient groups (PLP, non-PLP) for the three conditions (executed, mirrored and imagined movements) with the patients' individual amount of phantom limb pain as assessed by the MPI (for the amputees without pain the value 0 was entered) [44]. For the fMRI-data effect sizes were computed by using the t-values from the peak in the activated cluster with the following formula for comparisons between two groups: $e = t/\sqrt{[(n1 + n2)/(n1 * n2)]}$. We used the effect sizes as a measure to compare the amount of activation between groups and conditions. Statistical analyses of demographic and clinical data were performed using SPSS for Windows (version 12.0.1; SPSS Inc., 2003). Repeated measures analyses of variance (ANOVAs) and Bonferroni-corrected t-tests were employed.

3. Results

3.1. Clinical data

As expected, phantom limb pain and residual limb pain differed significantly between the three groups (see Supplementary data and Table 1 for details). Imagination skills measured by the Questionnaire upon Mental Imagery (QMI) and the vividness of imagery during measurement were not significantly different between the three groups. Neither the PLP nor the non-PLP group reported any phantom or residual limb pain during the experiment.

3.2. fMRI-data

No significant deactivations were found for any condition.

3.3. Condition 'mirror movement'

For movement of the right hand (which was the dominant hand in the controls and the intact hand in the patients with sagitally flipped data for right hand amputees [c.f. 30]) in front of the mirror box only the non-PLP and the HC showed significant activation in the primary motor and somatosensory cortex in the hemisphere contralateral to the hand that was perceived in the mirror (see Figs. 2 and 3 and Table 2). Group contrasts revealed significantly more activation for the non-PLP compared to the PLP group in primary somatosensory cortex ($t_{(12)} = 5.61$, $p = 0.013$), and primary motor cortex ($t_{(12)} = 3.93$, $p = 0.049$) contralateral to the hand seen in the mirror (and ipsilateral to the hand moved in front of the mirror) and primary somatosensory cortex ($t_{(12)} = 5.89$, $p = 0.03$) ipsilateral to it (contralateral to the executed movement, see Fig. 4 and Table 3). The non-PLP group also showed significantly more activation than the HC in primary motor cortex contralateral to the hand viewed in the mirror ($t_{(14)} = 5.34$, $p = 0.05$). All three groups equally activated primary motor cortex and primary somatosensory cortex in the hemisphere contralateral to the actually moved hand. Effect sizes in the hemisphere contralateral to the mirrored hand were highest for the non-PLP followed by the HC and could not be computed for the PLP group. The non-PLP group showed additional activation in the right secondary somatosensory cortex (contralateral to

