Neural correlates of evoked phantom limb sensations

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1. Introduction

Phantom limb phenomena include the persistent awareness of the amputated limb as well as specific sensory or kinesthetic sensations referred to the missing limb and are perceived by almost all amputees (Sherman, Devor, Casey Jones, Katz, & Marbach, 1996). Phantom sensations can be spontaneous or evoked by sensory inputs from the existing body parts (Hunter, Katz, & Davis, 2005). The sensations reported by the amputees can be diverse and may include feelings of warmth or cold, itching, tingling, electric sensations, and in some cases unpleasant or painful sensations (i.e. “phantom limb pain”).

Few studies have investigated neural changes related to non-painful phantom sensations. For example, Björkman et al. (2012) used functional magnetic resonance imaging (fMRI) during tactile stimulation of the residual limb and reported bilateral activation of the primary somatosensory cortex (SI), contralateral parietal and premotor cortices. However, they were not able to dissociate the neural activation induced by the stimulation of the residual limb from the percept of phantom sensations as they might have activated inputs from the residual limb to the brain region that formerly represented the amputated limb and they did not assess ratings of evoked phantom sensations. Similarly, Brugger et al. (2000) reported parietal and ventral premotor activation but not activation of SI related to the phantom percept in a congenital amputee.

However, transcranial magnetic stimulation of the sensorimotor cortex evoked phantom sensation. Roux et al. (2003) reported activations in the sensorimotor areas when phantom sensation was present as did Flor et al. (2000) although they did not observe reorganization of the cortical map as suggested by Ramachandran, Rogers-Ramachandran, and Cobb (1995) and Ramachandran, Brang, and McGeoch (2010). The current literature on tactile illusions in non-amputees also suggests the involvement of SI where the percept rather than the actual physical stimulus seems to be represented (Bufalari, Di Russo, & Aglioti, 2014). SI has also been involved in sensory illusions such as the cutaneous rabbit illusion (Blankenburg, Ruff, Deichmann, Rees, & Driver, 2006), the funneling or rubber hand illusion (Chen, Friedman, & Roc, 2003; Schaefer, Konczak, Heinze, & Rötte, 2013), or supernumerary phantom limbs, which are similar to a phantom sensation (Khatib et al., 2009; McGonigle et al., 2002).

However, it seems that not only SI but also the inferior frontal cortex (Brodmann area (BA) 44, BA45), premotor and posterior parietal areas (e.g. intraparietal sulcus, IPS) could be involved in the perception of abnormal somatosensory phenomena (Brancucci, Franciotti, D’Anselmo, & Della Penna, 2011; Ehrsson, Spence, & Passingham, 2004; Knapen, Brascamp, Pearson, van Ee, & Blake, 2011 Zaretskaya, Anstis, & Bartels, 2013). For example, illusion experience related to the rubber hand was associated with increased activity in brain areas related to the integration of multisensory representation of body parts, such as
the intraparietal and ventral premotor cortices (Brozzoli, Gentile, & Ehrsson, 2012). Hari et al. (1998) showed that a supernumerary limb is characterized by suppression of activity in contralateral secondary somatosensory cortex (SII). Similar neural networks might also be underlying evoked phantom sensations in amputees, although these networks might undergo cortical reorganization resulting from limb amputation. A main argument for such reorganization mechanisms is the sensory map of the body, which has been shown to change in amputees, such that touching the face could evoke illusions of tactile sensations on the phantom, in a stable, topographically organized manner (Ramachandran and Hirstein, 1998; Ramachandran et al., 2010). Plastic changes to the body map have also been related to altered interhemispheric interactions from the recruitment of horizontal connections of the intact limb representation to the deafferented cortex (Maclver, Lloyd, Kelly, Roberts, & Nurmiikko, 2008). For example, compared with two-handers, amputees have been shown to have a reduced interhemispheric structural (Xie et al., 2013) and functional connectivity in S1 (Makin et al., 2013). The neural correlates of nonpainful phantom sensations and the relationship between phantom sensations and interhemispheric reorganization have not yet been investigated.

