Amputees can move their phantom limb at will. These ‘movements without movements’ have generally been considered as motor imagery rather than motor execution, but amputees can in fact perform both executed and imagined movements with their phantom and they report distinct perceptions during each task. Behavioural evidence for this dual ability comes from the fact that executed movements are associated with stump muscle contractions whereas imagined movements are not, and that phantom executed movements are slower than intact hand executed movements whereas the speed of imagined movements is identical for both hands. Since neither execution nor imagination produces any visible movement, we hypothesized that the perceptual difference between these two motor tasks relies on the activation of distinct cerebral networks. Using functional magnetic resonance imaging and changes in functional connectivity (dynamic causal modelling), we examined the activity associated with imagined and executed movements of the intact and phantom hands of 14 upper-limb amputees. Distinct but partially overlapping cerebral networks were active during both executed and imagined phantom limb movements (both performed at the same speed). A region of interest analysis revealed a ‘switch’ between execution and imagination; during execution there was more activity in the primary somatosensory cortex, the primary motor cortex and the anterior lobe of the cerebellum, while during imagination there was more activity in the parietal and occipital lobes, and the posterior lobe of the cerebellum. In overlapping areas, task-related differences were detected in the location of activation peaks. The dynamic causal modelling analysis further confirmed the presence of a clear neurophysiological distinction between imagination and execution, as motor imagery and motor execution had opposite effects on the supplementary motor area–primary motor cortex network. This is the first imaging evidence that the neurophysiological network activated during phantom limb movements is similar to that of executed movements of intact limbs and differs from the phantom limb imagination network. The dual ability of amputees to execute and imagine movements of their phantom limb and the fact that these two tasks activate distinct
cortical networks are important factors to consider when designing rehabilitation programmes for the treatment of phantom limb pain.

Keywords: phantom limb; motor execution; motor imagery; brain imaging; connectivity
Abbreviations: M1 = primary motor cortex; SMA = supplementary motor area

Introduction

Amputees often continue to feel the presence of their missing limb and report that the phantom limb has certain sensory properties like touch and pain (Mitchell, 1872). The phantom limb may also be endowed with kinaesthetic properties like being able to be moved voluntarily (Jensen et al., 1983). Although scientists and medical doctors agree that phantoms are not imaginary but arise from physiological changes that occur after amputation (Melzack, 1992; Ramachandran and Hirstein, 1998) many scientific papers assume that since phantom movements cannot be observed they must be imaginary movements. However, there is increasing evidence that despite the absence of any visible movement, voluntary phantom limb movements are not imaginary, as they are accompanied by the distinct perception that the joints of the now-missing limb have moved—a perception that is not associated with imagined movements.

Patients suffering from phantom limb pain are sometimes offered a form of rehabilitation that involves visual feedback training in which they see modified visual feedback of their missing limb using either mirrors (Ramachandran and Rogers-Ramachandran, 1996; Chan et al., 2007) or pre-recorded and flipped videos of movements of their intact hand (Giraud and Sirigu, 2003; Mercier and Sirigu, 2009). During this training they are encouraged to move their phantom limb in synchrony with the movements they observe. Our conversations with these patients revealed that some of them spontaneously distinguish between executing and imagining a movement with the absent limb. Since neither execution nor imagination produces any overt movement we administered a series of behavioural tasks to assess the validity of these anecdotal reports and found that the distinct kinaesthetic sensations experienced during phantom limb execution and imagination were paralleled by performance differences (Raffin et al., 2011). Specifically, we found that motor execution with the phantom was substantially slower than with the intact limb, whereas the time to imagine a movement was similar for both the phantom and intact limbs. These results suggest that amputees can perform motor execution and motor imagery with their phantom limb and that limb loss differentially affects these two tasks. Patients with spinal cord injury also maintain the ability to perform motor imagery with paralysed body parts while at the same time experiencing modified kinaesthetic sensations during voluntary movements of their phantom limb (Hotz-Boendermaker et al., 2008). In contrast, a number of pathologies affecting either central or peripheral motor pathways lead to altered performance in both motor imagery and execution (Johnson, 2000; Fiorio et al., 2007; Helmich et al., 2007; Conson et al., 2008; Gieteling et al., 2008). In order to understand the differential effect of amputation on motor imagery and motor execution processes it is essential to examine the neurophysiological networks underlying these two tasks. The characterization of these networks will also help elucidate the mechanisms underlying visual rehabilitation therapies, the efficacy of which may depend upon whether patients make imagined or executed movements with their phantom limb.

A number of studies have examined the cerebral activity associated with phantom limb movements (Erland et al., 1996; Hugdahl et al., 2001; Lotze et al., 2001; Rosen et al., 2001; Roux et al., 2001, 2003), but in most of these it is unclear whether amputees were asked to execute or imagine, and little (if any) detail is provided concerning the amputees’ subjective movement perceptions. All of these studies, however, report activity in motor areas, such as the contralateral primary motor cortex (M1) and the supplementary motor area (SMA), which suggests that the default for amputees might be to make executed rather than imagined phantom limb movements. Further support for the idea that amputees perform motor execution by default comes from a functional MRI study by Roux et al. (2003) who found that executed movements of healthy subjects and ‘virtual movements’ of amputees (n = 3) activated a similar network, and that this network differed from that activated during imagined movements of healthy subjects (Roux et al., 2003). Taken together, these studies suggest that phantom limb movements are similar to executed movements in healthy subjects and are not a form of motor imagery, but since imagined and executed movements of the phantom limb have never been directly compared using neuroimaging techniques, the evidence for this argument remains indirect.