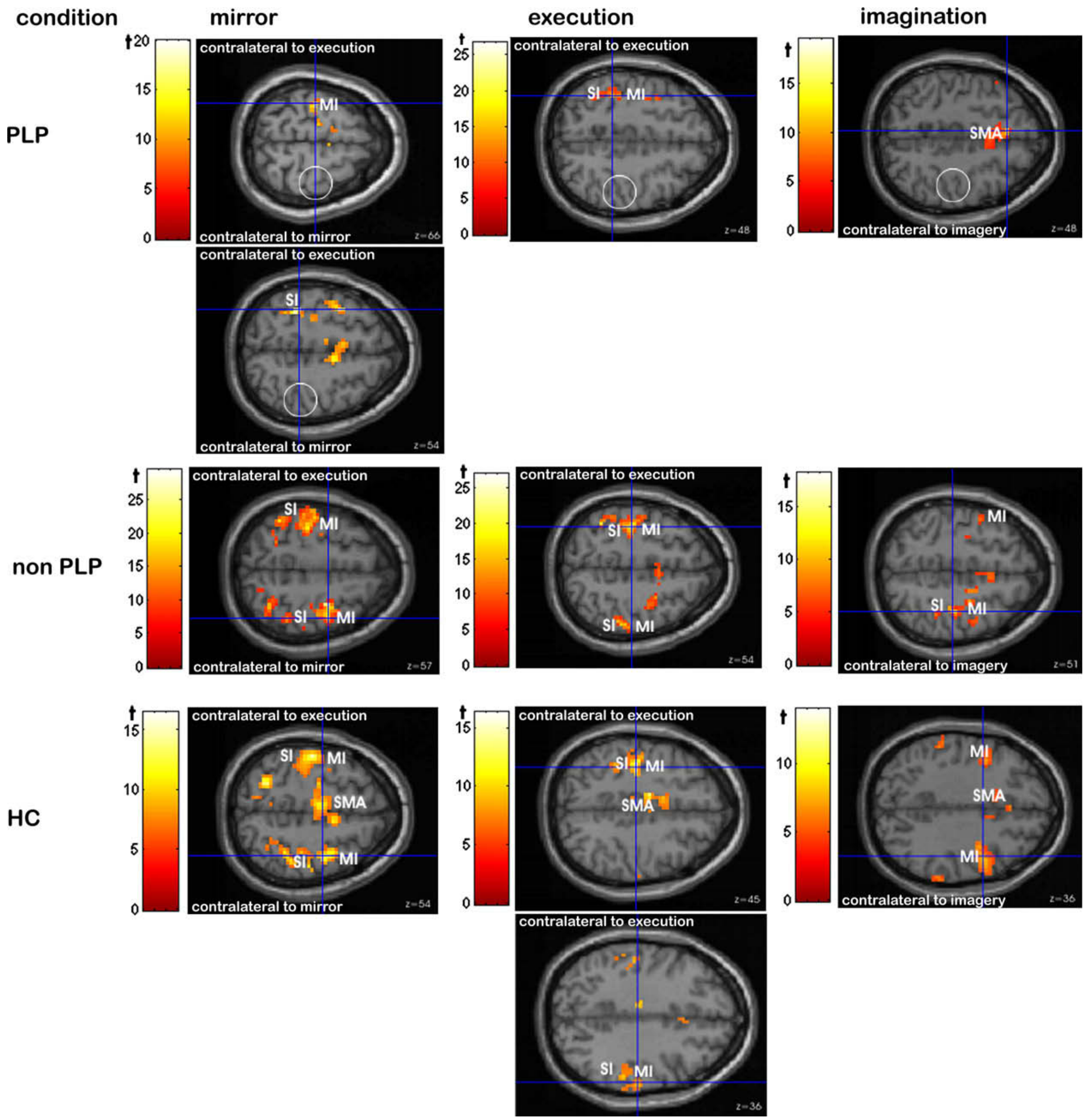

Fig. 2. Brain activation during hand movements in front of the mirror, executed movements and imagination of movement for the amputees with phantom limb pain (PLP), the amputees without phantom limb pain (non-PLP) and the controls (HC) The circle shows the missing activation in primary sensorimotor cortex in the PLP group. Montreal Neurological Institute (MNI) coordinates. Subjects executed movements with the right hand in the mirror and the executed movement conditions. The reflection in the mirror showed a left hand. In the imagination condition the left hand was moved in the HC and the phantom hand in the PLP and non-PLP.