We aimed to explore the brain representation of evoked non-painful phantom sensations (referred sensations) in limb amputees by using functional magnetic resonance imaging (fMRI) in five chronic limb amputees (selected from 156 patients) where phantom sensation could be reliably turned on and off while electrically stimulating body areas adjacent to or remote from the amputation site (Fig. 1). This permitted a passive activation of phantom sensation without any mental effort or physical induction such as hypnosis-, imagery- or movement-elicited phantoms (Raffin, Mattout, Reilly, & Giraux, 2012; Willoch et al., 2000). In addition, five controls matched for sex, age and stimulated body site (yoked controls) were included.

2. Materials and methods

2.1. Subjects

We selected five unilateral limb amputees out of 156 based on the criterion of showing phasic and reliable referred sensations in the phantom. These amputees were all male and included four upper- and one lower-limb amputee with a mean age of 42 years (range 35–59). Five yoked healthy controls were also tested (all male, right-handed, mean age 44 years, range 33–62). Patients with a history of neurological or mental disorder were excluded from the study. For a detailed description of the sample, see Table 1. Ethical committee approval was received from the Ethical Review Board of the Medical Faculty Mannheim, Heidelberg University, and written informed consent was obtained from all participants.

2.2. Laboratory screening: psychometric assessment of phantom phenomena

The amputees participated in a psychometric evaluation including a structured interview about the amputation and its consequences and included a detailed assessment of painful and non-painful phantom phenomena such as MPI (German version of the Multidimensional Pain Inventory adjusted to separately measure phantom and residual limb pain), (Flor, Rudy, Birbaumer, Streit, & Schugens, 1990; Flor et al., 1995; Winter et al., 2001).

2.3. Laboratory screening: procedure for detecting evoked phantom sensations in the missing limb

Referred sensations were assessed by using consecutive mechanical (cotton swabs, pin pricks) stimulation over 57 standardized sites spread over the entire body using a standardized procedure (Grüsser et al., 2004). Ten sites were located in the face, 23 on the upper body part and the remaining sites covered the lower body. The subjects had to indicate where they felt a sensation and described its quality and rated the intensity. They were naive to the procedure of testing evoked phantom sensations. The localization of sensations perceived at the site of stimulation as well as on the phantom was marked on the subject’s body and drawn on a body template. A total of 25 body sites which were responsive to mechanical stimulation and elicited phantom sensation in the amputee group, with five sites for amputee A1, three sites for A2, ten sites for A3, three sites for A4 and four sites for A5 (Fig. 1). When referred sensations were obtained, electrical stimulation was used to test if this would also elicit the sensation.

We applied monophasic constant current stimuli of 200 ms duration each (DS7A, Digitimer, Hertfordshire, England) using transcutaneous custom-designed foil electrodes. Using the method of limits, the perception threshold was determined as the mean of three series of ascending electrical stimuli, evoking sensations either at the stimulated body site or in the missing limb or both. Electrical stimulation over the selected body sites elicited phantom sensations in 10 body sites, with three sites for A1 and A2, two sites for A3, one site for A4 and for A5. The quality of phantom sensations was different across amputees (see Table 1). In the control group, no sensation was reported outside of the stimulated site.

Sites eliciting robust evoked phantom sensations that could be

![Fig. 1. Body templates of five limb amputees with phasic referred phantom sensations. Purple dots indicate the body sites that were electrically stimulated and green surfaces indicate areas in the missing limb in which amputees perceived phantom sensations. A1–A5: reference of amputee respective to data provided in Table 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)](image)
Table 1
Demographic and clinical data of the sample.