Studies of motor imagery in healthy subjects reveal that motor imagery systematically recruits the cerebellum, as well as parietal and premotor areas (including the SMA) (Roland et al., 1980; Decety et al., 1994; Lotze et al., 1999b; Hanakawa et al., 2003). Some studies also show activity in visual areas, especially if subjects perform visual rather than kinaesthetic motor imagery (Kosslyn, 1999; Solodkin et al., 2004). Activity in M1 is frequently absent during motor imagery of upper limb movement (Decety et al., 1994; Stephan et al., 1995; Gerardin et al., 2000; Romero et al., 2000; Hanakawa et al., 2003, 2008; Meister et al., 2004), and in those studies that do show M1 activity during imagination there is always less activity during imagination than execution (Porro et al., 1996; Roth et al., 1996; Lotze et al., 1999b; Dechent et al., 2004; Lacourse et al., 2005; Guillot et al., 2009; Berman et al., 2011; Lorey et al., 2011). Although less active during imagination than during execution, M1 is clearly implicated in motor imagery processes, as a number of transcranial magnetic stimulation studies have shown task-specific increases in
corticospinal excitability during motor imagery (Fourkas et al., 2006; Stinear et al., 2006; Bakker et al., 2008; Kumru et al., 2008; Pelgrims et al., 2010; Roosink and Zijdewind, 2010).

Thus, motor execution and motor imagery share a partially overlapping network, including parts of the SMA, the dorsal premotor cortex, the posterior parietal cortex and the cerebellum as well as M1 (under certain conditions). In healthy subjects the two tasks can be dissociated neurophysiologically by (i) the magnitude of task-specific activity in certain areas; for example, M1 during execution versus visual areas during imagination (Roland et al., 1980; Decety et al., 1994; Stephan et al., 1995; Gerardin et al., 2000; Hanakawa et al., 2003); and (ii) the location of activity within common brain areas; for example, more caudal activations during execution than imagination within the dorsal premotor cortex (Decety et al., 1994; Stephan et al., 1995; Grafton et al., 1996; Lotze et al., 1999b; Gerardin et al., 2000). Techniques examining the ‘effective connectivity’ networks underlying motor execution and motor imagery have also shown a neurophysiological dissociation between the two tasks (Solodkin et al., 2004; Chen et al., 2009; Gao et al., 2011). For example, a dynamical causal modelling study in normal subjects nicely illustrated that even though SMA is active during both imagination and execution, the functional connectivity between SMA and M1 differs during the two tasks, with a facilitatory effect during execution and an inhibitory effect during imagination (Kasess et al., 2008).

To date there are no data concerning the neurophysiological networks underlying executed and imagined phantom limb movements in amputees. Here, we used functional MRI and dynamical causal modelling to investigate the cerebral networks recruited during execution and imagination with the phantom limb. We hypothesized that executed movements of the phantom and intact hands would activate a similar network and that this network would differ from that activated during imagined movements of the phantom and intact hands. We further hypothesized that these execution and imagination networks would resemble those observed in healthy subjects. We also expected our dynamical causal modelling analysis to show that imagined movements (whether performed with the phantom or intact hand) exert an inhibitory influence over the M1-SMA part of the network whereas executed movements (intact or phantom hand) excite this part of the network.

**Subjects and methods**

**Subjects**

Fourteen upper limb amputees (two females and 12 males), ranging in age from 18 to 68 years [mean age: 36.5, standard deviation (SD): 11.1] participated in the study. Amputation was caused by a traumatic accident 5 months to 30 years before testing (mean: 7.3 years, SD: 8.1). Thirteen were right-handed before the amputation, and there were nine right amputations and five left amputations, 10 above the elbow and four below the elbow. None of the patients had a history of neurological or psychiatric disease; they were paid for their participation and full written consent was obtained from all subjects in accordance with the Declaration of Helsinki. The study was approved by the local ethics committee (A 09-115). Detailed subject characteristics are shown in Table 1.

**Behavioural assessment**

The examination began with the collection of general clinical data related to the amputation and the phantom limb. We also assessed each subject’s ability to make voluntary movements with their phantom limb. To do this we asked subjects to voluntarily move their phantom limb by opposing each of the four fingertips to their thumb. We told them to perform this task at a speed that was comfortable and induced no pain and to repeat the sequence five times. The experimenter gave a ‘go’ signal and recorded the time between this signal and completion of the five movement cycles (indicated verbally by the amputee). Phantom limb motor control was measured as the time taken to complete the five cycles (faster times = better control) and these times were later correlated with imaging data acquired during executed opening/closing movements of the phantom hand.

