Reassessing cortical reorganization in the primary sensorimotor cortex following arm amputation

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The role of cortical activity in generating and abolishing chronic pain is increasingly emphasized in the clinical community. Perhaps the most striking example of this is the maladaptive plasticity theory, according to which phantom pain arises from remapping of cortically neighbouring representations (lower face) into the territory of the missing hand following amputation. This theory has been extended to a wide range of chronic pain conditions, such as complex regional pain syndrome. Yet, despite its growing popularity, the evidence to support the maladaptive plasticity theory is largely based on correlations between pain ratings and oftentimes crude measurements of cortical reorganization, with little consideration of potential contributions of other clinical factors, such as adaptive behaviour, in driving the identified brain plasticity. Here, we used a physiologically meaningful measurement of cortical reorganization to reassess its relationship to phantom pain in upper limb amputees. We identified small yet consistent shifts in lip representation contralateral to the missing hand towards, but not invading, the hand area. However, we were unable to identify any statistical relationship between cortical reorganization and phantom sensations or pain either with this measurement or with the traditional Euclidian distance measurement. Instead, we demonstrate that other factors may contribute to the observed remapping. Further research that reassesses more broadly the relationship between cortical reorganization and chronic pain is warranted.

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Abbreviations: CoG = centre of gravity; SI = primary somatosensory cortex
**Introduction**

Brain reorganization is a key mechanism that enables adjustment to novel situations and injuries, but it had also been suggested to have maladaptive consequences (Flor et al., 2006). Amputation is a striking driver of plasticity, as it induces both sensory deprivation and altered behaviour. In monkeys, arm deafferentation drives massive cortical reorganization in the primary somatosensory cortex (SI), where the lower face representation takes over the cortical territory of the missing hand (Pons et al., 1991; Jain et al., 2008) (see Devor and Wall, 1978; Florence and Kaas, 1995; Kambi et al., 2014 for reorganization in subcortical structures). In humans, remapping of lower face representation was shown to correlate with phantom limb pain (Flor et al., 1995; Lotze et al., 2001; Foell et al., 2013). Subsequently, SI reorganization is increasingly assumed to play a key role in other syndromes of chronic pain (Maihöfner et al., 2003; Nava and Röder, 2011; Gustin et al., 2012), with important potential implications for designing clinical treatments (Moseley and Flor, 2012).

We recently reported that activity levels in the missing hands’ territory of amputees is not increased during lip movements (Makin et al., 2013b). Instead, we found that phantom pain is associated with maintained structure and function during phantom hand movements. Nevertheless, this approach was not suitable for studying reorganization along the sensorimotor homunculus outside the missing hand territory. Sensorimotor reorganization in humans is typically measured as the Euclidian distance between the centre of gravity (CoG), or peak, in activity associated with facial touch (Flor et al., 1995), or more recently lip movements (Lotze et al., 2001; Foell et al., 2013), and an ‘anchor’ (e.g. the mirror projection of the intact hand representation; Flor et al., 1995). However, these measurements are taken across a folded cortical volume, and therefore do not respect anatomical barriers (e.g. white matter), or take into account the unique cortical morphology of individuals. As such, the physiological relevance of these measurements for cortical reorganization is potentially limited.

Here, we assessed remapping of sensorimotor lip representations using an unfolded model of the cortex, allowing us to measure surface-based cortical distances while considering individual cortical folding patterns (Maeda et al., 2014) in 17 unilateral upper limb amputees and 21 intact controls. We found consistent shifts in lip representation along the homunculus contralateral to the missing hand in amputees (hereafter ‘deprived homunculus’) towards the hand area. However, this shift didn’t reflect full invasion of the lips into the hand territory as previously described, but rather a small local shift in the centre of gravity of the lips. This remapping was statistically independent of phantom pain ratings.

**Materials and methods**

**Participants**

Eighteen individuals with acquired unilateral upper limb amputation and varying degrees of phantom pain [mean age ± standard error of the mean (SEM) = 46 ± 3, six with absent right hand; Table 1] were recruited through the Oxford Centre for Enablement and Opcare. Twenty-two healthy controls, matched for handedness (seven left-hand dominant), age (41 ± 3 years) and education were also recruited. Procedures were in accordance with NHS national research ethics service approval (10/H0707/29), and written informed consent was obtained. Data from one amputee was discarded because of excessive head movements. One control and two amputees were discarded from subanalyses due to missing activity during feet (control) and phantom hand/arm (amputees) conditions (see below). The participants were studied intensively using a range of neuroimaging and behavioural tests and some of these data have been used to assess activity levels in the missing hand’s territory in previously published studies (Makin et al., 2013a, b).