| Subject | Age | Age at amp. | Amp. site | Stimulated side | Cause of Amp. | Intensity of habitual PLP as measured by MPI | Stimulation intensity (mA) | Sensation perception threshold (mA) | Rating of stimulation intensity at stim. site | Rating of sensation in the phantom | Rating of unpleasantness of stimulation at stim. Site | Unpleasantness of sensation in the phantom | Quality of referred sensation in the phantom limb |
|---------|-----|-------------|----------|----------------|---------------|---------------------------------------------|---------------------------|-----------------------------------|----------------------------------|---------------------------------|------------------------------------------|----------------------------------|------------------------------------------------|
| A1      | 39  | 15          | R        | L arm          | Cancer        | 0                                           | 6.9                       | 5.8                               | 4.0                               | 6.5                             | 2.0                                      | 5.0                               | Spasmodic sensation in thumb, index finger, and middle finger |
| A2      | 35  | 27          | R        | L arm          | Accident      | 1.66                                        | 3.2                       | 4.6                               | 4.0                               | 3.0                             | 4.0                                      | 5.0                               | Pleasant sensation in all fingers |
| A3      | 59  | 3           | L        | L arm          | Accident      | 0                                           | 2.5                       | 1.1                               | 5.0                               | 5.0                             | 7.0                                      | 7.0                               | Warming of the hand, hand begins to move, sensation of individual fingers differentiable |
| A4      | 37  | 25          | R        | R arm          | Accident      | 2.66                                        | 22.0                      | 10.9                              | 8.0                               | 5.0                             | 7.0                                      | 3.5                               | Fingers feel numb, like pins and needles, fingers start moving |
| A5      | 42  | 23          | L        | L leg          | Accident      | 0                                           | 6.0                       | 2.7                               | 7.5                               | 3.0                             | 7.0                                      | 3.0                               | Increasing sensation in the limb, sensation becomes more vivid |

Mean ± STD 42.4 ± 9.6 18.6 ± 9.8

* MPI: German version of the Multidimensional Pain Inventory adjusted to separately measure phantom and residual limb pain (Flor et al., 1990, 1995).

Ratings of the intensity of the electrical stimulation perceived at the stimulated site and in the phantom ranging from 0 ("just perceptible") to 10 ("it begins to hurt").

Ratings of the unpleasantness of the sensation perceived at the stimulated site and in the phantom, ranging from 0 ("very pleasant") to 10 ("very unpleasant").

Ratings were performed on a visual analogue scale (VAS) after the electrical stimulation.

PLP, phantom limb pain; Amp., amputation; R, right; L, left; stim., stimulated.
reliably turned on and off were then selected for the fMRI experiment. The selected body sites in the amputees were also stimulated in the yoked controls.

2.4. Magnetic resonance imaging (MRI) procedure

Electrical stimulation was applied in the MR scanner with an interstimulus interval that varied randomly between 70 ms and 140 ms resulting in a mean stimulus frequency of 10 Hz \((r = 105\, \text{ms})\). The electric stimulation procedure consisted of 5 blocks of 20 s each separated by 4 rest blocks of different length varying between 40 s and 1 min in order to rule out expectation effects. The stimulation current was set to twice the perception threshold (see above) and individually adjusted to be perceived as intense but not painful. The participants were told to keep their eyes open, to concentrate on the electrical stimulation and to lie in a relaxed manner.

A 1.5 T Siemens Vision Plus was used in combination with a 12-channel radiofrequency head-coil to obtain a functional gradient-echo Echo Planar Imaging (EPI) pulse sequence. Seventy-four volumes were acquired for each participant \((3 \times 3 \times 5 \, \text{mm voxels}, \text{TR/TE} = 3000/66\, \text{ms}, 24\, \text{slices})\) angulated in parallel to the anterior-posterior commissure line. For anatomical reference a high-resolution 3D Magnetization Prepared Rapid Gradient image (MPRAGE, 1 mm isotropic voxel, TR/TE = 22/10 ms) was obtained.

At the end of the fMRI session, ratings of perceived sensations were obtained using numeric rating scales. The participants were asked to rate the intensity and the unpleasantness of the sensations they felt at the site of stimulation as well as the sensations perceived elsewhere (e.g. in the phantom). But this was not explicitly mentioned to avoid bias. Ratings ranged from 0 (‘just perceptible’) to 10 (‘it begins to hurt’) for the intensity of sensation, and from 0 (‘very pleasant’) to 10 (‘very unpleasant’) for the unpleasantness of the sensation.

2.5. Data analysis

The ratings were analyzed with R package (“The R project for statistical computing”, http://www.r-project.org/). Between-group comparisons of the ratings were carried out using t-tests for independent samples. In addition, correlation analyses were computed between the ratings using Spearman’s rank correlation coefficients. The threshold for significance was set at \(p < 0.05\).