**Functional magnetic resonance imaging task paradigm**

Before entering the scanner all subjects were provided with detailed instructions about the experimental procedure and underwent a training session in which they practiced executing and imagining opening/closing their phantom and intact hands. For the motor imagery condition, we emphasized that they should ‘feel’ themselves moving their hand (kinesthetic as opposed to visual imagery) and we ensured that they did not contract their stump muscles. For the motor execution condition, we told subjects to make opening/closing movements with their hand at a pace that was comfortable and did not increase their phantom pain when using the phantom hand. Each subject chose their own pace but for a given subject the same pace was used for all four experimental conditions (phantom execution, phantom imagination, intact execution and intact imagination). Subjects were placed in the scanner once they felt comfortable making executed and imagined movements with both their intact and phantom hands (after ~15 min of practice).

The functional MRI experiment was conducted in the context of a conventional block-design. In a single run, subjects were required to execute a voluntary movement of the hand (execution—15 blocks) or imagine the same movement (imagination—15 blocks). Two consecutive 30-block runs were acquired for both the intact and phantom limbs and the order in which the limbs were tested was counterbalanced across subjects. Execution and imagination blocks lasted 10 s and were fully randomized within the run with variable length intertrial intervals (baselines) of between 5 and 15 s. Each block began by presenting a letter on the screen for 1 s to instruct the subject which task to perform in that block (‘M’ for ‘movement’ or ‘I’ for ‘imagination’). Within each block auditory beeps were presented every 2, 3 or 5 s depending on the movement pace chosen by the subject.

**Magnetic resonance imaging scanning**

Data were obtained on a 1.5-T Siemens Sonata MRI system fitted with a video imaging system for on-line monitoring of subject movements throughout the scan. Functional images were acquired using a gradient echo, echoplanar imaging sequence: scan repeat time = 3030 ms, echo time = 60 ms, field of view = 220 mm, flip angle = 90°, matrix size...
Table 1 Clinical and phantom limb characteristics of each subject

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (years)</th>
<th>Time since amputation (months)</th>
<th>Amputated side/dominant side</th>
<th>Amputation level (proximal/distal)</th>
<th>Phantom pain intensity (VAS: 0–10)</th>
<th>Prosthesis type (per cent time)</th>
<th>Phantom movement times (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40</td>
<td>102</td>
<td>R/R</td>
<td>P</td>
<td>4</td>
<td>Myoelectric (100)</td>
<td>33.4</td>
</tr>
<tr>
<td>2</td>
<td>22</td>
<td>38</td>
<td>R/R</td>
<td>P</td>
<td>6</td>
<td>No</td>
<td>21.4</td>
</tr>
<tr>
<td>3</td>
<td>33</td>
<td>204</td>
<td>L/R</td>
<td>D</td>
<td>3</td>
<td>Aesthetic (100)</td>
<td>51.7</td>
</tr>
<tr>
<td>4</td>
<td>39</td>
<td>360</td>
<td>R/R</td>
<td>P</td>
<td>8</td>
<td>No</td>
<td>44.7</td>
</tr>
<tr>
<td>5</td>
<td>41</td>
<td>150</td>
<td>R/R</td>
<td>P</td>
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<td>No</td>
<td>20.2</td>
</tr>
<tr>
<td>6</td>
<td>27</td>
<td>84</td>
<td>L/R</td>
<td>P</td>
<td>9</td>
<td>Myoelectric (100)</td>
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</tr>
<tr>
<td>7</td>
<td>29</td>
<td>44</td>
<td>L/L</td>
<td>P</td>
<td>6</td>
<td>No</td>
<td>36.1</td>
</tr>
<tr>
<td>8</td>
<td>33</td>
<td>12</td>
<td>L/R</td>
<td>P</td>
<td>5</td>
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</tr>
<tr>
<td>9</td>
<td>27</td>
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<td>R/R</td>
<td>P</td>
<td>5</td>
<td>No</td>
<td>39.2</td>
</tr>
<tr>
<td>10</td>
<td>47</td>
<td>37</td>
<td>L/R</td>
<td>P</td>
<td>0</td>
<td>Aesthetic (100)</td>
<td>13.5</td>
</tr>
<tr>
<td>11</td>
<td>55</td>
<td>12</td>
<td>R/R</td>
<td>P</td>
<td>6</td>
<td>Mechanic (50)</td>
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</tr>
<tr>
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<td>20</td>
<td>118</td>
<td>R/R</td>
<td>D</td>
<td>7</td>
<td>No</td>
<td>60.1</td>
</tr>
<tr>
<td>13</td>
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<td>P</td>
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<td>No</td>
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</tr>
<tr>
<td>14</td>
<td>55</td>
<td>30</td>
<td>R/R</td>
<td>D</td>
<td>1</td>
<td>No</td>
<td>42.1</td>
</tr>
</tbody>
</table>

D = distal; L = left; P = proximal; R = right; VAS = visual analogue scale.

64 × 64, slice thickness = 6 mm, 28 transversal slices per scan. Because we recorded electromyographic activity from the arm muscles we imposed a gap of 378.75 ms between scans to obtain a brief period during which there was no scan-related artefact within the EMG signal. The imaged volume covered the whole brain. Functional scanning was always preceded by 15.5 s of dummy scans to ensure tissue steadystate magnetization. Prior to obtaining the functional measurements, high resolution anatomical T1-weighted images were acquired using a 3D sequence (gradient-echo inversion recovery, repetition time 1600 ms, echo time 5 ms, matrix 256 × 256, field of view 220 × 220 mm²). Each functional MRI exam was composed of four runs, and each run consisted of 210 scans.