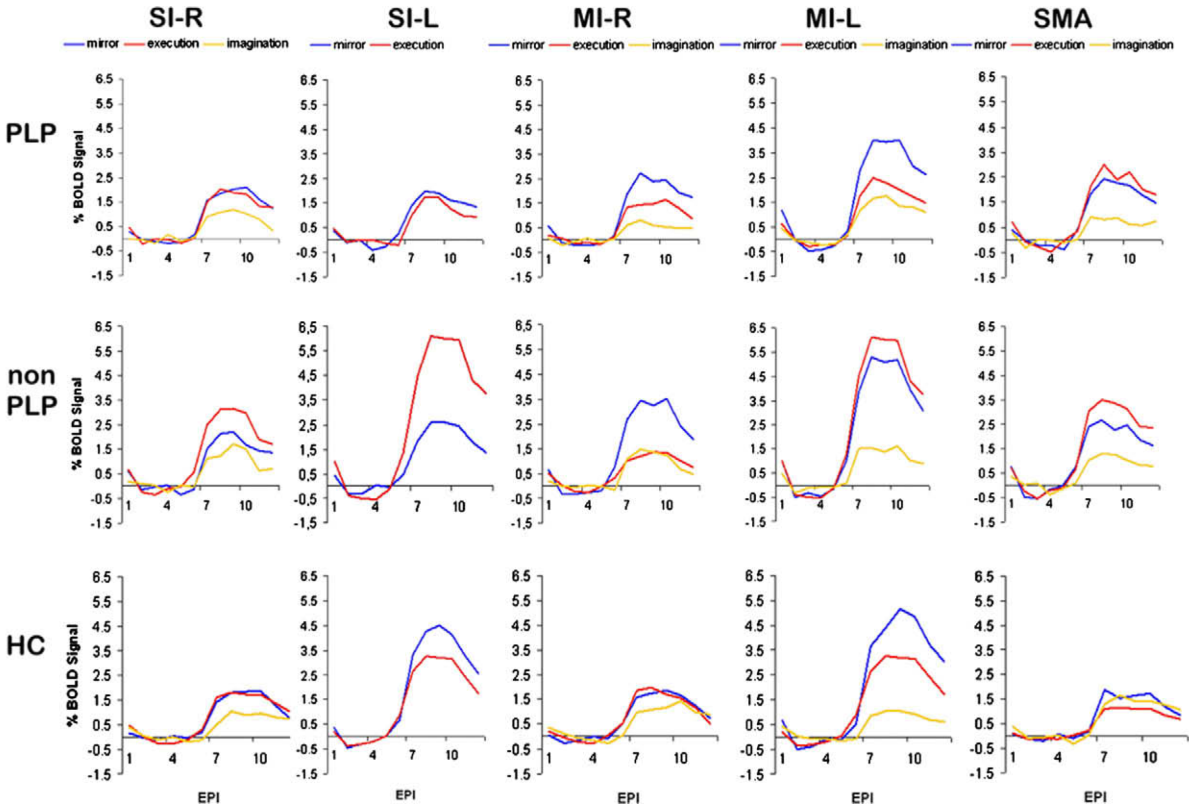

Fig. 3. Time courses of the maximally activated voxel during hand movements in front of the mirror, executed movements and imagination of movement for the amputees with phantom limb pain (PLP), the amputees without phantom limb pain (non-PLP) and the controls (HC).

the hand viewed in the mirror) and the PLP and the HC in the supplementary motor area (see Tables 2 and 3). The contrast between PLP and HC revealed no significant activation differences.

3.4. Condition ‘executed movement’

During execution of right hand movements (the dominant = right hand in the controls and the intact hand in the patients with sagitally flipped data for right hand amputees) all three groups activated primary motor cortex and primary somatosensory cortex contralateral to the moved hand (see Figs. 2 and 3 and Table 4). Effect sizes in the hemisphere contralateral to the moved hand were highest for the non-PLP followed by the PLP and the HC. The non-PLP group and the HC also significantly activated primary motor cortex and primary somatosensory cortex ipsilateral to the moved hand and the supplementary motor area with highest effect sizes for the non-PLP followed by the HC. Contrasts between the groups revealed significantly more activation for the non-PLP versus the PLP, but not the HC group in the contralateral primary somatosensory cortex ($t_{(12)} = 6.61$, $p = 0.03$) and the ipsilateral primary somatosensory cortex ($t_{(12)} = 6.54$, $p = 0.03$) compared to the PLP group (see Fig. 4 and Table 3). The contrast between PLP and HC and that between non-PLP and HC revealed no significant activation differences.

Table 2
Brain regions and coordinates of activation for the three groups in the mirror condition.