Functional imaging data were analyzed using the FMRIb’s Software Library (FSL) (Smith et al., 2004). Motion correction was applied using MCFLIRT (Jenkinson, Bannister, Brady, & Smith, 2002) and motion-correction parameters were used as nuisance regressors in the design matrix. Spatial smoothing was performed using a 5 mm isotropic Gaussian kernel of full-width at half maximum (FWHM) and a high pass temporal filtering was applied using a Gaussian-weighted least square straight line fitting at 100 s cut-off. Registration was performed using a 2-step procedure: EPI images from each scan were first registered to the high resolution T1-weighted structural image where non-brain structures were removed using Brain Extraction Tool (Smith, 2002). EPI images were then registered to the standard MNI 152 space using 12-parameter affine transformations.

The fMRI statistical analysis was carried out in three levels using FEAT (FMRI Expert Analysis Tool, Smith 2002). Data from each run of each participant were analyzed separately at a first level of analysis. Trials with electrical stimulation were modelled as a single factor of interest and were convolved with a canonical gaussian hemodynamic response function and were entered as a predictor into a general linear model (GLM). All runs for each participant were combined into a second level using a fixed effects analysis. Combined data from each participant were then subjected to the third level mixed-effects model in FLAME, which models and estimates the random-effects component of the measured intersession mixed-effects variance for group statistics. Areas of significant fMRI responses were determined using clusters identified by a \(z > 2.3\) threshold and a corrected cluster threshold of \(p < 0.05\).

Additionally, we defined eight independent regions of interest (ROI), right and left IPS, right and left SI, right and left frontal gyrus (pars opercularis, BA44 and pars triangularis, BA45) based on the FSL Harvard-Oxford Atlas (Nickollhoff et al., 2007), from which we extracted the percent bold signal change (%BSC) for each run across all participants using the FSL Featquery tool (Smith et al., 2004). We were especially interested in the right IPS because of its involvement in body representations and illusory own-body perception (Blanke, Ortigue, Landis, & Seeck, 2002; Hashimoto and Iriki, 2013). We also expected the involvement of SI regarding the nature of the stimuli used and its prior association with sensory illusions, and the involvement of the left and right BA44 and BA45 based on their role in body-related illusions (Brunacci et al., 2011; Knapen et al., 2011). Correlation analyses were carried out between the %BSC in the eight ROIs and the behavioural ratings. To preclude the contribution of the stump stimulation in the fMRI response related to referred sensation, we carried out correlation analyses between the %BSC in the eight ROIs and the intensity of referred sensations obtained in body sites remote from the stump. A Shapiro-Wilk test was performed and showed that the intensity ratings of referred sensations did not follow a normal distribution. Therefore, correlations between intensity ratings and % BSC in the ROIs were computed using Spearman’s rank correlation coefficient (Spearman’s rho).

The threshold for significance of rho was set at \(p < 0.05\) and corrected for multiple testing by controlling the false discovery rate (FDR; Benjamini & Hochberg, 1995). We then applied metric multidimensional scaling (MDS) to the correlation coefficients related to phantom perception using cmdscale in R package to obtain a model of the correlation structure (Shepard, 1980; Torgerson, 1958).

Based on the ROIs that showed significant relationships with ratings related to phantom sensations, we examined intra- and interhemispheric relationships between the selected ROIs in both amputees and controls using Spearman’s rank correlation coefficient. Intrahemispheric and interhemispheric interactions were examined in the selected ROIs using robust regression analyses using lmRob in R package.

3. Results

3.1. Phantom sensations and perceived stimulation

Physical electrical stimulus intensities were not significantly different between amputees and controls \((t(18) = -0.65, \ p = 0.52)\), consequently, neither the intensity ratings nor unpleasantness perceived at the stimulated site were significantly different \([\text{resp. } (t(18) = 1.6, \ p = 0.12); \ (t(18) = 1.55, \ p = 0.14)]\). Perception thresholds, defined as a mean intensity evoking sensations either at the stimulated site or in the phantom, were also not significantly different between amputees and controls \((t(18) = 0.3, \ p = 0.77)\). The intensity of evoked phantom sensations was rated as moderate in the amputee group \((4.7 \pm 2.4)\) and the unpleasantness as neutral \((4.0 \pm 2.4)\) (see Table 1).

