Limb amputation and chronic phantom limb pain (PLP) are both associated with neural alterations at all levels of the neuraxis. We investigated gray matter volume of 21 upper limb amputees and 14 healthy control subjects. Results demonstrate that amputation is associated with reduced gray matter in areas of the motor cortex representing the amputated limb. Additionally, patients show an increase in gray matter in brain regions that belong to the dorsal and ventral visual stream. We subdivided the patient group into patients with medium to high PLP (HPLP; \( N = 11 \)) and those with slight PLP (SPLP; \( N = 10 \)). HPLP patients showed reduced gray matter in brain areas involved in pain processing. SPLP patients showed a significant gray matter increase in regions of the visual stream. Results indicate that all patients may have an enhanced need for visual control to compensate the lack of sensory feedback of the missing limb. As we found these alterations primarily in the SPLP patient group, successful compensation may have an impact on PLP development. Therefore, we hypothesize that visual adaptation mechanisms may compensate for the lack of sensorimotor feedback and may therefore function as a protection mechanism against high PLP development.

Keywords: amputation, chronic pain, morphometry, phantom limb pain, visual stream

Introduction

After amputation and deafferentation of a limb, up to 98% of individuals report vivid sensations in the missing part of the body (Ramachandran and Hirstein 1998). In turn, 50–80% of amputees describe these sensations as painful (Flor 2002). This phenomenon is known as phantom limb pain (PLP) (Jensen et al. 1985). PLP includes a variety of different qualities such as burning, stabbing, or cramping (Giummarra et al. 2007). PLP must be differentiated from stump pain, which is characterized by painful sensations located in the residual limb. PLP should also be differentiated from nonpainful sensations (phantom limb sensations, PLS) and the feeling of the enduring presence of the missing part of the body (phantom limb awareness) (Flor et al. 2006). High PLP (HPLP) seems to have more impact on patient's daily life than slight PLP (SPLP) (Jensen et al. 2001).

Previous studies have described neural alterations in all parts of the neuraxis. Especially, neural alterations in the brain were found in patients with chronic pain (May 2011), chronic back pain (Apkarian et al. 2004; Schmidt-Wilcke et al. 2006), headache (May et al. 1999; Schmidt-Wilcke et al. 2005, 2008; Rocca et al. 2006; Kim et al. 2008; Valfrè et al. 2008), fibromyalgia (Kuchinad et al. 2007; Schmidt-Wilcke et al. 2009; Puri et al. 2010), chronic complex regional pain syndrome (Geha et al. 2008), irritable bowel syndrome (Davis et al. 2008; Seminowicz et al. 2010), painful hip osteoarthritis (Gwilym et al. 2010), chronic facial pain (DaSilva et al. 2008; Schmidt-Wilcke et al. 2010), and pain disorder (Valet et al. 2009). In these studies, a significant reduction of gray matter volume was observed. Changes in density or thickness were reported for the insular cortex (IC), areas of the medial pain system including the anterior cingulate cortex (ACC) and the prefrontal cortex (PFC) (Treede et al. 1999).

Studies focusing on neural alterations caused by amputation are rare and the results are not uniform. A single case study reports a continuous loss of gray matter in the primary somatosensory cortex (S1), superior parietal cortex, supplementary motor area, primary motor cortex (M1), and cerebellum covering a 21-week period after upper limb amputation (Gaser et al. 2004). Neurological alterations in S1 are in line with previous studies focusing on the underlying central mechanisms of PLP. Cortical reorganization of S1 is thought to play a major role in the genesis and maintenance of PLP (Flor et al. 1995, 2006; Birbaumer et al. 1997; Preißler et al. 2011). Neurological alterations were also observed in patients with deafferentation following complete thoracic spinal cord injury. Here, loss of gray matter was observed in the motor cortex, motor pathways, the ACC, anterior temporal cortex, lateral hypothalamus, and IC (Wrigley et al. 2009; Freund et al. 2011). In contrast, phantom limb patients with either upper or lower limb amputation and different pain levels exhibited only subcortical gray matter reduction located in the thalamus contralateral to the side of amputation (Draganski et al. 2006). Taken together, these findings suggest that limb amputation is followed by neural alterations in different brain areas. However, it is still not known whether such neural alterations are due to the amputation or to the chronic pain condition that is experienced by most amputees. In the present study, we aimed to evaluate these 2 potential causes of cortical alterations by comparing the morphology of different brain structures of patients with upper limb amputation who experience high chronic PLP with those of patients who did not develop PLP at all or only to a very mild extent. Additionally, we compared the brains of both groups with those of a matched healthy control group.

Materials and Methods

Participants

Twenty-one patients with right upper limb amputation and 14agematched healthy controls participated in this study. Except for 3 female
patients, all subjects were male. Exclusion criteria were plexus avulsion, amputations of another part of the body, congenital malformation, and/or any neurological or psychiatric disease. Sociodemographic and clinical data of patients and controls are presented in Table 1 (for a more detailed view, refer to Supplementary Material Table S1). Amputation was undertaken following trauma in 19 patients and sarcoma in 2 patients.

