Abnormal Access of Axial Vibrotactile Input to Deafferented Somatosensory Cortex in Human Upper Limb Amputees

JOHN J. M. KEW, PETER W. HALLIGAN, JOHN C. MARSHALL, RICHARD E. PASSINGHAM, JOHN C. ROTHWELL, MICHAEL C. RIDDING, C. DAVID MARSDEN, and DAVID J. BROOKS.

1 Medical Research Council Cyclotron Unit, Hammersmith Hospital, London W12 0HS; 2 Medical Research Council Human Movement and Balance Unit, Institute of Neurology, London WC1N 3BG; 3 Neuropsychology Unit, Radcliffe Infirmary, and Rivermead Rehabilitation Centre, Oxford OX1 4XD; and 4 Department of Experimental Psychology, University of Oxford, Oxford OX1 3UD, United Kingdom

Kew, John J. M., Peter W. Halligan, John C. Marshall, Richard E. Passingham, John C. Rothwell, Michael C. Ridding, C. David Marsden, and David J. Brooks. Abnormal access of axial vibrotactile input to deafferented somatosensory cortex in human upper limb amputees. J. Neurophysiol. 77: 2753–2764, 1997. We studied two human subjects with total deafferentation of one upper limb secondary to traumatic multiple cervical root avulsions. Both subjects developed a phantom limb and underwent elective amputation of the paralyzed, deafferented limb. Psychophysical study revealed in each subject an area of skin in the pectoral region ipsilateral to the amputation where vibrotactile stimulation (VS) elicited referred sensations (RS) in the phantom limb. Positron emission tomography was then used to measure regional cerebral blood flow changes during VS of the pectoral region ipsilateral to the amputation with RS and during VS of a homologous part of the pectoral region adjacent to the intact arm without RS. A voxel-based correlation analysis was subsequently used to study functional connectivity. VS of the pectoral region adjacent to the intact arm was associated with activation of the dorsal part of the contralateral primary somatosensory cortex (S1) in a position consistent with the S1 trunk area. In contrast, VS of the pectoral region ipsilateral to the amputation with RS was associated with activation of the contralateral S1 that extended from the level of the trunk representation ventrally over distances of 20 and 12 mm, respectively, in the two subjects. The area of S1 activated during VS of the digits in a normal control subject was coextensive with the ventral S1 region abnormally activated during VS of the ectopic phantom representation in the two amputees, suggesting that the deafferented digit or hand/arm area had been activated by sensory input from the pectoral region. Correlation analysis showed an abnormal pattern of intrinsic connectivity within the deafferented S1 hand/arm area of both amputees. In one subject, the deafferented S1 was functionally connected with 3 times as many S1 voxels as the normally afferented S1. This abnormal functional connectivity extended in both the rostrocaudal and ventrodorsal dimensions. The results demonstrate that sensory input delivered to the axial body surface may gain access to the S1 hand/arm area in some humans who have suffered extensive deafferentation of this area. The findings are consistent with the hypothesis that deafferentation of an area of S1 may result in activation of previously dormant inputs from body surfaces represented in immediately adjacent parts of S1. The results also provide evidence that changes in functional connectivity between these adjacent areas of the cortex may play a role in the somatotopic reorganization.

INTRODUCTION

The dramatic results of a study on the functional effects of long-term, extensive deafferentation of the primary somatosensory cortex (S1) in adult primates (Pons et al. 1991) have reopened the debate about the extent to which the cortex of fully developed mammals can reorganize. In the light of these findings a reappraisal of the upper spatial limit of cortical reorganization has been necessary. In their studies on monkeys with longstanding total deafferentation of the S1 hand/arm area, Pons et al. (1991) showed that neurons throughout the deafferented S1, rather than remaining unresponsive, became responsive to cutaneous stimuli delivered to the face. These results unexpectedly demonstrated a spatial extent of cortical reorganization that was 1 order of magnitude greater than any encountered before. At the time, researchers could only speculate on the perceptual correlates of this large-scale reorganization.