Correlation analyses between the ratings combining both groups showed a significant association between the stimulation intensity and the rating of intensity perceived at the stimulation site \([\text{rho} = 0.62, \ p < 0.005]\) as well as between the rating of intensity perceived at the stimulation site and the unpleasantness of stimulation at the stimulated site \([\text{rho} = 0.75, \ p = 0.0005]\), and a statistical trend \((p < 0.06)\) for an association between the stimulation intensity and the mean perception threshold. The other correlation analyses \(\text{i.e. between the unpleasantness of stimulation at the stimulated site and the stimulation intensity, or between the mean perception threshold and the rating of intensity perceived at the stimulation site} \) did not reach significance \([\text{rho} < 0.31, \ p > 0.20]\).
In the amputee group, there was no significant relationship between the physical stimulation intensity and the perceived intensity or unpleasantness perceived in the phantom (\( \rho < 0.49, p > 0.20 \)).

### 3.2. fMRI results

The amputee group showed neural activity during electrical stimulation in the inferior parietal lobule, IPS, inferior frontal cortex (BA44, BA45), premotor cortex as well as in SI and SII bilaterally (Fig. 2A and Table 2A). In the control group, we found neural activity during electrical stimulation in the right inferior parietal lobule and the right SII (Fig. 2B and Table 2B). Based on our hypotheses of the role of SI and IPS in the perception of referred sensations, we separately extracted the percent of BOLD signal change (%BSC) from these areas in both the left and the right hemispheres. We also extracted the %BSC from the left and right BA44 and BA45 based on their role in body-related illusions.

In the amputee group, the intensity of referred sensations in the missing limb was significantly correlated with the %BSC in the right IPS (\( \rho = 0.89, p = 0.0005 \), with \( p < 0.005 \) corrected) and with the right and left SI (resp. \( \rho = 0.75, p = 0.01 \), with \( p < 0.05 \) corrected; \( \rho = 0.75, p = 0.01 \), with \( p < 0.05 \) corrected), but not with the %BSC in the left IPS (\( \rho = 0.03, p = 0.95 \) corrected), (Fig. 3A). The coefficients of correlation related to phantom sensations are also represented as distance matrices using metric multidimensional scaling (MDS), (Fig. 3B). There was no significant correlation between the intensity of referred sensations and the %BSC in the right or left BA44 (resp. \( \rho = 0.06, p = 0.95 \) corrected; \( \rho = 0.41, p = 0.33 \) corrected) or in the right or left BA45 (resp. \( \rho = 0.44, p = 0.33 \) corrected; \( \rho = 0.49, p = 0.30 \) corrected). Therefore BA44 or BA45 were not taken into account for further analyses.

Compared with the right amputees (n = 3), the two left amputees (n = 2; upper limb A3, lower limb A5) with perceived phantom sensations on their left side had a comparable %BSC in the right IPS (A3 (0.11), A5 (−0.15), range [−0.15 to 0.22]), the left IPS (A3 (0.05), A5 (0.15), range [−0.04 to 0.15]), the right SI (A3 (0.26), A5 (−0.27), range [−0.27 to 0.45]), and the left SI (A3 (0.24), A5 (0.11), range [−0.15 to 0.36]).

In order to test that the relationship between the intensity of referred sensations in the missing limb and brain activations was not related to the stimulation of the stump, we carried out additional correlation analyses by removing the data sets in which stimulation was applied over the stump (n = 2). The correlation between the intensity of referred sensations and the %BSC in the right IPS, the right and the left SI remained significant (resp. \( \rho = 0.88; p < 0.02 \) corrected; \( \rho = 0.88; p < 0.02 \) corrected; \( \rho = 0.88; p < 0.02 \) corrected).