The study was approved by the ethics committee of the Friedrich Schiller University. Informed consent was obtained from each subject prior to examination.

Assessment of PLP

The amputees were asked to rate the intensity of their PLP. Assessment of PLP was performed using a 10-cm Visual Analog Scale (VAS) with "no pain at all" presented by the left end point and "the strongest pain I can imagine" by the right end point of the scale (Scott and Huskisson 1976).

Assessment of Depression Symptoms

Depression symptoms were measured with the Beck Depression Inventory-II (BDI-II) (Beck et al. 1996). The BDI-II is a 21-item questionnaire that assesses somatic, affective, and cognitive symptoms due to depression. Participants answer questions based on a 4-point scale ranging from 0 (not at all) to 3 (extreme form of each symptom) for the last 2 weeks. Scores range from 0 to 63. Scores of 30 and higher point to a "severe depression."

Assessment of Life Interference

For the assessment of life interference, "The life interference scale" of the German version of the Multidimensional Pain Inventory (MPI) was used (Kerns et al. 1985; Flor et al. 1990). The MPI is a questionnaire for a multidimensional assessment of chronic pain. It consists of 52 items, which consists of 3 sections (Kerns et al. 1985). The first section includes 5 scales; they measure patient's perception of pain severity, life interference caused by chronic pain, experienced life control, affective distress, as well as social support. Patients have to respond on a 7-point scale ranging from 0 to 6.

Magnetic Resonance Imaging Data Acquisition

Two T1-weighted sagitally oriented sequences for morphometric analyses were acquired with all subjects (192 slices; flip angle: 30°; matrix: 256 × 256; voxel size: 1 × 1 × 1 mm). As participants began to be studied in 2005, the first 16 subjects were measured on a 1.5-T magnetic resonance imaging (MRI) scanner (Siemens Magnetom Vision Plus, Erlangen, Germany; time echo [TE]: 5 ms; time repetition [TR]: 15 ms). For the remaining 18 participants, a 3-T MRI scanner (Siemens Magnetom Trio, Erlangen, Germany; TE: 3.03 ms; TR: 2.3 ms) was used (see Table 1). All acquisitions were done with a standard head coil to acquire whole brain MRI data. Head movement was minimized using a vacuum pad.

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Patients with high phantom limb pain (N = 11)</th>
<th>Patients with slight phantom limb pain and phantom limb sensations, and healthy controls, respectively (N = 13)</th>
<th>Group difference test</th>
</tr>
</thead>
<tbody>
<tr>
<td>N male/female</td>
<td>8/3</td>
<td>10/0</td>
<td>X² (2) = 2.71 (P = 0.03)</td>
</tr>
<tr>
<td>Age in years, M (SD)</td>
<td>49.82 (10.52)</td>
<td>38.20 (16.01)</td>
<td>F (2,34) = 2.52 (P = 0.10)</td>
</tr>
<tr>
<td>MRI scanner 1.5T/3T</td>
<td>3/8</td>
<td>5/5</td>
<td>X² (2) = 2.32 (P = 0.31)</td>
</tr>
<tr>
<td>Time since amputation in month, M (SD)</td>
<td>130.09 (112.16)</td>
<td>143.55 (198.97)</td>
<td>T₁0 = −0.19 (P = 0.85)</td>
</tr>
<tr>
<td>Mean age at amputation in years, M (SD)</td>
<td>38.64 (12.46)</td>
<td>26.55 (7.86)</td>
<td>T₂ = 2.86 (P = 0.01)</td>
</tr>
<tr>
<td>Handedness (before amputation) right/left</td>
<td>11/0</td>
<td>10/0</td>
<td>X² (1) = 0.005 (P = 0.94)</td>
</tr>
<tr>
<td>Side of amputation right/left</td>
<td>11/0</td>
<td>10/0</td>
<td>T₃ = 1.51 (P = 0.15)</td>
</tr>
<tr>
<td>Traumatic amputation yes/no</td>
<td>10/1</td>
<td>9/1</td>
<td>T₄ = 7.32 (P &lt; 0.001)</td>
</tr>
<tr>
<td>BDI, M (SD)</td>
<td>15.09 (12.83)</td>
<td>7.80 (8.65)</td>
<td>T₅ = 4.99 (P &lt; 0.001)</td>
</tr>
<tr>
<td>VAS, M (SD)</td>
<td>5.46 (1.64)</td>
<td>0.91 (1.31)</td>
<td></td>
</tr>
<tr>
<td>MPI, M (SD)</td>
<td>4.70 (1.25)</td>
<td>1.78 (1.30)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: MRI = magnetic resonance imaging; BDI = Becks Depression Inventory, VAS = pain intensity ratings with visual analog scale; MPI = life interference scale of the MPI.