Electromyography recordings

During the functional MRI scanning we monitored the activity of stump muscles on the amputated side (triceps and biceps for above-elbow amputees and extensor digitorum communis and flexor digitorum superficialis for below-elbow amputees), and forearm muscles on the intact side (extensor digitorum communis and flexor digitorum superficialis). The absence of activity in these muscles during imagination compared to execution was taken as a sign that the subject had correctly performed the imagination task. For each muscle, two silver-silver chloride surface electrodes of 10-mm diameter were positioned on the muscle with an interelectrode distance (centre to centre) of 2 cm (with the skin previously shaved and/or cleaned with alcohol). The reference electrode was placed on the lateral epicondyle. EMG data were recorded using a Biopac MP150 data acquisition system and an EMG100C electromyogram amplifier (BIOPAC Systems Inc.) with MEC MRI cable and filter components for use in an MRI environment. EMG signals were amplified (×2000), recorded at a frequency of 1000Hz, and band pass filtered (20–400Hz). Only data recorded between successive repetition times (i.e. in the artefact-free gap) were included in further analyses, which were performed using a custom-made MATLAB® program.

For each muscle we computed the mean activation level (root mean square) in each gap (378.75 ms period in which there was no scan-related artefact) and calculated the average for each condition. Separate one-way ANOVAs were performed for each muscle with three conditions (rest, imagination and execution) and post hoc tests were conducted using Student’s paired t-tests. The results are presented as means and SDs. The alpha level was set at $P < 0.05$.

Functional magnetic resonance imaging analysis

All functional MRI analyses were performed in SPM8 (http://www.filion.ucl.ac.uk/spm/).

Preprocessing of functional magnetic resonance imaging data

The first five volumes of a session and the three last volumes (dummy images) were discarded from all analyses. All functional volumes were spatially realigned. As we had an inhomogeneous sample of patients in terms of side of amputation, data from patients with a right-side amputation were flipped, such that all patients were considered as ‘left hand amputated’ (same method in MacIver et al., 2008; Diers et al., 2010). To do this, the echoplanar imaging mean images of each time series were flipped sagittally for the nine patients with a right-side amputation. Before pooling the data, we performed a random effect t-test analysis comparing flipped right hand amputees with the left hand amputees (unflipped) to check for the absence of hemisphere-specific activation. This analysis revealed no differences between the two groups, which prompted us to proceed with a single group analysis. All echoplanar imaging images were normalized to fit a symmetric echoplanar imaging template, consistent with the MNI (Montreal Neurological Institute) reference brain provided by SPM. Finally, the normalized functional images ($2 × 2 × 2$ mm³) were spatially smoothed by a Gaussian kernel of full width at half maximum 8 mm.

Single subject analysis

We estimated the parameters of a general linear model for each participant to generate voxel-wise statistical parametric maps of brain activation. For each participant, we constructed a functional MRI design matrix by modelling the following regressors for each session: ‘execution’, ‘imagination’ (the two regressors being modelled as a 10-s duration event at the onset of each trial of the experimental task), and the rest condition. The regressors were convolved with a canonical
haemodynamic response function and the time series were high-pass filtered with a cut-off at 128 s. For each comparison of interest and for each subject, a contrast of parameter estimates was calculated in a voxel-wise manner to produce a ‘contrast image’. Contrasts were defined as: (i) execution of phantom/intact hand movements versus rest; (ii) imagination of phantom/intact hand movements versus rest; and (iii) execution versus imagination of phantom/intact hand movements. For all single subject analyses, the statistical threshold was set at \( p < 0.05 \) corrected for multiple comparisons using family-wise error (FWE) correction.

**Group analysis**

The parameter estimates from this first-level analysis were then entered into a random (between-subject) effect analysis, and linear contrasts were used to identify responsive regions. Thresholded Z-maps were obtained for execution (with a threshold of \( p < 0.05 \), corrected for multiple comparisons using FWE) and for imagination (with a threshold of \( p < 0.001 \) uncorrected). A lower threshold was applied for imagination to account for the fact that individual subjects used different motor imagery strategies, which resulted in more widespread activations than for execution.

**Functional magnetic resonance imaging analysis 1**

An *a priori* region of interest analysis was performed. First, we anatomically defined two broad regions of interest that we expected to distinguish between execution and imagination. All regions of interests were defined using Wake Forest University (WFU) PickAtlas software (http://fmri.wfubmc.edu), which provides a method for generating region of interest masks based on the Talairach Daemon database (Lancaster et al., 1997, 2000). The Talairach Daemon atlases include Brodmann areas (BAs) and a lobar atlas. The first region of interest ‘M1S1’ (for primary motor and sensory cortices) included the contralateral primary somatosensory and motor cortices (BA 1, 2, 3 and 4). The second region of interest ‘PO’ (for parieto-occipital cortex) included the bilateral superior and inferior parietal cortex and the primary visual cortex (BA 5, 7, 39, 40 and 17, 18, 19). We chose this very large region of interest because we expected that some amputees might encounter difficulties performing kinesthetic imagery and perform visual imagery instead. Since these two types of motor imagery recruit different brain networks (Solodkin et al., 2004; Guillot et al., 2009), we chose a region of interest that included areas specifically activated during visual imagery (primary BA 17 and associative (BA 18 and 19) visual areas), as well as kinaesthetic imagery (bilateral inferior and superior parietal lobules (BA 5, 7, 39 and 40)). All region of interest analyses were performed on data normalized according to the number of voxels within each region of interest. For the cerebellum analysis, we used the bilateral anterior lobe (Larsell’s lobules I–III) and the bilateral posterior lobe (Larsell’s lobules IV–X) of the cerebellum. The percentage of signal change relative to the mean signal of the four regions of interest was calculated for each subject and each session using MarsBar (http://marsbar.sourceforge.net) (Brett, 2002). The percentage of activated voxels was computed using a custom-built program implemented in MATLAB®. Wilcoxon tests were conducted using STATISTICA 8.0 (StatSoft, Inc.). Medians are reported with the range indicated in brackets.