Group	BA		H[a]	x	y	z	t-Value	z-Value	p-Value[b]	Voxel[c]
PLP	1–3	Primary somatosensory cortex	L	−36	−36	54	19.27	4.85	0.01	54
	4	Primary motor cortex	L	−33	−21	66	19.52	4.86	0.01	51
	6	Supplementary motor area	M	9	−6	54	14.07	4.46	0.01	124
Non-PLP	1–3	Primary somatosensory cortex	R	60	−21	33	18.86	4.82	0.005	158
	1–3	Primary somatosensory cortex	L	−54	−21	54	24.85	5.14	0.005	466
	4	Primary motor cortex	R	42	−6	57	25.04	5.15	0.005	131
	4	Primary motor cortex	L	−36	−24	57	19.68	4.87	0.005	466
		Secondary somatosensory cortex	R	54	−12	12	16.56	4.66	0.005	16
HC	1–3	Primary somatosensory cortex	R	36	−42	57	10.29	4.50	0.002	214
	1–3	Primary somatosensory cortex	L	−48	−18	54	14.80	5.06	0.002	355
	4	Primary motor cortex	R	39	−9	54	15.09	5.08	0.002	214
	4	Primary motor cortex	L	−42	−24	63	13.28	4.89	0.002	355
	6	Supplementary motor area	M	−9	−12	48	14.35	5.01	0.002	439

Non-PLP, no phantom limb pain ($n = 7$); PLP, phantom limb pain ($n = 7$); HC, healthy controls ($n = 9$); BA, Brodman area; H, hemisphere.

[a] Data sagittaly flipped for right hand amputees, R, right hemisphere (contralateral to the mirrored hand); L, left hemisphere (ipsilateral to the mirrored hand); M, medial.

[b] False discovery rate corrected.

[c] Voxel 3 * 3 * 3; Montreal Neurological Institute (MNI) coordinates.

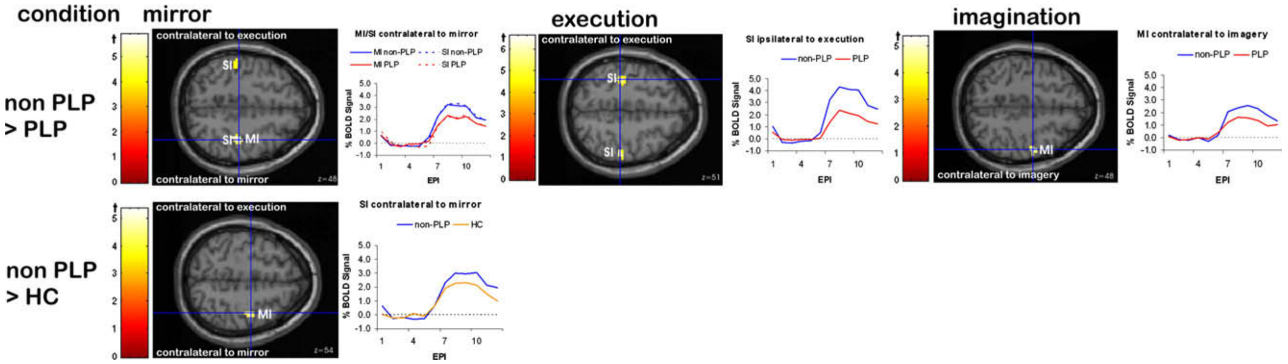

Fig. 4. Brain activation and time courses of the maximally activated voxel for significant contrasts between groups during the movement in front of the mirror, executed movements and imagination of movement. Only the significant contrasts and the time courses in these regions between the amputees with phantom limb pain (PLP), the amputees without phantom limb pain (non-PLP) and the controls (HC) are displayed. Montreal Neurological Institute (MNI) coordinates.

3.5. Condition ‘imagined movement’

For movement imagery of the left hand (which was the non-dominant hand in the controls and the amputated hand in the patients with sagittaly flipped data for right hand amputees) all three groups activated the supplementary motor area (see Figs. 2 and 3 and Table 5). The HC and the non-PLP also activated primary motor cortex bilaterally to the imagined movements. Effect sizes in both hemispheres were highest for the non-PLP followed by the HC. Additionally, the non-PLP group showed activation in primary somatosensory cortex and the secondary somatosensory cortex contralateral to the imagined movements. Contrasts between the PLP and the non-PLP group showed significantly more activation for the non-PLP group ($t_{(12)} = 5.34$, $p = 0.03$) in primary motor cortex contralateral to the imagined hand/phantom than for the PLP group (see Fig. 4 and Table 3). The contrast between PLP and HC and that between non-PLP and HC revealed no significant activation differences.