Cortical Reconstruction and Volumetric Segmentation


Briefly, the processing includes motion correction and averaging (Reuter et al. 2010) of the 2 volumetric T₁-weighted images, removal of nonbrain tissue using a hybrid watershed/surface deformation procedure (Segonne et al. 2004), automated Talairach transformation, segmentation of the subcortical white matter, and deep gray matter volumetric structures (Fischl et al. 2002; Fischl, Salat, et al. 2004), intensity normalization (Sled et al. 1998), tessellation of the gray matter-white matter boundary, automated topology correction (Fischl et al. 2001; Segonne et al. 2007), and surface deformation. The last procedure is accomplished by following intensity gradients to optimally place the gray/white and gray/cerebrospinal fluid borders at the location where the greatest shift in intensity defines the transition to the other tissue class (Dale and Sereno 1993; Dale et al. 1999; Fischl and Dale 2000). Once the cortical models are complete, a number of deformable procedures can be performed for further data processing and analysis including surface inflation (Fischl, Sereno, and Dale 1999), registration to a spherical atlas which utilizes individual cortical folding patterns to match cortical geometry across subjects (Fischl, Sereno, Tootell, et al. 1999), parcellation of the cerebral cortex into units based on gyral and sulcal structure (Fischl, Salat, et al. 2004; Desikan et al. 2006), and creation of a variety of surface-based data, including maps of curvature and sulcal depth. This method uses both intensity and continuity information from the entire 3D MR volume in segmentation and deformation procedures to generate representations of cortical thickness, calculated as the closest distance from the gray/white boundary to the gray/cerebrospinal fluid boundary at each vertex on the tessellated surface. FreeSurfer morphometric procedures have been demonstrated to show good test-retest reliability across scanner manufacturers and across field strengths (Han et al. 2006). Volume measures may be mapped on the inflated surface of each participant’s reconstructed brain. Maps are smoothed using a circularly symmetric Gaussian kernel across the surface with a standard deviation (SD) of 10 mm and averaged across participants using a nonrigid high-dimensional spherical averaging method to align cortical folding patterns. This procedure provides accurate matching of morphologically homologous cortical locations among participants, resulting in a mean measure of cortical thickness for each group at each point on the reconstructed surface. The entire cortex in each participant was visually inspected and any inaccuracies in segmentation were manually corrected by experts in brain anatomy who were blind to group membership.

Statistical comparisons of global data and surface maps were generated by computing a general linear model of the effects of each variable (group membership, demographic and neuropsychological variables) on volume at each vertex.

Cortical volume clusters were first displayed using a threshold that shows all vertices with P values below 0.05. To avoid type I error inflation, a Monte Carlo simulation was then conducted to correct for...
multiple comparisons on the significant clusters, using a vertex-wise threshold of $P < 0.05$ (Hayasaka and Nichols 2003; Hagler et al. 2006). Additionally, only clusters with a minimum size of 10 vertices were accepted (Lieberman and Cunningham 2009).

From a generated cluster, we created an image of the cluster on the group average brain that was mapped back to each individual subject using spherical morphing to find homologous regions across subjects and yield a mean volume score over the location for each subject (http://www.mail-archive.com/freesurfer@nmr.mgh.harvard.edu/msg17209.html). These individual volume values were used for correlation analyses computed by IBM SPSS Statistics (version 19.0, SPSS Inc., an IBM Company, Chicago, IL, USA).

**Results**

**Patients with Amputation versus Healthy Controls**

To examine the effect of amputation on morphological brain structures, brains of healthy controls were compared with those of upper limb amputees. Brains of amputees showed reduced total gray matter volume, exhibiting significant differences for the right (patients: $M = 232197.57$ mm$^3$, SD = 35448.54 mm$^3$; controls: $M = 255797.50$ mm$^3$, SD = 25985.19 mm$^3$; $F_{1.33} = 4.55, P = 0.040$) and the left hemisphere (patients: $M = 230827.43$ mm$^3$, SD = 34615.14 mm$^3$; controls: $M = 253095.21$ mm$^3$, SD = 24594.69 mm$^3$; $F_{1.33} = 4.32, P = 0.046$).

A significant increase in gray matter volume was found for the left temporal pole, left dorsolateral prefrontal cortex (DLPFC), left fusiform cortex, right middle temporal cortex (mTC), and the right superior parietal cortex of amputees' brains. For the left hemisphere, patients showed reduced gray matter in the primary motor cortex (M1) (see Fig. 1). A significant decrease was also found for the right DLPFC (see Table 2).

To ensure that these results are not caused by the different field power of the 2 different MRI scanners, age or gender, we performed 3 additional MANCOVAs with scanner type, age, or gender as covariate, respectively. None of these covariates had a significant influence on the results (MRI scanner type: $F_{7,26} = 1.93, P = 0.11$; age: $F_{7,26} = 1.42, P = 0.24$; gender: $F_{7,26} = 1.54, P = 0.20$).