**Phantom sensations rating**

Amputees rated intensities of phantom/stump pain and non-painful phantom sensations, using a 0–10 scale, as well as the frequency of these experiences, as follows: (i) intensity of worst pain/most vivid sensation experienced during the last week (or in a typical week involving such sensations); (ii) intensity of phantom pain on average over the last week (or in a typical week if last week was atypical); and (iii) current intensity/vividness of phantom pain and sensations, during scanning day. In addition, participants were asked to rate the intensity of an inventory of pain sensations (see Table 2 legend). ‘Pain magnitude’ was calculated by dividing pain intensity by frequency (1, all the time; 2, daily; 3, weekly; 4, several times per month; and 5, once or less per month). An analogous measure was obtained for vividness of non-painful phantom sensations. See Table 1 for individual ratings and Supplementary Table 1 for dependencies between these various measurements.

**Functional MRI sensorimotor task**

We used an active motor paradigm, similar to previous studies of reorganization and phantom pain (Lotze et al., 2001; Maclver et al., 2008; Foell et al., 2013). In different conditions, participants were visually instructed to flex and extend their fingers, elbows, toes or smack their lips, resulting in six conditions: left/right hand; left/right arm, feet and lips. The protocol comprised of alternating 12-s periods of movement and ‘rest’, with each condition repeated four times, in a counterbalanced manner. It was stressed to the amputees that they should attempt to perform actual phantom hand movements, rather than imagined movements (see Makin et al., 2013b for further details).
amputees. Task-based statistical parametric maps were computed for each condition using a voxel-based general linear model (GLM) based on the gamma function of the experimental time course and its temporal derivatives. To minimize any potential contribution of secondary somatosensory cortex to lip clusters, body-part-specific representation was identified using a contrast between each of the body parts and feet movements (whereas feet were compared to baseline). We note that similar results to those reported in Fig. 1 were also identified when lip activation was compared to baseline). We note that similar results to those reported in Fig. 1 were also identified when lip activation was contrasted against baseline.

The active task involved both motor and somatosensory elements (e.g. resulting from contact between body parts or with clothes during movement execution, proprioceptive inputs, etc).

Functional MRI data analysis

MRI data acquisition, preprocessing and preliminary analysis followed standard procedures, as detailed in the Supplementary material. Spatial resolution was 3 mm isotropic; temporal resolution was 2000 ms, with a total of 300 whole-brain samples (volumes). All functional MRI analysis was carried in individual’s native anatomical space. Functional data were processed using FSL FMRIB’s expert analysis tool (FEAT, version 5.98), using a Gaussian kernel of full-width at half-maximum of 2.5 mm for spatial smoothing. Task-based statistical parametric maps were computed for each condition using a voxel-based general linear model (GLM) based on the gamma function of the experimental time course and its temporal derivatives. To minimize any potential contribution of secondary somatosensory cortex to lip clusters, body-part-specific representation was identified using a contrast between each of the body parts and feet movements (whereas feet were compared to baseline). We note that similar results to those reported in Fig. 1 were also identified when lip activation maps were contrasted against baseline.

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**Table 1** Demographic and clinical details of the amputees, and individual imaging values