Using the whole sample (n = 5), we found no significant correlation between the %BSC in the left IPS and unpleasantness of sensations in the phantom (\( \rho = 0.50, p = 0.10 \)) or the stimulated site (\( \rho = 0.40, p = 0.30 \)). Likewise, the other ratings (stimulation intensity, intensity of sensations at the stimulated site or mean perception threshold) did not show an association with the %BSC within the left and right IPS or with the left and right SI (\( \rho < 0.40, p > 0.30 \)).

In the control group (n = 5), the correlation analyses between the ratings (stimulation intensity, intensity or unpleasantness of sensations at the stimulated site or mean perception threshold) and the %BSC in
sensations, MPI) and the %BSC in the four regions of interest depicted in (A), shown in blue. Superimposed on the MDS plots are the corresponding signif

cant positive relationship between the %BSC in the right IPS and the right SI in the control group (rho = 0.83, p < 0.005) but not in the amputee group (rho = 0.38, p > 0.05), Fig. 4A. We did not find a significant relationship between the %BSC in the left IPS and the left SI in the amputee group (rho = 0.51, p = 0.5), nor in the control group (rho = 0.33, p = 0.35).

3.3. Intrahemispheric interaction

Robust regression analyses showed a significant positive association between the %BSC in the right IPS and the right SI in the amputees (rho = 0.84, p < 0.005), but not in the control group (rho = −0.30, p = 0.41), Fig. 4A. We did not find a significant relationship between the %BSC in the left IPS and the left SI in the amputee group (rho = 0.51, p = 0.5), nor in the control group (rho = −0.33, p = 0.35).

3.4. Interhemispheric interaction

Robust regression analyses showed a significant positive association between the %BSC in the right IPS and the left SI in the control group (rho = 0.91, p < 0.0005) but not in the amputee group (rho = −0.20, p > 0.50), Fig. 4B. Similarly, there was a significant positive association between the %BSC in the right SI and the left SI in the control group (rho = 0.83, p < 0.005) but not in the amputee group (rho = 0.38, p > 0.20), Fig. 4C.

4. Discussion

We aimed to determine the neuronal correlates of evoked phantom sensations in a highly selective group of unilateral limb amputees, where referred sensations could be reliably elicited for limited amounts of time. We found a significant positive relationship between the intensity of non-painful phantom sensations and functional activity in the right IPS and bilateral SI but not the left IPS. There seems to be a specificity of the right IPS and bilateral SI for non-painful phantom sensations, as there was no significant relationship with other ratings related to the quality and quantity of the stimulation itself. Such findings could also be caused by the stimulation procedure used, as different body sites were stimulated in the same participant, however, amputees and controls showed different patterns of activation suggesting that this activation relates to phantom sensations. Previous studies on non-painful phantom sensations did not provide the intensity of evoked phantom sensations, which could therefore not be linked with functional activations (Björkman et al., 2012; Brugger et al., 2000; Schmalzl, Kalckert, Ragni, & Ehrsson, 2014). In the present study, the ratings were assessed at the end of the fMRI session. Although this the left and right IPS, and left and right SI did not reach significance (rho < 0.45, p > 0.20).

Table 2
Mean coordinates (mm) of the areas activated during electrical stimulation in Amputees (A) and Controls (B).

| Brain areas | MNI coordinates in mm | Z-score | Extent (voxels) |
|-------------|------------------------|---------|-----------------|
|             | x   y   z               |         |                 |
| **Right hemisphere** |                         |         |                 |
| Inferior parietal lobule, inc. Primary somatosensory cortex | 52  -34  36 | 5.17  | 517            |
| Anterior intraparietal sulcus Secondary somatosensory cortex | 36  0   18 | 3.99  | 165            |
| Inferior frontal cortex (BA44) Premotor cortex | 54  14  24 | 3.99  | 165            |
| Inferior frontal cortex (BA44) |                         |         |                 |
| Inferior parietal lobule inc. | -52  16  12 | 4.49  | 1747           |
| Lateral intraparietal sulcus | -38  -52  64 | 4.31  | 223            |
| Secondary somatosensory cortex | -62  -10  16 | 4.19  | 46             |
| Inferior frontal cortex (BA45) |                         |         |                 |
| Inferior parietal lobule inc. | -58  -40  48 | 3.96  | 258            |
| Anterior intraparietal sulcus | -50  -36  44 | 3.6   |                 |
| Premotor cortex | -14  6   60 | 3.92  | 11             |
| Primary somatosensory cortex | -64  -14  34 | 3.83  | 46             |
| **Left hemisphere** |                         |         |                 |
| Inferior parietal lobule, inc. | 50  -30  26 | 5.16  | 542            |
| Secondary somatosensory cortex | 52   -28  26 | 4.99  |                 |