Figure 1. Cortical gray matter volume differences for patients after amputation compared with healthy controls. Inflated presentation of a standardized brain from different sides. Areas shown in blue are regions with decrease in gray matter volume for patients after amputation. Red areas mark an increase in gray matter volume for patients after amputation. Only areas with a vertex-wise threshold of $P < 0.05$ are shown. Light blue surrounded areas belong to the visual stream. 1: superior parietal; 2: middle temporal; 3 and 4: DLPFC; 5: precentral; 6: temporal pole; 7: fusiform cortex.
Patients with High Phantom Limb Pain versus Patients with Slight Phantom Limb Pain

To further differentiate cortical effects of limb amputation from the effects of chronic pain following amputation, we performed a second group comparison focusing only on those patients having intense PLP and patients with PLS and slight PLP. Based on a study by Jensen et al. (2001), we divided our patient group in 2 groups, one with no to slight PLP (SPLP) and the other with moderate to severe PLP (HPLP), as severe PLP seems to have more impact on life and daily activities as mild or no PLP. We performed 4 ANOVAs with life interference as independent variable and mild versus severe pain as grouping variable. The grouping variable was based on the pain VAS score 1) less than 2, 2) less than 3, 3) less than 4, 4) less than 5. Each model reached significance, with the second model with border VAS = 3 reaching the highest F-score (F19,1 = 24.86).

The groups did not differ significantly in the time since amputation (t0 = -0.19; P = 0.85), depression scores (t0 = 1.51; P = 0.15), or reasons for amputation (χ²(1) = 0.005; P = 0.94). However, they showed a significant difference in mean age at amputation (t0 = 2.85; P = 0.01) (see Table 1).

Total gray matter volume did not significantly differ between the 2 patient groups (right hemisphere: HPLP patients: M = 220809.64 mm³; SD = 18321.26 mm³; SPLP patients: M = 244724.30 mm³; SD = 45679.53 mm³; t11,60 = 1.55; P = 0.149; left hemisphere: HPLP patients: M = 219706.64 mm³; SD = 19607.30 mm³; SPLP patients: M = 243603.30 mm³; SD = 43796.09 mm³; t12,21 = 1.55; P = 0.146).

Except for an increased gray matter volume in the caudal part of the left ACC, HPLP patients showed a continuous picture of gray matter reduction compared to SPLP patients. Additional analyses revealed that the volume in the caudal ACC in patients with HPLP is not only higher with respect to patients with SPLP, but also compared to healthy controls (for results of the additional group contrasts - refer to Supplementary Material Tables S2 and S3).

We found a bilateral decrease of gray matter volume for PLP patients in the fusiform cortex, the inferior temporal cortex, the DLPFC, the lateral orbitofrontal cortex (OFC), and the posterior cingulate. Moreover, HPLP patients showed reduced gray matter volume in the isthmus of the left cingulate, the left supramarginal gyrus, the lateral and inferior part of the left M1, the left superior temporal lobe, the right M1, the right insula with extension to pars opercularis (see Fig. 2) and the right pars opercularis. In the left frontal lobe, we also found a reduction of gray matter volume for HPLP patients located in the superior frontal and the caudal part of the middle frontal lobe in contrast to the SPLP patient group (see Table 3 and Supplementary Material Fig. S1). Again we tried to ensure that these results are not caused by the different field power of the 2 different MRI scanners, age, or gender. We performed 3 additional multiple analyses of covariance (MANCOVAs) with scanner type, age, or gender as covariate, respectively. None of these covariates had a significant influence on the results (MRI scanner type: F19,1 = 2.05, P = 0.51; age: F19,1 = 80.6, P = 0.09; gender F19,1 = 5.2, P = 0.33).

Correlation Analyses

To further specify the underlying relation between cortical structural alterations and chronic pain (VAS ratings), depression (BDI-II), life interference (MPI), age at amputation, time since amputation, and stump length, we applied nonparametric correlation analyses (http://www.mail-archive.com/freesurfer@nmr.mgh.harvard.edu/msg17209.html). In the patient group with HPLP, we found a positive correlation for VAS scores with gray matter volume in the caudal ACC (r = 0.697; P = 0.003). Additionally, gray matter volume in the insula region (right hemisphere) correlated negatively with VAS rating (r = -0.477; P = 0.042). Furthermore, there is a positive correlation between the gray matter volume of the isthmus cingulate cortex and individual pain rating (r = 0.514; P = 0.029).

Also we found a positive correlation between the volume of the inferior temporal region and life interference (r = 0.630; P = 0.018), between the volume of the supramarginal gyrus (left hemisphere) and life interference (r = 0.580; P = 0.030). Time since amputation in this group had a negative association with gray matter volume of the fusiform gyrus (r = -0.527; P = 0.024). In the patient group with SPLP, we found a negative correlation between pain ratings and gray matter volume (r = -0.529; P = 0.044) in the caudal ACC. Time since amputation was in this group negatively associated with the gray matter volume in the caudal middle frontal cortex (left hemisphere) (r = -0.822; P = 0.001) and posterior cingulate cortex (left hemisphere) (r = -0.689; P = 0.006). In this patient group, the following regions showed an association with the BDI-II sum score: lateral OFC (left hemisphere) (r = -0.568; P = 0.024), DLPFC (left hemisphere) (r = -0.523, P = 0.038), and fusiform cortex (right hemisphere) (r = -0.659; P = 0.009). The lateral OFC (right hemisphere) showed a negative association with age at amputation (r = -0.511; P = 0.040).
Discussion

In this study, we differentiated between alterations of cortical volume due to amputation and those associated with HPLP.