<table>
<thead>
<tr>
<th>Age</th>
<th>Age at amp.</th>
<th>Amputation level</th>
<th>Side/ dominant</th>
<th>PLS Mag./scan</th>
<th>PLP Mag./scan/ave.</th>
<th>Cause of amp.</th>
<th>Lips cortical shift (distance mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A01</td>
<td>43</td>
<td>38</td>
<td>4</td>
<td>L/R</td>
<td>10/10</td>
<td>7/8/7</td>
<td>Trauma</td>
</tr>
<tr>
<td>A02</td>
<td>42</td>
<td>22</td>
<td>4</td>
<td>R/L</td>
<td>1.74/5</td>
<td>2.5/0/7</td>
<td>Nerve</td>
</tr>
<tr>
<td>A03</td>
<td>21</td>
<td>18</td>
<td>4</td>
<td>R/L</td>
<td>8/9</td>
<td>3.3/0/5</td>
<td>Trauma</td>
</tr>
<tr>
<td>A04</td>
<td>46</td>
<td>37</td>
<td>2</td>
<td>L/R</td>
<td>3/2</td>
<td>2/0/1</td>
<td>Nerve</td>
</tr>
<tr>
<td>A05</td>
<td>48</td>
<td>20</td>
<td>1</td>
<td>R/R</td>
<td>10/7</td>
<td>4.5/1/9</td>
<td>Trauma</td>
</tr>
<tr>
<td>A06</td>
<td>58</td>
<td>11</td>
<td>2</td>
<td>R/R</td>
<td>1.2/6</td>
<td>1.7/0/2</td>
<td>Trauma</td>
</tr>
<tr>
<td>A07</td>
<td>31</td>
<td>2</td>
<td>2</td>
<td>L/R</td>
<td>0/0</td>
<td>0/0/0</td>
<td>Trauma</td>
</tr>
<tr>
<td>A08</td>
<td>54</td>
<td>20</td>
<td>5</td>
<td>L/L</td>
<td>10/10</td>
<td>4/4/4</td>
<td>Trauma</td>
</tr>
<tr>
<td>A09</td>
<td>47</td>
<td>45</td>
<td>2</td>
<td>L/L</td>
<td>9/9</td>
<td>8/4/5</td>
<td>Tumour</td>
</tr>
<tr>
<td>A10</td>
<td>60</td>
<td>34</td>
<td>2</td>
<td>R/R</td>
<td>8/6</td>
<td>1/0/5</td>
<td>Trauma</td>
</tr>
<tr>
<td>A11</td>
<td>51</td>
<td>35</td>
<td>4</td>
<td>L/R</td>
<td>5/2</td>
<td>1.7/1/7</td>
<td>Infection</td>
</tr>
<tr>
<td>A12</td>
<td>47</td>
<td>19</td>
<td>2</td>
<td>L/R</td>
<td>4.5/6</td>
<td>4.5/4/4</td>
<td>Trauma</td>
</tr>
<tr>
<td>A13</td>
<td>57</td>
<td>48</td>
<td>4</td>
<td>R/L</td>
<td>3.5/7</td>
<td>1.5/6/3</td>
<td>Infection</td>
</tr>
<tr>
<td>A15</td>
<td>22</td>
<td>18</td>
<td>5</td>
<td>L/R</td>
<td>10/10</td>
<td>1/0/2</td>
<td>Trauma</td>
</tr>
<tr>
<td>A16</td>
<td>43</td>
<td>33</td>
<td>4</td>
<td>L/R</td>
<td>2.6/4</td>
<td>2.3/0/6</td>
<td>Trauma</td>
</tr>
<tr>
<td>A17</td>
<td>50</td>
<td>28</td>
<td>4</td>
<td>L/R</td>
<td>5/2</td>
<td>3/0/4</td>
<td>Trauma</td>
</tr>
<tr>
<td>A18</td>
<td>52</td>
<td>45</td>
<td>4</td>
<td>L/R</td>
<td>1.33/0</td>
<td>0/0/0</td>
<td>Trauma</td>
</tr>
</tbody>
</table>

**Table 2** Pearson’s partial coefficients of determination ($r^2$) between phantom sensations and lip cortical distances in amputees.

<table>
<thead>
<tr>
<th>Lip-to-feet cortical distance (partial)</th>
<th>PLS Mag.</th>
<th>PLS Scan</th>
<th>PLP Mag.</th>
<th>PLP Scan</th>
<th>PLP Ave.</th>
<th>PLP Mechanic</th>
<th>PLP Thermal</th>
<th>PLP Other</th>
<th>PLP Stump pain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euclidian (classical)</td>
<td>0.03</td>
<td>0.08</td>
<td>0.17</td>
<td>0.08</td>
<td>0.10</td>
<td>0.05</td>
<td>0.11</td>
<td>0.04</td>
<td>0.09</td>
</tr>
<tr>
<td>Surface-base</td>
<td>0.01</td>
<td>0.02</td>
<td>0.03</td>
<td>0.10</td>
<td>0.00</td>
<td>0.01</td>
<td>0.03</td>
<td>0.00</td>
<td>0.04</td>
</tr>
</tbody>
</table>

To account for interindividual variation in structural and functional anatomy, cortical distances of the intact hand were included as a control variable (see Supplementary Table 2 for bivariate correlations). This table demonstrates that none of the phantom sensations and pain ratings significantly explains variation in lip-to-feet cortical distances, even when wavering correction for multiple comparisons.