3.6. Correlations between fMRI-data and the amount of phantom limb pain

We correlated brain activations with the presence and amount of phantom limb pain as assessed by the MPI for the amputees. The presence and magnitude of phantom limb pain were significantly negatively correlated with the activation during the mirror movement condition in primary somatosensory and motor cortex contralateral to the hand seen in the mirror and primary somatosensory cortex ipsilateral to the hand seen in the mirror (see Fig. 5 and Supplementary material Table S1). Phantom limb pain was also significantly negatively correlated with the activation during the executed movement condition in primary motor cortex ipsilateral to the moved hand. During the imagined movement condition there was no significant correlation with phantom limb pain.

4. Discussion

This study yielded several important results. First, it was shown that viewing movements of one’s own hand in a mirror evokes activity in SI and MI contralateral to the hand that is perceived in the mirror, in addition to similar activations in the hemisphere contralateral to the hand that is actually moving. This finding is in accordance with the literature on illusory perceptions, which has shown that the brain represents the perception rather than the actual physical stimulus [2,6]. The mirror-related activation pattern in both SI and MI was highest for the non-PLP group with a focus in MI when a direct comparison between the conditions was made, followed by the HC and was nonexistent in the PLP group. Amputees with PLP thus failed to activate the SI/MI contralateral to the hand perceived in the mirror whereas persons without PLP activated it even more than the HC. A transcranial magnetic stimulation (TMS) study in healthy persons found that viewing a mirror reflection of one’s hand that conducted unilateral finger–thumb opposition movements facilitated activity in the motor cortex ipsilateral to the moved hand [15]. It was previously shown that amputees with PLP in contrast to those without PLP show coactivation of the deafferented zone in SI and MI when neighboring regions are stimulated in the periphery (e.g. [12,25]). The larger the shift of neighboring activations into the cortical

Table 3
Brain regions and coordinates of activation for significant contrasts between the three groups for the three conditions as well as significant contrasts between condition mirror and execution.

Group comparisons	Con	BA		H[a]	*x*	*y*	*z*	*t*-Value	*z*-Value	*p*-Value[b]	Voxel[c]	Effect size
Non-PLP > PLP	Mir	1–3	Primary somatosensory cortex	R	48	−27	51	5.61	3.86	0.049	16	3.50
	Mir	1–3	Primary somatosensory cortex	L	−39	−24	48	5.89	3.96	0.03	42	3.53
	Mir	4	Primary motor cortex	R	39	−15	57	3.93	3.09	0.049	42	3.00
	Exe	1–3	Primary somatosensory cortex	R	51	−30	54	6.54	4.19	0.033	39	3.15
	Exe	1–3	Primary somatosensory cortex	L	−36	−30	51	6.61	4.21	0.025	55	2.10
	Ima	4	Primary motor cortex	R	51	−9	48	5.34	3.75	0.029	20	2.85
Non-PLP > HC	Mir	4	Primary motor cortex	R	39	−12	54	5.34	3.88	0.05	17	2.69

Non-PLP, no phantom limb pain ($n = 7$); PLP, phantom limb pain ($n = 7$); HC = healthy controls ($n = 9$); Con, condition; Exe, executed movements; Mir, mirrored movements; Ima, imagined movements; BA, Brodman area.

[a] Data sagittaly flipped for right hand amputees, H, hemisphere; R, right hemisphere (ipsilateral to the moved hand, contralateral to mirrored and imagined movements); L, left hemisphere (contralateral to the moved hand, ipsilateral to mirrored and imagined movements).

[b] False discovery rate corrected.

[c] Voxel 3 * 3 * 3; Montreal Neurological Institute (MNI) coordinates.