Cortical Alterations due to Amputation

Global Changes

Compared with healthy adults, amputees showed a significantly reduced total gray matter volume. This result does not support observations of Draganski et al. (2006). These authors studied structural alterations of the brain in 28 amputees and did not detect global gray matter differences in comparison to control subjects. Although patients in the study of Draganski et al. (2006) were comparable to our patients in age and time since amputation, the difference between their and our study might be due to different methods used in assessing brain integrity. Draganski et al. (2006) used voxel-based morphometry (VBM). It is possible that the kind of VBM used in 2006 was not as sensitive as the FreeSurfer algorithm implemented in the present study (Fornito et al. 2008). This algorithm is capable of detecting submillimeter differences between groups (Fischl and Dale 2000). So the differences between the studies might be explained by the more sensitive approach used in our study.

Alternative explanations might be a differential use of functional prostheses. Prosthesis use has been shown to have an influence on functional reorganization and PLP (Lotze et al. 1999; Weiss et al. 1999). So it is possible that the amount of prosthesis use in our group influenced anatomical reorganization.

The observed reduction of gray matter may represent a progressive degenerative process that is caused by the loss of afferent input from an important part of the body, that is, one hand. This result is in line with previous reports demonstrating that deprivation of afferent input will lead to functional and structural reorganization in the adult human brain (Pascual-Leone et al. 1993, 2005; Elbert et al. 1994; Flor et al. 1995; Weiss et al. 2004; Draganski et al. 2006). As an illustration, patients with amputations early in life have shown to develop an

Figure 2. Cortical gray matter volume differences for patients with high PLP compared to patients with slight PLP and PLS. Inflated presentation of a standardized brain from different sides. Areas shown in blue are regions with decrease in gray matter volume for patients with high PLP. Only areas with a vertex-wise threshold of \( P < 0.05 \) are shown. Light blue surrounded areas belong to the visual stream. 1: pars opercularis; 2: insula (with extension to pars opercularis); 3: DLPFC; 4: lateral orbitofrontal cortex (OFC); 5: middle temporal cortex; 6: inferior temporal cortex; 7: caudal middle frontal cortex; 8(a,b): precentral cortex; 9: supramarginal; 10: fusiform cortex; 11(a,b): inferior temporal cortex; 12: superior temporal cortex; 13(a,b): lateral OFC; 14: DLFFC; 15: posterior cingulate cortex (PCC); 16: fusiform cortex; 17: isthmus cingulate cortex; 18(a-c): PCC; 19: caudal ACC.
abnormal organization of the primary motor cortex (Dettmers et al. 1999).

**Neural Alterations in the Precentral Gyrus**

Compared to the control subjects, we found a decreased cortical volume in the left primary motor cortex (M1) in amputees compared to controls. This finding is in line with data from Dettmers et al. (1999). Our results show a decrease in the volume of the left primary motor cortex (M1) in patients with amputation of the right upper limb. According to the Talairach space (Lancaster et al. 2000) and the localization of the hand knob according to Yousry et al. (1997), this region corresponds to the motor region of the removed hand. This observation does not support findings reported by Draganski et al. (2006), who showed no alterations in M1. One explanation for these discrepant observations is that the patients in the study of Draganski had different locations of amputation. Our result consisting of a cluster of 25 vertices in the left primary motor cortex (M1) in amputees compared to controls, increased gray matter density was also observed in different parts of the patients' temporal lobe (temporal pole, middle temporal lobe, fusiform cortex) and in the superior parietal lobe. The cortical visual-to-motor network includes 2 pathways of visual information processing (Rossetti et al. 2003; Cardoso-Leite and Gorea 2010). The dorsal stream including occipital, posterior temporal, and especially parietal regions is responsible for the processing of the spatial orientation of objects and the detection of movements; while the ventral stream, located mainly in occipital and temporal lobes, is involved in object perception (Ellison and Cowey 2006, 2007, 2009; Cavina-Pratesi et al. 2007; Rice et al. 2007; Cohen et al. 2009). Our findings indicate alterations in both the ventral and the dorsal pathways of the visual stream.

The temporal lobe is involved in movement planning that is based on memory of an object relative to other items (Milner and Goodale 1995; Goodale et al. 2004, 2005) as well as in linking visual information with memory (Godinho et al. 2006). The fusiform cortex, located at the very inferior part of the temporal lobe, exhibits a high specialization in body and face recognition (Pecalen and Downing 2006). The superior parietal lobe is an anatomical core structure of the dorsal part of the visual stream. It is connected with somatosensory and motor-related regions (Margulies et al. 2009) and receives visual input as well (Rizzolatti and Matelli 2003). The superior parietal lobe