**Functional magnetic resonance imaging analysis 2**

We conducted the same analysis as Gerardin et al. (2000), who compared individual subject activation peaks in the antero-posterior axes during execution and imagination in normal subjects. Y MNI coordinates for maximal activity in all individual maps associated with motor execution and motor imagery were computed for both limbs in several areas that were activated bilaterally during both tasks. These areas were the dorsal premotor cortex (lateral part of BA 6), the SMA (medial part of BA 6), the inferior parietal cortex (BA 39 and 40) and the superior parietal cortex (BA 5 and 7). These four regions of interest were defined using automated anatomical labelling (Tzourio-Mazoyer et al., 2002) as implemented in Wake Forest University PickAtlas. Wilcoxon paired tests \( (p < 0.05) \) were performed on Y coordinates to find differences between imagination peak locations and execution peak locations.

**Functional magnetic resonance imaging analysis 3**

We used dynamic causal modelling (Friston et al., 2003) to assess effective connectivity between the SMA and M1 during motor execution and motor imagination. Note that we used a long repetition time (3030 ms) in order to acquire data from the whole brain and to allow us to insert a substantial gap in between scans during which we could obtain artefact-free EMG recordings. Although SMA and M1 are closely situated in the brain, slice-timing corrections are needed to obtain proper estimates of effective connectivity between these regions. Such corrections are implemented in the dynamical causal modelling for functional MRI toolbox included with SPM8 (Kiebel et al., 2007).

**Definition of the dynamic causal models**

An additional general linear model was especially estimated for the dynamical causal modelling analysis in which two regressors were added to execution and imagination: PREPA was modelled as the 1-s long period during which subjects were visually instructed about the task and BEEP corresponding to the auditory pacer for each movement. These two sensory stimuli were considered as the causal inputs into the network, while execution and imagination were taken as modulatory (contextual) factors.

Here, we specifically aimed to investigate the influences of execution and imagination on the reciprocal connections between the SMA (bilateral) and M1 (contralateral) network. For each subject, blood oxygen level-dependent signal time courses were manually extracted from spherical shapes of 5-mm radius for the contralateral primary motor cortex (contralateral M1) and 6-mm radius for the caudal part of the SMA using the ‘eigenvariate’ extraction tool in SPM8. All subjects demonstrated activation in these regions. Sphere centres for each subject were set as the closest maxima within a distance of 4 mm from the group peak obtained from the random effects analysis (individual map thresholds were set at \( p < 0.05 \) and adjusted to the \( F \) contrast of each participant) (see mean coordinates in Supplementary Table 1).

All models included a direct and a reciprocal connection between SMA and contralateral M1. We compared a large number of possible models using Bayesian model comparison and a factorial-like design in model space. The latter was first partitioned into three different families depending on the inputs given to the system (Penny et al., 2010). Family I included input BEEP only, Family II included both inputs BEEP and PREPA, while Family III included PREPA only (Supplementary Fig. 1A). The target regions for each of these input families were chosen based on Kassess et al. (2008). Each of the model families was then further partitioned into \( 7 \times 7 \) modulatory families. According to the pattern of models used by Kassess et al. (2008), the Families A–G varied as a function of execution’s input location(s) whereas the Families 1–7 varied as a function of imagination’s input location(s). This resulted in 147 different models (Supplementary Fig. 1B), fitted to all the data from \( n = 14 \) subjects.
Each model was estimated separately for each of the two functional MRI sessions and for both hemispheres.

Model selection and parameter testing

Since we expected to observe some between-subject variability, we used a 'random effect' approach to Bayesian model selection in order to compare model families and models within the best family (Stephan et al., 2009). The random effect approach accounts for putative differences between subjects in the relative model evidence within each family (Stephan et al., 2009, 2010; Kasess et al., 2010). Given the factorial nature of our technique for comparing the different models, we started by comparing the three families of sensory inputs over subjects and for each limb independently. Then, within the best family out of the three, we compared families of contextual inputs (execution or imagination). Finally, having identified the best subset of models, we compared models within that subset based on their posterior and exceedance probabilities and proceeded with inference on model parameters across models and subjects using Bayesian model averaging. Bayesian model averaging uses the optimal family of models and computes a weighted average of each model parameter, where the weighting is given by the posterior probability for each model. Parameters resulting from the Bayesian model averaging analysis were considered as significant only if their posterior probabilities were significantly different from zero (one sample $t$-test $P < 0.05$).