Table 4
Brain regions and coordinates of activation for the three groups for the condition execution.

Group	BA		H[a]	x	y	z	t-Value	z-Value	p-Value[b]	Voxel[c]
PLP	1–3	Primary somatosensory cortex	L	−39	−36	48	19.28	4.85	0.013	122
	4	Primary motor cortex	L	−36	−3	54	16.61	4.67	0.013	49
Non-PLP	1–3	Primary somatosensory cortex	R	51	−33	51	18.35	4.79	0.007	60
	1–3	Primary somatosensory cortex	L	−36	−24	54	26.91	5.23	0.007	241
	4	Primary motor cortex	R	36	−9	48	21.74	4.99	0.007	55
	4	Primary motor cortex	L	−36	−24	54	26.91	5.23	0.007	241
	6	Supplementary motor area	M	6	0	57	19.35	4.85	0.007	51
HC	1–3	Primary somatosensory cortex	R	60	−21	36	9.79	4.42	0.003	278
	1–3	Primary somatosensory cortex	L	−39	−21	45	16.31	5.20	0.003	419
	4	Primary motor cortex	R	63	6	21	10.51	4.53	0.003	50
	4	Primary motor cortex	L	−39	−21	45	16.31	5.20	0.003	419
	6	Supplementary motor cortex	M	−12	−12	54	15.14	5.09	0.003	305

Non-PLP, no phantom limb pain ($n = 7$); PLP, phantom limb pain ($n = 7$); HC, healthy controls ($n = 9$); BA, Brodman area; H, hemisphere.

[a] Data sagittaly flipped for right hand amputees, R, right hemisphere (ipsilateral to the moved hand); L, left hemisphere (contralateral to the moved hand); M; medial.

[b] False discovery rate corrected.

[c] Voxel 3 * 3 * 3; Montreal Neurological Institute (MNI) coordinates.

amputation zone, the more phantom limb pain was present. Conversely, the use of myoelectric prostheses or sensory discrimination training both of which are thought to restore the activation of the cortical representation of the amputated limb, led to the activation of these zones and reduced phantom limb pain (e.g. [11,28]). The lack of activation in sensorimotor areas contralateral to the mirror in the mirror condition in the PLP patients and the enhanced activation in the non-PLP patients is in accordance with the assumption that this cortical reorganization may be an important covariate for the presence of PLP. This was supported by the significant negative correlation between PLP and activation in MI contralateral to the hand viewed in the mirror, which was due to the high activation in the non-PLP compared to the PLP since the correlation in the PLP group alone was not significant ($r = -.06$). This is in accordance with our previous findings on the relationship of PLP, cortical reorganization and prosthesis use [12,28].

Previously a mirror box was used to loosen cramps and the sensation of touch and pain in the phantom limb [37,38]. The findings by Moseley et al. [34], Brodie et al. [3] and Chan et al. [5] who demonstrated reduction in phantom limb pain after mirror training suggest that pain and cortical reorganization can potentially be altered by visual feedback. It is well known that vision tends to take precedence over the other senses (touch included) when conflicting information is presented to vision and another sense [20,40]. However, it should be noted that both Ramachandran and Rogers-Ramachandran [38] who originally reported on the effects of mirror training on phantom pain and Chan et al. [5] employed concurrent imagery of phantom hand movement. Possibly, the coactivation of the brain region involved in sensation and movement of the limb may be needed (see below our discussion of the effects of imagined movement).

Execution of hand movements led to the activation of the contralateral SI and MI in all three groups. These results are consistent with the previous findings [27]. In addition, the non-PLP and the HC activated the ipsilateral motor and somatosensory cortex similar to but less pronounced than the mirror condition. Activation in the MI and pre-motor cortex is associated with self and other attribution of movements [9,24] as well as movement simulation or preparation for imagined movement. Fadiga et al. [8] postulated that the sensorimotor neurons located in the ventral part of the monkey pre-motor cortex motor system not only are involved in the execution of actions but also internally represent them in terms of ‘motor ideas’. Neurons in the same area responded to the position of a visible, realistic false arm [19] and it is thought that they bind visual and proprioceptive cues [18]. The fact that executed movement also showed differential activation for the PLP and non-PLP groups is in accordance with the findings in complex regional pain syndrome type 1 that show that movement and stimulation of one hand also transfer to the other hand [1] and are in accordance with the finding of Brodie et al. [3] that mere movement of the intact hand without a mirror also led to a change in phantom pain and phantom sensation.