**Table 3**

<table>
<thead>
<tr>
<th>Region</th>
<th>Subregion</th>
<th>Talairach coordinates</th>
<th>Change direction</th>
<th>Cluster size</th>
<th>z-score of peak voxel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temporal lobe</td>
<td>LH fusiform cortex</td>
<td>−40.8 −72.5 −15.5</td>
<td>−</td>
<td>243</td>
<td>5.73</td>
</tr>
<tr>
<td></td>
<td>RH fusiform cortex</td>
<td>−30.6 −68.3 −14.5</td>
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<td>30</td>
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<tr>
<td></td>
<td>LH inferior temporal</td>
<td>−48.4 −24.2 −26.5</td>
<td>−</td>
<td>293</td>
<td>2.78</td>
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<tr>
<td></td>
<td>RH inferior temporal</td>
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<td>2.37</td>
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<td></td>
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<td>2.82</td>
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<td>−</td>
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<td>5</td>
<td>2.34</td>
</tr>
<tr>
<td></td>
<td>RH lateral OFC</td>
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</tr>
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<td>−</td>
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<td>2.52</td>
</tr>
<tr>
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<td>40.1 6.2 22.1</td>
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<td>LH caudal anterior cingulate</td>
<td>−9.6 −15.5 38.6</td>
<td>−</td>
<td>36</td>
<td>2.60</td>
</tr>
<tr>
<td></td>
<td>LH isthmus cingulate</td>
<td>−13.7 −26.9 36.5</td>
<td>−</td>
<td>117</td>
<td>3.21</td>
</tr>
<tr>
<td></td>
<td>LH precentral</td>
<td>−2.9 10.1 25.1</td>
<td>+</td>
<td>40</td>
<td>−3.27</td>
</tr>
<tr>
<td></td>
<td>RH precentral</td>
<td>−2.3 −51.2 27.3</td>
<td>+</td>
<td>189</td>
<td>3.20</td>
</tr>
<tr>
<td></td>
<td>LH superiorfrontal</td>
<td>−40.2 2.8 22.5</td>
<td>−</td>
<td>222</td>
<td>4.05</td>
</tr>
<tr>
<td></td>
<td>RH superiorfrontal</td>
<td>−43.0 −5.1 16.2</td>
<td>−</td>
<td>26</td>
<td>2.59</td>
</tr>
<tr>
<td></td>
<td>LH supramarginal</td>
<td>−50.2 −53.2 42.2</td>
<td>−</td>
<td>210</td>
<td>3.07</td>
</tr>
<tr>
<td></td>
<td>LH extension to pars opercularis</td>
<td>−34.8 15.0 12.7</td>
<td>−</td>
<td>395</td>
<td>4.16</td>
</tr>
</tbody>
</table>

Note: Abbreviations: LH = left hemisphere; RH = right hemisphere; PFC = prefrontal cortex; M1 = primary motor cortex; DLPFC = dorsolateral prefrontal cortex; OFC = orbitofrontal cortex; + = increase of gray matter volume for phantom limb pain (PLP) patient group; − = decrease of gray matter volume for PLP patient group.
et al. (1998) suggest that an internal estimation of the state of the world and one's own body is gained from continuous sensory and visual inputs in combination with motor output. The right superior parietal gyrus matches the dorsodorsal pathway (Pisella et al. 2006). This is the most immediate visual pathway for action by means of online control (Rossetti et al. 2003) and direct goal-directed movements, such as reaching and grasping (Rizzolatti and Matelli 2003). Absolute metrics and position of visual objects are computed in real time by this area (Cardoso-Leite and Gorea 2010). Taking into account that upper limb amputees lack an important resource of sensory feedback (Dietrich et al. 2012), that is, somatosensory feedback from the hand, we hypothesize that the lack of somatosensory feedback from the hand will lead to a need to shift attention during manipulation of objects away from somatosensory toward visual feedback.

Three arguments are in line with our hypothesis. First, our results demonstrate an increase in gray matter in regions that are activated by different kinds of visual information (Cardoso-Leite and Gorea 2010). It has been shown for this system that extensive training might result in an increase of gray matter volume in these regions (Draganski et al. 2004). Based on these observations, it seems likely that the increase in gray matter volume in the structures observed in our patients results from an increased use of visual information in these patients. Second, 11 of our patients used a myoelectric prosthesis regularly at an advanced level. There is a special need to visually control the proper action of the prosthesis, for example, during grasping. This enhanced need for visual control might lead to (training-induced) cortical plasticity resulting in gray matter increase. Third, the same kind of inverted mechanism has been observed in blind subjects. The loss of visual input enhances the development of other sensory abilities. This holds especially true for somatosensory abilities in Braille readers (Sterr et al. 1998).

Previous studies have shown that activation in DLPFC occurs when there is a mismatch between motor intention and sensory or visual feedback (Fink et al. 1999). Upper limb amputees may experience a permanent mismatch and this may lead to persistent DLPFC activation. In the long term, this continuous over activation could result in anatomical changes in this region. Another possible explanation focuses on the DLPFC as a region involved in spatial working memory tasks (Funahashi 2006). As our result is located contralaterally to the amputation site, continuous gray matter decrease may be the consequence of a reduced use of spatial memory tasks involving the right upper limb.

In summary, the observed regional increase of gray matter in parts of the visual stream in the patient group as compared to healthy controls points to the increased importance of the visual system in upper limb amputees with a possible use-dependent increase of gray matter volume both in the dorsal and ventral visual streams.