Results

A post-scan debriefing revealed that all amputees were aware of having performed two distinct tasks with both their intact and amputated limbs. This was supported by the EMG data which showed a significant increase in EMG activity in the monitored muscles (relative to baseline) during execution but not during imagination (Supplementary Fig. 2). While executed movements of the intact and phantom hands were associated with significant muscle activity these contractions were not accompanied by movement of either the forearm or the stump.

Brain areas recruited during motor execution and motor imagery

We performed an individual first level analysis, which revealed that for each subject the statistical maps obtained for the execution and imagination tasks corresponded roughly to the cerebral networks generally reported to be active during execution and imagination. Next, we performed a random effect group analysis. Figure 1 shows that for motor execution of both the intact and phantom limbs there were significant signal changes in the hand area of the contralateral M1, in the hand area of the contralateral primary somatosensory cortex, in the contralateral dorsal premotor areas, in the bilateral medial premotor areas, and in the ipsilateral anteromedial part of the cerebellum. The bilateral superior and inferior parietal areas were activated during intact limb execution, whereas only the inferior part of the bilateral parietal area was activated during phantom limb execution.

Figure 1 shows that for motor imagery of both limbs there were significant activations in the contralateral dorsal premotor areas, in the bilateral parietal areas, and in the bilateral inferior parietal areas. The group results did not exhibit significant activation in contralateral M1 during motor imagery, but seven subjects showed some activity for the phantom limb and six for the intact limb. Cerebellar activity during imagination was more posterior and inferior (centred in Larsell's lobule H-VII) than during execution (centred in Larsell's lobule H-IV).

While both networks included some common areas each was unique and could be clearly differentiated from the other. Notably, a group comparison between execution and imagination showed that for both limbs contralateral M1, contralateral primary somatosensory cortex, the contralateral secondary somatosensory cortex and the ipsilateral anterior cerebellum were more active during execution than during imagination (Fig. 2).

While there were clear differences between execution and imagination for both hands, the network activated by each of these tasks did not appear to change according to which hand (phantom or intact) performed the task; a group comparison between phantom and intact limbs revealed no differences for either execution- or imagination-related activations (separate two sample $t$-tests $P < 0.05$ corrected for multiple comparisons, FWE).

In order to quantify the spatial distribution of activations associated with execution and imagery depicted in Fig. 1, we conducted a complementary region of interest analysis. Figure 3A shows that for both limbs the percentage of active voxels was significantly greater in the primary motor and sensory cortices (M1S1) when amputees made an executed movement than when they imagined. In contrast, for the parietal and occipital lobes (PO), the imagination percentages were higher than the execution percentages. The same pattern of results was found for signal intensity (Fig. 3B). We also examined whether a similar switch occurred in the cerebellum and found that for both limbs the percentage of activated voxels as well as the relative percentage signal change were greater in the anterior lobe during execution compared with imagination (statistically significant), and in the posterior lobe during imagination compared with execution (non-significant) (Fig. 3C and D).

Differences between execution and imagination within common brain areas and changes in the interactions within cortical networks

For those brain areas that were activated during both motor execution and imagination we calculated the $Y$ coordinates of the maximal activity associated with each task and each limb for all individual maps. Figure 4 shows that in these brain areas the activation peaks for each task often formed two separate clusters. In the hemisphere contralateral to the moving hand (imagined or executed) activation peaks in the SMA and dorsal premotor area (BA 6) were more anterior during imagination than execution, whereas they were more posterior in the parietal areas (BA 5–7, 39–40) (Wilcoxon paired $t$-tests: $P < 0.05$ for all contralateral hemisphere comparisons and $P > 0.05$ for all ipsilateral hemisphere comparisons) (Fig. 4 and Supplementary Table 3).

In agreement with the conventional brain activation analyses described above, the results of our dynamical causal modelling
analysis also showed a dissociation between imagination and execution in the form of a switch in effective connectivity within the network composed of the SMA proper and M1 contralateral to the moving hand. Imagination always exerted a negative (suppressive) influence on the network [mean significant Bayesian model averaging parameters for the phantom limb: $-0.05$ (SD = 0.11) and for the intact limb: $-0.59$ (SD = 0.1)]. In contrast, execution inputs always showed a positive (enhancing) effect on the system [mean Bayesian model averaging parameters for the phantom limb: 0.05 (SD = 0.05) and for the intact limb: 0.89 (SD = 0.06)] (one sample t-test, $P < 0.05$; Fig. 5).

While the suppressive effect of imagination and enhancing effect of execution was present for both intact and phantom limb movements the dynamical causal modelling analysis revealed several subtle differences between the effective connectivity patterns for phantom and intact limbs. During intact limb movements (both imagined and executed), the network was modulated at the level of the connection between SMA and M1, whereas during phantom limb movements the modulation directly affected either M1 (imagined) or SMA (executed). Furthermore, the modulation recorded during both phantom limb tasks was weaker than with the intact limb. Additional dynamical causal modelling analyses (not shown in Fig. 5) revealed that the reciprocal paths linking the contralateral M1 and SMA regions, [which can be thought of as models of bottom-up (contralateral M1 to SMA) and top-down (SMA to contralateral M1) control] differed between the intact and phantom limbs. While the bottom-up and top-down modulations were nearly equal for the intact limb [mean Bayesian model averaging parameters for the contralateral M1 to SMA connection: 0.23 (SD = 0.09) and for the SMA to contralateral M1 connection: 0.27 (SD = 0.04)], this was not the case for the phantom limb in which the top-down connection strength was greater [mean Bayesian model averaging parameters: 0.09 (SD = 0.18) and 0.93 (SD = 0.59)] (Supplementary Material and Supplementary Table 7). To investigate whether the unequal connection strengths between contralateral M1 → SMA and SMA → contralateral M1 for the phantom hand were related to the limited ability of amputees to make voluntarily phantom limb movements we correlated an objective measure of phantom limb motor control (time to execute the finger-to-thumb opposition task) with the difference between the SMA → contralateral M1 and contralateral M1 → SMA neural coupling rates. There was a significant positive correlation between the difference in coupling rates and the time taken to perform the finger-to-thumb opposition task ($r = 0.70$, $P = 0.006$); the greater the imbalance the slower the movement time. In a separate analysis, for each subject