Curious perceptual phenomena had been recognized in human amputees with phantom limbs for over a century (Cronholm 1951; Katz 1992; Mitchell 1872). It had been established that in some amputees, stimulation of areas of skin adjacent, and sometimes distant (Case 13 of Cronholm 1951), to the amputation reproducibly elicited sensations in the phantom limb. However, it was not until referred phantom sensations were reexplored and reevaluated in a systematic way that their significance started to become apparent. Ramachandran et al. (1992a,b) reported two human upper limb amputees in whom stimuli applied to the lower face ipsilateral to the amputation were referred to the phantom limb in an apparently topographic manner. These findings were later replicated and extended independently (Halligan et al. 1993, 1994), and similar perceptual mislocalizations were subsequently reported in lower limb amputees (Aglioti et al. 1994a) and patients who had undergone mastectomy (Aglioti et al. 1994b). Ramachandran et al. (1992a,b) postulated that the referred phantom sensations reported by their amputees could represent the perceptual correlate of the physiological “facial remapping” demonstrated by Pons et al. (1991) in primates, and hypothesized that similar remapping should be demonstrable in these subjects (Yang et al. 1994). Studies with magnetic source imaging, a technique combining magnetic resonance imaging of brain anatomy with magnetoencephalographic localization of functional activity, have recently provided evidence in support of this hypothesis (Elbert et al. 1994; Knecht et al. 1996; Yang et al. 1994). However, although the changes reported in these studies were dramatic, with shifts of S1 facial sites into the...
deafferented hand/arm area on the order of 15–30 mm, their overall spatial extent could not be determined because of limitations imposed by the imaging technique, and the results did not allow any firm conclusions about the route by which the reorganized facial input gained access to the deafferented cortex. The objective of the present study was to provide greater insight into the cortical substrate of referred phantom sensations by using positron emission tomography (PET) to study reorganized patterns of activation and connectivity in the deafferented S1 of human amputees with ectopic representations of the phantom limb.

METHODS

Subjects

SUBJECT 1. Subject 1 was a 31-yr-old male who sustained multiple left-sided cervical nerve root avulsions as a result of a motorcycle accident in 1980. Myelography confirmed avulsion of all cervical roots between C2 and T1. The subject was left with a paralyzed, anesthetic left arm that was subsequently amputated above the elbow in 1982, and a left Horner’s syndrome. Subject 1 became aware of a phantom left arm and hand, which was supernumerary to the paralyzed arm, within a few weeks of the original injury. The experience of the phantom limb had remained complete. In the hand, the thumb and the index and middle fingers were particularly prominent. The subject had noticed intermittent “telescoping” of the hand toward the stump, and had become aware of two areas on the body surface tactile stimulation of which elicited referred sensations (RS) in the phantom limb. The first was a large area centered around the left upper chest and pectoral region, from which RS in the thumb and forearm could be elicited. The second was smaller and centered around the left upper scapula; stimulation here induced referred pain in the medial aspect of the phantom forearm. The subject had had an unsuccessful trial of a myoelectric arm and was using a body-powered prosthesis at the time of the study.

SUBJECT 2. Subject 2 was a 49-yr-old male who sustained multiple, myelographically proven right-sided cervical nerve root avulsions (C2–T1) as a result of a motorcycle accident in 1963. The subject was left with a paralyzed, anesthetic right arm that was subsequently amputated above the elbow four months after the injury. Subject 2 had become aware of a phantom right arm immediately after the injury. In the early postinjury period this was felt to be contained within the paralyzed, anesthetic limb while the subject was awake, but during drowsiness the phantom limb would drift out of the paralyzed limb to become supernumerary. The phantom limb had gradually telescoped to the extent that the phantom hand was felt to be attached to the amputation stump. The subject had become aware that RS in the phantom hand could be elicited by tactile stimulation of the right pectoral region. The subject used a cosmetic prosthesis.

SUBJECT 3. Subject 3, a healthy 31-yr-old male with no history of neurological injury, was employed as a control subject.

Sensory activation paradigm

The somatosensory paradigm consisted of the application of a vibrotactile stimulus (series 200 vibrotactile stimulator and TPO 25 power oscillator, Ling Dynamic Systems, Royston, UK). The stimulus frequency was set at 100 Hz, with a stimulus amplitude of 2 mm. The stimulus was applied throughout the period of data acquisition in each PET scan. This type of vibrotactile stimulation (VS) paradigm has been shown to induce robust and reproducible regional cerebral blood flow (rCBF) responses in the human S1 (Fox et al. 1987). In accordance with the findings of Meyer et al. (1991), subjects were instructed to direct attention toward the stimulus throughout each scan to maximize the rCBF changes induced by the sensory paradigm. Subjects were also instructed to close the eyes during all stimulation conditions.