Fig. 3. (A) fMRI activation averaged over five amputees during electrical stimulation of ten body sites plotted on a 3D brain. The red lines delineate the postcentral sulcus. (B) Individual data points with, on the ordinate scale, the perceived intensity of evoked phantom sensations, and on the abscissa scale, the %BSC in left SI (top, left), right SI (top, right), left IPS (bottom, left) and right IPS (bottom right). Points with identical colors indicate the same participant. (B) Three-dimensional configuration of the modelled metric multidimensional scaling (MDS), showing distances matrices between variables related to phantom sensations shown in green (i.e. perceived intensity of evoked phantom sensations, unpleasantness of phantom sensations, MPI) and the %BSC in the four regions of interest depicted in (A), shown in blue. Superimposed on the MDS plots are the corresponding significant pairwise correlations using Spearman’s correlation coefficients rho (corrected for multiple testing using FDR). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
procedure did not take into account the variability of the intensity and unpleasantness of evoked phantom sensations, the ratings were in accordance with the previously obtained ratings in the laboratory.

In accordance to previous studies reporting brain areas involved in experience of referred sensations (Björkmann et al., 2012; Condés-Lara et al., 2000; Flor et al., 2000) we found significant activation in bilateral IPS, SI and SII as well as premotor areas during the perception of phantom sensations. Involvement of SI and SII during referred sensations has previously been shown in Condés-Lara et al. (2000) in a right leg amputee, after soft brush stimulation over the intact leg who reported to perceive phantom sensations. By including ratings in our analyses, we could show that both the intensity and the unpleasantness of evoked phantom sensations were unrelated to the stimulation intensity used.

Furthermore, the intensity of referred sensations was significantly correlated with activation in the right IPS and bilateral SI demonstrating the specificity of these regions for the experience of evoked phantom sensations. These findings are in line with Schmalzl et al. (2014), reporting the involvement of IPS during experience of ownership sensations of an artificial hand. Interestingly, these findings also fit with studies using non-invasive brain stimulation techniques (transcranial magnetic stimulation or transcranial direct current stimulation) showing that stimulation of SI or IPS modulated the phantom percept (Brugger et al., 2000; Bolognini, Olgiati, Maravita, Ferraro, & Fregni, 2013). Additionally, we reported the contribution of bilateral inferior frontal areas (BA44, BA45) in the amputee group, which have been shown to be involved in sensory integration, recognition of movement, action observation and motion perception (Johnson-Frey et al., 2003; Servos, Osu, Santi, & Kawato, 2002) as well as the perception of body-related movement illusions (Naito and Ehrsson, 2006). The activity in BA44 and BA45 did not significantly correlate with the ratings of referred sensations, suggesting that their role may not be directly related to phantom sensations, but might be activated due to the percept of touch on the phantom (Grafton, Arbib, Fadiga, & Rizzolatti, 1996; Rizzolatti et al., 1996). BA44 and BA45 have been discussed to be important for action recognition (de Lussanet et al., 2008; Wurm & Schubotz, 2012). Three of the amputees reported to have kinesthetic evoked phantom sensations with their phantom performing specific movements. Thus, significant activation of BA44 and BA45 might be driven by the recognition of action in the phantom. The control group showed strong SII activity during electrical stimulation, which is inline with an fMRI study by Khosheijad, Piché, Saleh, Duncan, and Rainville (2014), also reporting SII activation in a group of healthy subjects during transcutaneous electrical stimulation. We did not find significant SI activation in the controls, which could be related to the electrical stimulation of different body sites which are both left and right lateralized (see Fig. 1A) might have diluted the fMRI signal related to sensory stimulation. The use of a less conservative threshold did show activation in bilateral SI. In contrast, the amputee group mostly felt phantom sensations in the hand. Therefore, convergence of sensory percept in the hand could have led to an increased fMRI signal in SI.