PLS = phantom limb sensations; Mag. = magnitude; Scan = score of sensation vividness/pain intensity on scanning day; PLP = phantom limb pain; Ave. = score of average pain; Nerve I = nerve injury; asterisk indicates potential partial spinal damage. Lip cortical shift is the difference between lip-to-feet cortical distances of the intact homunculus minus the deprived homunculus (positive values mark a medial shift). Euclid = Euclidian distance, measured in the folded brain. Surface = inflated surface analysis.
and was designed to activate the primary somatosensory, as well as the primary motor cortices (SI and M1, respectively). While SI contains detailed body maps, M1 topography is relatively crude (Schieber, 2001; Graziano and Aflalo, 2007). Indeed, as can be seen in Fig. 1B, lip activations were centred on the central sulcus, suggesting that the resulting lip-specific representations had a strong somatosensory component. It should be noted that due to partial sampling, which is a consequence of the standard spatial resolution and smoothing applied, it is impossible to reliably dissociate SI and M1 contributions to the resulting clusters. Representations resulting from this task were therefore termed ‘sensorimotor’. However, to verify that the results are not restricted by the inclusion of M1 activation, the analysis reported in Fig. 1 was repeated while excluding the precentral gyrus, with comparable results.

Cortical distances analysis

Automated reconstruction and segmentation of individual subjects’ T1 scans into surface mesh representations were carried out using FreeSurfer (http://surfer.nmr.mgh.harvard.edu/). The surface mesh of each cerebral hemisphere was inflated to a sphere while minimizing distortions to facilitate registration and maintain individual differences in cortical topology (Dale et al., 1999). The body-part-specific statistical parametric maps were registered and projected to the individual sphere mesh. To allow us to specifically focus on topographic shifts in body-part representation, individual maps were masked by FreeSurfer’s pre- and postcentral gyrus labels. Maps were thresholded by a false discovery rate (FDR) of q < 0.05 and a minimal use area of 100 mm². The resulting maps were
visually inspected to verify that the clusters were located along the sensorimotor strip. To minimize the potential contribution of neighbouring sensorimotor representations beyond the homunculus, clusters centred in the secondary somatosensory cortex and in the posterior parietal cortex surviving the contrastimg and masking procedures were discarded from this analysis (see Makin et al., 2015 for a study of whole-brain reorganization in amputees). As the aim of this analysis was simply to locate the spatial position of activation centres, the threshold criteria were waived when clusters did not survive the thresholding criteria (feet 2/3; lips 2/1; hands 1/0; arms 2/1 for amputees/controls, respectively). A CoG approach was applied to identify the location of each cluster. This approach was previously proven to be reliable across sessions, independently of functional MRI procedures (e.g. thresholding, clustering) for hand and lip movement representations (Fesl et al., 2008). The CoG of a surface cluster was defined as the average position of all its vertices on the sphere. Therefore, the distance between the CoG was defined as the shortest distance between the points along the surface of the sphere (i.e. the great circle distance). Distances between the feet CoG and each of the body part representations were measured for each participant bilaterally. In addition, distances were measured for each hemisphere between the centres of gravity of hand and lip clusters, and between lip clusters CoGs and the intercept of the central sulcus with the medial wall (as specified manually by the experimenter). These measurements of lip distances from these various anchors strongly correlated with each other (all r’s > 0.74, all P-values < 0.001), even when accounting for differences in brain size (by controlling for hand-to-feet distances). The clusters from the surface analysis were further projected back to a folded brain, and the CoG of a 3D cluster was defined as the average position of the voxels in each axis. The 3D distance was defined as the Euclidian distance across the three axes (Lotz et al., 2001).

We also devised a preliminary analysis paradigm to visualize the spatial distribution of lip representation along the central sulcus. This analysis and (null) results are detailed in the Supplementary material and in Supplementary Fig. 2.

**Statistical comparisons**

Statistical analysis was carried with SPSS version 22. Cortical distances along the deprived homunculus in amputees were compared with the intact homunculus in amputees and the hemisphere contralateral to the non-dominant hand in controls, initially using a mixed-model ANOVA (accounting for both hemispheres and groups) and then using a priori planned comparisons using independent-samples or paired two-tailed t-tests.

Another common measurement for assessing lip reorganization is made by comparing the ipsilateral and contralateral lip representation (Lotz et al., 1999, 2001; Karl et al., 2001; Foell et al., 2013). In an effort to respect individuals’ unique brain topology, rather than ‘flipping’ brain representations across hemispheres (as previously practiced) we calculated the difference of lip-to-feet distance across the two hemispheres for each individual participant (Table 1).

To account for interindividual variations in structural and functional anatomy, correlations with phantom sensations and pain were assessed using a two-tailed Pearson partial correlation test, with distances in the deprived homunculus as the variable of interest, and the intact homunculus distances as the control variable (Table 2, see Supplementary Table 2 for bivariate correlations). As none of the phantom sensation or pain measurements were independently shown to underlie lip remapping, and as these measurements were interrelated, we next selected a subset of the phantom sensation and pain ratings, to feed into a multiple regression analysis, as described below.