Psychophysical study

Before the PET study, both amputees were subjected to a detailed somatosensory examination to determine the spatial extent of the cutaneous area from which RS in the phantom limb could be obtained. In addition to the vibrotactile stimulus a second stimulus, firm pressure stimulation (PS) of the skin with a probe, was employed to determine whether the extent of the ectopic phantom representation was specific to the mode of stimulation.

PET study

In the two amputees studied the stimulus was applied to two corresponding regions of the trunk: one in the upper pectoral region ipsilateral to the amputated upper limb from which the most consistent and intense referred phantom sensations had been obtained in the psychophysical study (the optimal site); the other in a homologous part of the upper pectoral region ipsilateral to the normal intact arm from which no referred phantom sensations could be obtained. In the normal subject, the stimulus was delivered to a part of the right upper pectoral region corresponding to that stimulated in the amputees, and to the fingers of the right hand, to map the normal somatotopic representation of these body parts in S1. A ball applicator was used for pectoral stimulation in all three subjects. A bar applicator was used for the simultaneous stimulation of all four fingers of the right hand in the normal subject.

Measurement of rCBF

Measurements of rCBF at rest and during VS were made with the use of PET. Scans were performed with the use of a CTI/Siemens 953B PET scanner (CTI, Knoxville, TN) operating in three-dimensional mode with septa retracted, allowing simultaneous acquisition of a 31-transaxial-plane three-dimensional image volume (Spinks et al. 1992). Images were reconstructed with a Hanning filter with a cutoff frequency of 0.5 cycles per pixel, giving a spatial resolution in the reconstructed image of 8.5 × 8.5 × 4.3 mm full width at half maximum (FWHM). Twelve dynamic scans were performed in each subject during repeated 2-min infusions of H15O administered via an antecubital vein canula at a flow rate of 10 ml/min. Data were acquired for 165 s after the start of each infusion. Measured attenuation correction of the emission scans was made with the use of transmission scans performed with three external 68Ge rod sources.

In the amputees, four scans were performed in the resting state (condition A), four during stimulation of the pectoral region ipsilateral to the intact arm (condition B), and four during stimulation of the pectoral region ipsilateral to the amputated arm with referred phantom sensations (condition C).

In the normal subject, four scans were performed in the resting state (condition A), four during stimulation of the right pectoral region (condition B), and four during stimulation of the right fingers (condition C). Scans were conducted with eyes closed and ears plugged in a counterbalanced sequence (ABCBCAABBCBA) to avoid order and time effects.

Image analysis

Image analysis was performed on a SUN Sparstation 2 (Sun Microsystems Europe, Surrey, UK) with the use of interactive image display software (ANALYZE, Biodynamics Research Unit,
Statistical analysis

ACTIVATION STUDY. Global cerebral blood flow across the 12 scans was normalized to a value of 50 ml per 100 g per min with the use of a voxel-based analysis of covariance (Friston et al. 1990). Adjusted mean rCBF maps were derived for each of the three experimental conditions. Planned comparisons between rCBF at rest and rCBF during stimulation of the pectoral region ipsilateral to the intact or amputated arm were then performed. Significant changes in the distribution of rCBF between the rest condition and each stimulation condition were identified by statistical parametric mapping (Friston et al. 1991b). The significance of the mean change in rCBF at each voxel location was determined with the use of the adjusted error variance generated by the analysis of covariance to compute the t statistic. The t distribution was transformed into a Gaussian distribution before projection in three orthogonal planes as a statistical parametric map (SPM). By the use of this approach it is possible to specify the location of a region of functionally activated brain to within a few millimeters (Fox et al. 1986; Mintun et al. 1986).

In subjects 1 and 3 the significance threshold was set at the conventional level of $P < 0.05$ per plane after application of a Bonferroni-like correction for multiple, nonindependent comparisons; this threshold corresponded to a Z score of $\pm 3.75$. An uncorrected significance threshold of $P < 0.01$ (corresponding to a Z score of $\pm 2.33$) was, however, necessary to demonstrate quantitatively smaller rCBF responses in the contralateral S1 during VS of the right pectoral region in subjects 1 and 3. Although not significant by conventional (Bonferroni-corrected) criteria traditionally used in PET activation studies, these responses were felt to be of clear physiological relevance. To facilitate interpretation of the results, these responses will be referred to as “low-amplitude rCBF increases.” Because these data were obtained with the use of an exploratory ( albeit hypothesis-led ) approach, it is appropriate to report them descriptively in the text, but not in tabular or SPM format.