Cortical Alterations due to Chronic HPLP

Global Changes

We found no differences in global volume between patients with HPLP and patients with SPLP. This is in line with results of other studies focusing on gray matter changes due to chronic pain. Earlier studies observed regional alterations but no global changes due to chronic pain (Schmidt-Wilcke et al. 2006; Geha et al. 2008; Kim et al. 2008; Valfré et al. 2008). Thus, chronic HPLP is not related to global gray matter volume changes in HPLP patients.

Anatomical Changes in the Visual Stream

However, by contrasting patients with HPLP and those with SPLP, we found cortical alterations in the visual stream. Patients with HPLP showed reduced gray matter volume in the ventral visual system, that is, the ventral temporal lobe. This is in line with a number of recent morphometric studies that have also found gray matter reductions in different parts of the temporal lobe of chronic pain patients. Reduced gray matter in the inferior, middle, and/or superior temporal cortex was reported for patients suffering from irritable bowel syndrome (Seminowicz et al. 2010), headache (Rocca et al. 2006; Valfré et al. 2008; Holle et al. 2011), chronic back pain (Schmidt-Wilcke et al. 2006), fibromyalgia (Schmidt-Wilcke et al. 2007), and pain disorder (Valet et al. 2009). Furthermore, patients with painful hip osteoarthritis show increased gray matter bilaterally in the fusiform cortex (Gwilym et al. 2010), while pain patients with cyclic menstrual pain demonstrate increased gray matter volume in the left superior middle temporal gyrus (Tu et al. 2010). Despite these findings, the temporal lobe is not generally viewed as an established area that processes nociceptive information or pain. In line with our hypothesis about the functional significance of the increased volume of gray matter in the visual stream of patients, we hypothesize that patients with HPLP might use their affected arms less often due to PLP. Thus, patients with HPLP showed no adaptation compared with healthy controls, but less adaptation in the visual stream than patients with SPLP. This leads to a highly speculative hypothesis for the development of PLP. The adaptability of the visual system may indicate a cortical compensation mechanism counteracting the genesis of PLP. More precisely, if proper adaptation to the loss of selective somatosensory input in form of a modality shift toward the visual system fails, then incongruities between neurosignatures of different systems might occur resulting in the sensation of pain (Melzack 1990, 2005). A similar hypothesis for the DLPFC postulates that incongruence between motor intention and movement due to inappropriate representation of proprioception can cause pathological pain (Harris 1999).

Alternatively, these cortical alterations may be simply caused by inhibited use of the affected arm due to high PLP in the HPLP group as compared with lower PLP in the SPLP group. Reduction in use is probably accompanied by a reduced need to visually control movements of the arm or the prosthesis. As a result, structures belonging to the visual stream might increase their gray matter volume in SPLP patients due to an increased use of visual information, a phenomenon that might be absent in HPLP patients. This hypothesis is in line with previous research showing that a therapy using visual feedback with a mirror box can be used to reduce PLP (Ramachandran et al. 1996; Ramachandran and Rogers-Ramachandran 2006). It can be suggested that pain and cortical reorganization can potentially be altered by visual feedback (Chan et al. 2007; Flor et al. 2008; Diers et al. 2010). Nevertheless, from our data it cannot be decided whether the cortical alterations in the visual stream going together with visual feedback are cause or a possible consequence of chronic pain.
Anatomical Changes in the Pain Processing Network

The volumetric alterations associated with chronic pain were also found in areas known to be involved in the processing of nociceptive information and pain (right IC, DLPFC, OFC, caudal ACC; Weiss et al. 2008; Craig 2009; Iannetti and Mouraux 2010). Our observations thus support the results of a series of other studies on chronic pain patients (May 2011). Specifically, structural alterations have been described in chronic back pain (Apkarian et al. 2004; Schmidt-Wilcke et al. 2006), fibromyalgia (Kuchnad et al. 2007; Schmidt-Wilcke et al. 2007; Hsu et al. 2009), migraine (Kim et al. 2008; Schmidt-Wilcke et al. 2008; Valfré et al. 2008), and PLP (Draganski et al. 2006). These alterations of brain structures were related to central plasticity (Flor 2003) and considered as “maladaptive plasticity” (Katz and Melzack 1990; May 2011).

Focusing on regions with cortical alterations, we found reduced cortical volume in parts of the posterior/middle IC in HPLP patients compared with SPLP patients. The IC plays a prominent role in the processing of acute pain (Brooks and Tracey 2007). Electrical stimulation of the posterior IC seems to produce pain in distinct sites on the contralateral body (Ostrowsky et al. 2002). The existence of a pain representation system in the posterior IC is also in line with anatomical connectivity studies and electrophysiological properties of this area (Ostrowsky et al. 2002; Garcia-Larrea et al. 2010).

We also found significant changes in the OFC. This structure is known to be involved in the processing of nociceptive information and pain. According to Kringelbach and Rolls (2004), the OFC is an important brain region for the processing of rewards and punishments. OFC receives input from all sensory modalities. The authors claim that the OFC represents the “subjective affective value” of reinforcers (Kringelbach and Rolls 2004). It is assumed that the negative affective value of a painful stimuli or a chronic painful experience may be coded in this region.