We found a significant positive relationship between the stimulation intensity and the rating of intensity perceived at the stimulation site, as well as between the stimulation intensity and the unpleasantness of the stimulation at the stimulated site in both controls and amputees, indicating that both groups perceived the stimulation in a similar manner, despite of the deafferentation in amputees. In the amputee group, there was no significant relationship between the ratings related to the electrical stimulation and the intensity and unpleasantness of the phantom sensations. This shows that the sensations perceived in the phantom can be triggered but cannot be modulated remotely.

Moreover, we observed significant differences in the intra- and interhemispheric communication between amputees and controls as revealed by ROI-analyses. Amputees showed a significant positive association between neural activity in the right IPS and right SI, indicating intrahemispheric interaction mechanisms. The dual contribution of the right IPS, involved in egocentric body representation (Rousseaux, Honoré, & Saj, 2014) and the right SI, involved in tactile illusions (Schaefer et al., 2014) could be related to the perception of phantom sensations.

The controls showed strong interhemispheric interaction mechanisms between the right IPS and its homologous contralateral left IPS as well as between the right SI and its homologous contralateral left SI. Although SI did not show significant activation during electrical stimulation in controls, possibly because of a lack of power related to the various body points being stimulated, this functional connectivity between left and right SI suggests that sensory information is being processed and shared between the two hemispheres. These interhemispheric interaction mechanisms have previously been reported in primary somatosensory and motor areas in non-amputees using transcranial magnetic stimulation (Chen, 2004; Meehan, Lindsell, Handy, & Boyd, 2011). In addition, similar findings have been reported in an animal study where interhemispheric sensorimotor connectivity was disrupted following peripheral nerve injury (Pawela et al., 2010).

The present findings suggest that “normal” interhemispheric communication in SI may not occur in amputees, and evoked phantom sensations may be related to a decoupling of left and right SI. These interhemispheric interaction mechanisms could be mediated by impaired transcalsal sensorimotor integration, resulting from the absence of sensory inputs from the missing limb. These data are inline with the Hari et al. (1998) study on supernumerary limbs where contralateral inhibition in SII was related to the limb presence.

We found that the neuronal correlates of non-painful phantom sensations seem to be related to activity in the right IPS and in bilateral SI. The temporal dynamics of these phantom sensations could not be
established with this study, therefore we could not determine if the involvement of SI is a cause or a consequence or a parallel process of IPS activation. The use of TMS should enable to establish a causal link between the role of IPS and SI and phantom sensations.

4.1. Limitations

Our sample of amputees was heterogeneous and relatively small, even though comparable to previous studies (Bolognini et al., 2013; Brugger et al., 2000; Grüsser et al., 2004). This is related to the circumstance that only few amputees show easy to evoke and modulate (an in-onf manner suitable for fMRI) referred sensations. It is therefore worth to mention that the analyses performed might have resulted in overestimated power, and replicability and generalizability of the results needs to be determined in further studies with larger samples.

We also combined left and right amputees in our group analysis. In some studies, the functional data of some amputees were flipped to obtain homogeneity of the sample (Björkman et al., 2012; Makin et al., 2013; Simões et al., 2012), even though the two hemispheres of a single subject are neither anatomical nor functionally equivalent. Although we have not flipped our data, our fMRI results are comparable to previous studies using tactile stimulation of the hand in amputees (Björkman et al., 2012; Schmalzl et al., 2014).

Another potential limiting factor could be the application of electrical stimulations over different sites in the left and right body sides, which could decrease the signal to noise ratio of fMRI maps when averaging across all stimulated sites. We aimed to determine the neural correlates of evoked phantom sensations, which should be similar across all amputees despite the side of amputation and the site of stimulation. Further research should be carried out to examine the relationship between side of amputation and evoked phantom sensations.

Conflict of interest

The authors declare no conflict of interest.

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