**Figure 1** Group results during execution ($P < 0.05$, FWE corrected) and imagination ($P < 0.001$, not corrected) for the intact and phantom limbs overlaid on the PC-CC (PALS Cerebral, Colin Cerebellar) template which has been partially inflated using Caret 5.56 software (http://brainmap.wustl.edu/caret/).
we correlated the voxel-wise activation levels recorded during the phantom limb execution condition with the time to execute the finger-to-thumb opposition task. This revealed significant negative correlations between movement time and activity in contralateral M1: the greater the activity, the faster the movement [contralateral M1 correlation cluster: \( k = 85, P \) (small volume corrected) \( < 0.002, \text{mean } r = -0.74 \)]; as well as positive correlations between movement time and activity in contralateral SMA: the greater the activity the slower the movement [SMA correlation cluster: \( k = 59, P \) (small volume corrected) \( < 0.003, \text{mean } r = 0.8 \)].

**Discussion**

This study provides the first physiological evidence that amputees perform two distinct tasks when executing and imagining a movement with their phantom limb. We show that imagination and execution activate two distinct brain networks that partially overlap, and that these networks are similar to those activated during imagination and execution with the intact limb. We also show that motor execution with both the phantom and intact limbs recruits brain areas typically activated during movement execution in healthy subjects, including the primary motor and sensory cortices, the SMA, the dorsal premotor cortex and the cerebellum (Grafton et al., 1996; Gerardin et al., 2000). These findings are consistent with previous amputee studies in which movements of the phantom limb activated a network similar to the one activated by contralateral intact limb movements (Enslin et al., 1996; Lotze et al., 2001; Roux et al., 2003), and reinforce the idea that movement execution with the phantom limb is not a form of motor imagery. Further evidence that executed and imagined phantom movements are two distinct tasks comes from our finding that phantom and intact hand imagination recruited brain areas typically recruited during motor imagery in healthy subjects; the inferior and superior parietal lobules, the SMA, the dorsal premotor cortex and the cerebellum (Gerardin et al., 2000; Naito et al., 2002; Nair et al., 2003). Our dynamical causal modelling analysis further supports the idea that there is a clear neurophysiological distinction between imagination and execution in amputees, as it shows that each task (regardless of hand) had an opposite effect on the SMA–M1 network. Furthermore, the direction of this effect was identical to that reported by effective connectivity studies in healthy subjects; a suppressive influence of motor imagery and an enhancing influence of motor execution (Solodkin et al., 2004; Kasess et al., 2008).

While the networks activated by imagination and execution were distinct they did share some common brain areas. Interestingly, the location of the activation peaks within these common brain areas depended on the task (imagination or execution) but was similar for both hands (phantom or intact). Compared with execution, activation peaks recorded during imagination were more rostral in the SMA and the dorsal premotor cortex, and more posterior in the inferior and superior parietal
lobes. A similar spatial dissociation within these areas has been reported in studies examining imagination and execution in healthy subjects (Stephan et al., 1995; Lotze et al., 1999b; Gerardin et al., 2000; Hanakawa et al., 2003).

There are a number of previous studies showing that phantom and intact limb execution activate similar networks, but this is the first to show that phantom and intact limb imagination activate similar brain areas, and that the phantom limb motor imagery network is preserved even in long-term deafferented patients. This is consistent with the finding that patients with spinal cord injury have an intact imagination network (Hotz-Boendermaker et al., 2008), and with our recent behavioural results in amputees showing no performance differences between the intact and amputated hands on motor imagery tasks (Raffin et al., 2011). While the similarity between the phantom and intact limb execution networks replicates the results of previous studies, it appears to contradict the results of neuroimaging studies demonstrating asymmetries in movement-related activity in the primary motor and sensory cortices of amputees. Such asymmetries are, however, primarily due to an enlargement and displacement of activations associated with stump and face movements (Lotze et al., 1999a, 2001; Karl et al., 2001, 2004). Thus, the similarity between our execution networks for the intact and phantom hands is not inconsistent with the presence of post-amputation reorganization. Instead, it supports the idea that the hand’s representation maintains its original position within M1 despite the reorganization of other body part representations (Ersland et al., 1996; Roux et al., 2001, 2003; Mercier et al., 2006).