To explore the potential contribution of phantom sensation and pain alongside other clinical and behavioural parameters that might associate with lip remapping, we used a backward-elimination linear regression. The following independent variables were added to the regression: (1–2) phantom sensations and pain magnitude (accounting for both intensity and frequency of experience); (3) averaged phantom pain intensity; (4) extent of residual arm (stump) usage, using motor activity questionnaires (as validated in Makin et al., 2013a); (5) intact hand dexterity, measured using the pegboard task (Otten et al., 2012); (6) tactile acuity of the intact index finger, measured using the grating orientation task (Bleyenheuft and Thonnard, 2007); (7) mouth and chin usage, measured using a customised questionnaire; (8) age at which amputation occurred; and (9) total brain volume, measured based on the anatomical scan. (See Supplementary Table 4 for bivariate correlations with lip reorganization and Supplementary material for details about assessment of the behavioural parameters). One participant was excluded from the tactile acuity analysis because of difficulties with task performance. Two further amputees didn’t complete the mouth usage questionnaire. The regression proceeds by considering different combinations of independent variables and eliminating the variable that explains least variance at each step. For each model, an r2-value quantifies the variance explained by the model and an F-value quantifies the model significance, which takes into account the number of independent variables in order to favour more simple models.

**Results**

Lip mapping was initially measured as the cortical distance between the CoG of lip-selective (Fig. 1B) and feet (Fig. 1C) activations. Using the surface-based approach, we identified reliable lip reorganization in amputees, as reflected in a significant interaction between hemisphere (deprived, intact) and group (amputees, controls) [F(1,36) = 7.16, P = 0.011; Fig. 1D]. Lip distance was significantly shorter in the amputees’ deprived homunculus, compared with both their intact homunculus, [t(16)=2.37, P = 0.031] and controls’ non-dominant hand homunculus [t(36) = 3.11, P = 0.004]. This confirms remapping (i.e. shorter lip-to-feet distances) in the amputees’ deprived homunculus. On average, lips in the deprived homunculus were shifted medially by 7.8 mm, compared to the intact homunculus (Table 1, see also Supplementary Fig. 2).

We also applied the ‘traditional’ Euclidian distance approach in the folded brain (Gustin et al., 2012; Foell et al., 2013). Cortical distances measured with this approach correlated significantly with the surface-based values.
that no single clinical factor explains changes in cortical distances and that a combination of behavioural and methodological factors should be considered when interpreting these measurements.

**Discussion**

Using a surface-based approach, which takes into account individual brain morphology, we identified reliable lip remapping in the deprived homunculus of amputees. This shift may reflect invasion of the lip representation towards the missing hand cortex, as described in seminal electrophysiology studies (Pons et al., 1991). However, this shift was only partial (8 mm), and did not reflect full invasion of the lips into the hand territory (which is located some 63 mm from the lips in the controls’ homunculus, see also Supplementary Fig. 2 for complementary analysis). This result is consistent with our previous findings, showing maintained activity of the phantom hand in the missing hands’ territory of amputees (Makin et al., 2013b) (Supplementary Fig. 1).

Contrary to previous studies and despite our relatively large sample size, we were unable to identify any statistical relationship between cortical reorganization and phantom sensations or pain. This could be attributed to differences in the underlying assumptions of the different experimental approaches. For example, in their seminal paper, Flor et al. (1995) used electrical source estimates of lip and cheek foci, distances were measured with respect to the intact hand, and phantom pain was assessed with respect to pain intensity and suffering. To bridge this gap, it is important that further studies are carried out, while taking into consideration the methodological and conceptual constraints highlighted here, namely the usage of physiologically realistic measurements of reorganization and attention to other clinical factors that could be driving brain plasticity. Our current results are consistent with a recent study in patients with carpal tunnel syndrome that identified correlations between surface-based SI reorganization and paraesthesia severity, but no correlation with pain (Maeda et al., 2014). The view that multiple factors may shape reorganization is also in accordance with our recent findings for use-dependent plasticity in the deprived cortex of amputees (Makin et al., 2013a; Hahamy et al., 2015). Therefore, our results call for a reassessment of maladaptive plasticity theories ascribing a specific causal role to cortical remapping in driving chronic pain and the associated treatments targeting this remapping to alleviate pain.

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Supplementary material
Supplementary material is available at Brain online.

References