Imagination of movement in the phantom led to activity in motor areas such as the supplementary motor area (all three groups) and in the contralateral and ipsilateral MI in the area representing the imagined hand in the non-PLP and the HC. These findings are in

Table 5
Brain regions and coordinates of activation for the three groups in the imagery condition.

Group	BA		H[a]	x	y	z	t-Value	z-Value	p-Value[b]	Voxel[c]
PLP	6	Supplementary motor area	M	−6	24	48	15.77	4.60	0.124	162
Non-PL	1–3	Primary somatosensory cortex	R	39	−39	54	11.48	4.20	0.056	295
	4	Primary motor cortex	R	36	−24	51	15.47	4.58	0.056	295
	4	Primary motor cortex	L	−48	3	39	9.28	3.92	0.056	43
	6	Supplementary motor area	M	9	12	54	9.87	4.00	0.056	30
		Secondary somatosensory cortex	R	54	6	−3	10.94	4.14	0.056	164
HC	4	Primary motor cortex	R	45	3	36	8.32	4.15	0.028	217
	4	Primary motor cortex	L	−39	6	33	7.47	3.97	0.028	129
	6	Supplementary motor area	M	9	3	57	12.39	4.79	0.028	368

Non-PLP, no phantom limb pain ($n = 7$); PLP, phantom limb pain ($n = 7$); HC, healthy controls ($n = 9$); BA = Brodman area; H, hemisphere.

[a] Data sagittaly flipped for right hand amputees, R, right hemisphere (contralateral to imagined movements); L, left hemisphere (ipsilateral to imagined movements); M, medial.

[b] False discovery rate corrected.

[c] Voxel 3 * 3 * 3; Montreal Neurological Institute (MNI) coordinates.