While amputation-related reorganization does not necessarily lead to the expectation of asymmetries between intact and phantom hand execution networks, some asymmetries could be expected given the difficulty that amputees experience when trying to move their phantom hand and the fact that phantom movements are generally more effortful than movements of intact body parts (Ramachandran and Rogers-Ramachandran, 1996; Gagné et al., 2009; Raffin et al., 2011). Surprisingly, our

Figure 3 Percentage of activated voxels (A) and relative percentage of signal change (B) in the contralateral primary motor and sensory cortices (M1S1) and in the bilateral parieto-occipital cortex (PO) during execution (EXE) and imagination (IMA) with the intact and phantom limbs. Percentage of activated voxels (C) and relative percentage of signal change (D) in the anterior (ANTE) and posterior (POST) lobes of the cerebellum during execution and imagination with the intact and phantom limbs. All percentages were computed using a statistical threshold set at P < 0.05, corrected for multiple comparisons using FWE correction.
comparison between the amputated and intact sides did not reveal any statistical difference between the two networks at the level of the group, and we also found no between-side differences in the intensity or area of movement-related activations, particularly in the caudal-SMA and in various parietal areas (Deiber et al., 1991; Grafton et al., 1992; Winstein et al., 1997), suggesting that our subjects exerted similar levels of effort when making intact and phantom hand movements. This might have been due to the fact that phantom and intact hand movements were performed at the same pace, defined as the speed at which opening–closing movements of the phantom hand were comfortable, required little effort and did not cause any pain.

Although we detected no between-limb differences during execution for the group, the between-subject variability in M1 activity levels during executed phantom hand movements was related to the ability to move the phantom hand: our correlation analyses revealed that the time to perform the finger opposition task was negatively correlated with the overall level of activity in contralateral M1 and positively correlated with the contralateral SMA activity level. If we consider the speed at which an amputee can perform finger-to-thumb opposition with their phantom hand as an indirect indicator of the ease/difficulty of moving the phantom limb, then the negative correlation with contralateral M1 activity suggests that the easier it is to move the phantom the greater the activity level in contralateral M1. Similarly, the positive correlation with contralateral SMA suggests that the more difficult it is to move the phantom the greater the activity in contralateral SMA. Overactivity in premotor areas has also been observed in patients with focal hand dystonia and complex regional pain syndrome (Ceballos-Baumann et al., 1995; Playford et al., 1998; Maihofner et al., 2003, 2007), suggesting that other motor deficiencies that do not involve any apparent injury to the CNS are also associated with overactivity in SMA. This overactivity might reflect a compensatory phenomenon in which M1 receives a...
sustained input from premotor cortex, the role of which is to increase the probability of 'triggering' a movement. This hypothesis is supported by our effective connectivity analysis, which demonstrated differences in the connectivity between SMA and contralateral M1 for the phantom limb compared to the intact limb, as well as a significant correlation between reciprocal connection strengths and the ability to voluntarily move the phantom.

Alterations in cortical function are not specific to amputation, but have also been reported in patients with focal hand dystonia (Koch et al., 2006; Huang et al., 2010) and in two patients who underwent a unilateral heterotopic hand replantation (Eickhoff et al., 2008). Thus, pathological interactions among key motor regions might partially account for the motor impairment and neuropathic pain that frequently occur after a peripheral lesion or injury.

Clinical implications

Our demonstration that executed and imagined phantom limb movements activate two distinct cerebral networks is of particular importance given the increasing interest in treating neuropathic pain using various combinations of visuomotor feedback, voluntary movements and motor imagery (Ramachandran and Rogers-Ramachandran, 1996; Moseley, 2006; Chan et al., 2007; Murray et al., 2007; Mercier and Sirigu, 2009; Beaumont et al., 2011). Despite the increasing number of such studies, the evidence is too sparse (and sometimes conflicting) to conclude whether or not these techniques are effective.

It is now clear that M1 plays a key role in reducing neuropathic pain. Evidence for this comes from several sources, including
studies using implanted (e.g. Saitoh and Yoshimine, 2007) or non-invasive stimulation (O’Connell et al., 2010), as well as the fact that M1 stimulation can enhance the endogenous opioid system (Maarrawi et al., 2007). The link between M1 and pain also appears to be present in amputees, as those with more phantom limb pain are less able to move their phantom (Gagné et al., 2009; Raffin et al., 2011), and those with poorer phantom motor control have lower contralateral M1 activity levels (present results). This link is further supported by the finding that phantom pain can be reduced and M1 (re)activated after visuomotor training (Giraux and Sirigu, 2003). These results suggest that the most efficient behavioural treatments for the relief of neuropathic pain will probably be those that use tasks which recruit M1. There is some (weak) evidence that motor imagery alone can effectively treat neuropathic pain (Coslett et al., 2010; Beaumont et al., 2011; McAvinue and Robertson, 2011), and while this analgesic effect could be due to M1 activation, the fact that we only observed imagination-related M1 activity in half of our amputee population suggests that the physiological mechanisms underlying pain relief from motor imagery might involve structures other than M1 (e.g. the sensory thalamus) (for a review see Levy et al., 2010). Given this, we suggest that future studies investigating the efficacy of behavioural techniques for pain reduction monitor the motor task actually performed by the patients (execution versus imagination) and include functional imaging in order to clarify the physiological mechanisms underlying their analgesic effects.

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Supplementary material

Supplementary material is available at Brain online.

References