We also found significant differences between HPLP and SPLP patients in the DLPFC. The DLPFC seems to play an important role in chronic pain. Wand and O’Connell (2008) described a biochemical profile of patients with chronic lower back pain with strong evidence of neurodegeneration within the DLPFC. This is supported by the results from morphometric studies (Apkarian et al. 2004; Schmidt-Wilcke et al. 2006). While morphometric data support the role of the DLPFC in chronic pain, functional MRI studies support a general role of this structure in the processing of noxious input. For example, activity in this region has been shown to be negatively correlated with pain intensity and unpleasantness (Lorenz et al. 2003) and with the degree of pain-related catastrophizing (Seminowicz and Davis 2006). Thus, structural and functional alterations of the DLPFC are consistent findings in patients with chronic pain (Grachev et al. 2003; Apkarian et al. 2004; Schmidt-Wilcke et al. 2006). In line with our study, DLPFC changes were more often localized in the right hemisphere (Grachev et al. 2003; Schmidt-Wilcke et al. 2006). Our finding of lateralization in processing chronic pain is also in line with studies on cerebral lateralization in spatial as well as in attention processes and many aspects of emotion (Mesulam 1981; Silberman and Weingartner 1986). Taken together, our findings indicate that, in line with current theories on the role of DLPFC in the genesis of chronic pain (Wand and O’Connell 2008; Apkarian et al. 2009), there is a reduction of gray matter in DLPFC in chronic PLP patients.

We found that VAS pain ratings were significantly negatively related to gray matter volume in an cortical area involved in pain processing, the right IC. The more pain was experienced the more gray matter in the right IC was decreased. A similar negative correlation between pain intensity and gray matter density in the DLPFC was previously reported for patients with chronic back pain (Apkarian et al. 2004). So this correlation seems to support the concept of "maladaptive plasticity" in areas belonging to the pain-processing network (Katz and Melzack 1990; Apkarian et al. 2009).

Speculating about the observed reduction of gray matter, they may represent a progressive degenerative process, which may be caused by permanent (nociceptive) input. Patients with HPLP experience permanent PLP. While training effects associated with rhythmic or temporary input has been shown to increase the representation (Sterr et al. 1998; Liepert et al. 2000; Dragnanski et al. 2004), persistent possibly inadequate input seems to result in a reduction of representations and volumes (Flor et al. 1995, 2006). In the long term, this continuous over activation might result in anatomical changes of the affected region, possibly due to cell death, for example, by glutamatergic over activation (Diemer et al. 1993). Cell atrophy, synaptic loss, decrease in cell size, or decrease blood volume are also discussed as possible reasons for cortical volume reduction (Dragnanski and May 2008; May 2008, 2009). These alterations may go in line with transsynaptical microglia activation, subcortical or cortical in patients after amputation with chronic PLP (Banati 2002).

There is only one region where patients with HPLP showed an increase of cortical volume compared to both patients with SPLP and healthy controls. This result indicates the importance of caudal ACC in chronic PLP. It is in line with the hypothesis pointing to a role of the caudal ACC in anticipatory attention toward emotionally salient (e.g., painful) stimuli (Bentley et al. 2003). This region is also associated with response selection, motor inhibition, and with the rating of the unpleasantness of pain (Vogt et al. 2003). Moreover, the caudal ACC is the most commonly activated structure after nociceptive stimulation in imaging studies (Peyron et al. 2000). These results are in line with a recent meta-analysis by Friebel et al. (2011), showing a consistent activation of this area in chronic neuropathic pain. As pain in our study is positively associated with the volume of this area, the volume of the caudal ACC may be a vulnerability factor for the development of chronic pain before amputation. An alternative explanation may be that the ACC increases in volume due to the permanent unpleasant pain experience or permanent tendency to escape from pain or both.

Limitations

It is still a matter of debate whether the alterations in cortical volume are the cause or consequence of chronic pain (Kuner 2010). The relationship may be interpreted in terms of a type of pain-related atrophy (Apkarian et al. 2004; Rocca et al. 2006; Kim et al. 2008). Cell atrophy, synaptic loss, decrease in cell size, or decreased blood volume are possible reasons for this cortical volume reduction (Dragnanski and May 2008; May 2008, 2009). Nevertheless, it has been shown that the decrease in gray matter in association with chronic pain is partially reversible. Proper treatment may countermand the alterations mentioned.
Our data have several limitations. We only included patients with unilateral limb amputation who were otherwise in good physical condition. Bilateral amputations might result in different morphological entities, especially with respect to contralateral M1 and the changes in structures of the visual stream. Furthermore, the variance of time since amputation is relatively large. Since most of our amputees were only examined years after amputation, we have no hint from the present data for the possible time course of gray matter changes reported here. Additionally, VAS pain ratings on the day of imaging cannot capture the level of pain for longer times or even since the onset of PLP. So more longitudinal studies are needed that examine the PLP experience and cortical volume beginning at the time of amputation.

**Supplementary Material**

Supplementary material can be found at: http://www.cercor.oxfordjournals.org

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