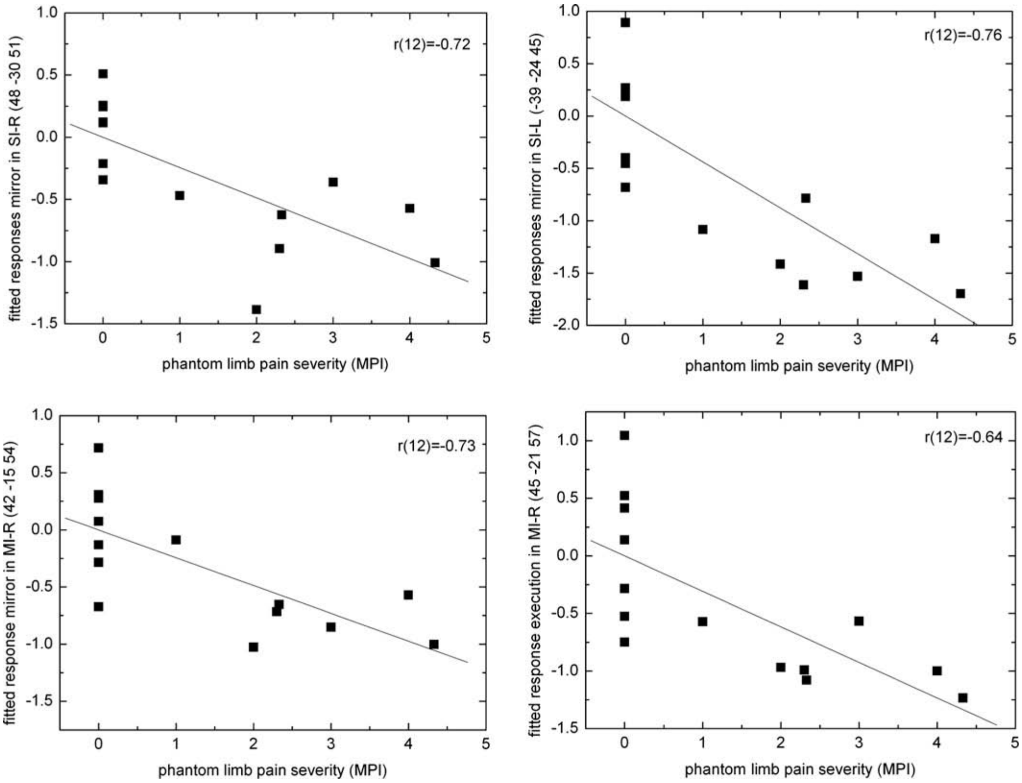

Fig. 5. Correlation plot between functional magnetic resonance imaging data for the conditions mirror and executed movements and the West Haven-Yale Multidimensional Phantom Limb Pain Inventory Pain Severity scale pooled for all amputees. Montreal Neurological Institute (MNI) coordinates.

accordance with the previous reports on imagined phantom movements in amputees [7,27,29,41,42] as well as the results from a TMS study [32]. Activation in SI was reported in some studies [29,41,42], whereas others [7,29] reported only activation in MI. It must be noted that some of these studies were case studies [7,41] or only performed in HC [29]. As noted before prolonged imagery reduced phantom limb pain and led to reactivation of the cortical area representing the amputated limb or a symmetrical representation of activity in neighboring zones [16,30]. In our study, PLP compared to non-PLP patients showed a lack of activation in MI ipsi- and contralateral to the imagined limb in accordance with these findings. The large effect sizes for all conditions suggest that all three interventions may affect PLP and cortical reorganization, however, the mirror condition may add extra activation due to the added visual input [35].

This study has several limitations. First, we had only a small and inhomogeneous sample of patients in terms of side of amputation. For this reason we flipped the data of patients with a right amputated hand to create a sample of "left hand amputated" patients like MacIver et al. [30]. Since brain activations can be hemisphere specific, we also analyzed the data in a non-flipped manner to determine hemisphere-specific activations. This analysis revealed comparable results, which prompted us to use flipped results and to include all subjects in one analysis. Our groups differed in time since amputation and age since amputation. It is well known that amputees without phantom pain are usually amputated at an earlier age than those with phantom pain. It has been suggested that compensatory reorganizational changes are more easily achieved at an earlier age. This fact should, however, not have influenced our results. Another factor is the reliability and validity of pain measurements in amputees. It has been shown that phantom limb pain is relatively stable over the time. A longitudinal study by Jensen et al [23] found that the incidence of PLP remained constant over 2 years following lower extremity amputation and Hunter et al. [22] found that the initial phantom limb pain in upper extremity amputees correlated significantly with the phantom limb pain at 2-year follow-up. This suggests that our pain data reflect the status of these patients quite well. Future studies need to employ larger and stratified samples with multiple measurements. In addition, we did not use concurrent EMG assessments but only assessed arm movements during the imagery condition outside the scanning session. Future studies need to use simultaneous assessments of muscular activity since this can influence brain activation [c.f. 39]. Moreover, we only asked the patients to view the intact arm in the mirror and did not ask them to actually move the phantom along with the hand seen in the mirror. Addition of this imagery condition might have strengthened the results.

Our data show a lack of activation in MI and SI in the mirror and the imagery as well as the executed movement conditions in amputees with PLP compared to those without pain. Even though this effect was obvious in all three conditions, mirrored movements seemed to have the greatest effect on the activation in this area and were most closely related to phantom pain, most likely due to the multisensory integration present in this condition [17]. A longitudinal study of these effects is still lacking and it has not yet been determined how a combination of interventions such as in graded motor imagery therapy might affect brain activation and pain in amputees with phantom limb pain and other persons with chronic pain.

Conflict of interest

None of the authors declares any conflict of interest for this paper.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.pain.2010.02.020.

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