Because of quantitatively smaller rCBF increases in subject 2 during both stimulation conditions, use of a conventional (Bonferroni-corrected) significance threshold resulted in the detection of very few rCBF responses. Detection of rCBF responses was improved by the use of an uncorrected significance threshold of $P < 0.01$ (corresponding to a Z score of $\pm 2.33$ as above), and all results in this subject were therefore thresholded at this level.

To determine the magnitude of the rCBF changes between the resting and active states, percentage changes in rCBF at voxels of maximal significance were determined from the statistical maps.

CONNECTIVITY STUDY. To determine the functional connectivity within cortical regions of interest, rCBF at a reference voxel location was correlated across all 12 scans within each subject. The confounding effects of global activity were removed with the use of linear regression to produce adjusted activity values for each of the 12 scans. An SPM of the correlation coefficient between activity at the reference voxel and all remaining voxels was constructed after the correlation coefficient was transformed to the normal distribution with the use of Fisher’s Z transformation. The resulting SPM of the Z score was thresholded at $P < 0.001$ (uncorrected); across a series of 12 scans, with 10 degrees of freedom, this corresponded to a voxel-to-voxel correlation of $R = 0.823$. The theory behind this analysis is that if two or more voxels are functionally connected, rCBF at these two voxels should be mutually correlated within a subject across different brain conditions.

RESULTS

Physiological study

Subject 1 was found to have a large ectopic phantom representation over the left anterior thorax in the pectoral region ipsilateral to the amputation (Fig. 1A). This phantom extended mediolaterally between the sternum and the nipple, and superiorly from 5 cm below the nipple to the level of the clavicle. The ectopic representation was more extensive inferiorly, superiorly, and medially during VS than during PS (Fig. 1A). However, there was considerable overlap of VS and PS sites. Furthermore, the wider spatial distribution of VS sites may simply have reflected stimulus spread. A second ectopic representation of the phantom limb was found over the left posterior thorax in the upper scapular region close to the midline (Fig. 1B). This site was more clearly responsive to PS than VS. The subject’s face was carefully explored with both stimuli but no ectopic phantom representation was found.

The optimal site for stimulation was found to be the first left intercostal space 4 cm from the midline. RS elicited from this site consisted of paresthesias spreading down the medial aspect of the phantom forearm, into the hand, and diffusely into the fingers. Gentle movement of the ball applicator elicited more intense RS than a stationary stimulus. This moving stimulus was therefore used for stimulation of the optimal site in the PET study. For control purposes, a similar moving stimulus was applied to the opposite (right) pectoral region in the PET study in this subject.

Subject 2 was found to have a smaller ectopic representation of the phantom hand in the right pectoral region ipsilateral to the amputation, superior and lateral to the nipple (Fig. 2). A second, even smaller ectopic representation, which the subject had not previously noticed, was found just proximal to the stump in an area of skin in which there was some remaining cutaneous sensation. The ectopic phantom representation in the pectoral region was responsive to both VS and PS. However, phantom sensations elicited by stimulation of this region were much more intense with VS than with PS, to the extent that VS of the optimal site induced vibratory phantom sensations that were of sufficient intensity to cause extinction of the vibratory sensation at the point of
application. RS were elicited from the ectopic representation proximal to the stump by PS but not by VS.

The optimal site for stimulation lay near the axilla in the lateral pectoral region adjacent to the amputated arm. This site was situated 5 cm lateral and 8 cm superior to the nipple. Stationary VS applied to this area was found to elicit an intense, diffuse vibratory sensation in the subject’s phantom hand. A stationary rather than a moving stimulus was therefore used for stimulation of the optimal site in the PET study in this subject.

**PET activation study**

**SUBJECT 1.** VS of the subject’s right pectoral region (ipsilateral to the intact arm) was associated with significant ($P < 0.05$, Bonferroni-corrected) activation of the second somatic sensory cortex (SII) bilaterally and of the contralateral anterior thalamus. Additional low-amplitude rCBF increases (significance threshold $P < 0.01$, non-Bonferroni-corrected) were also present in the contralateral S1 60 mm (coordinates: $X = -8$, $Y = -42$, $Z = 60$; rCBF increase 2.8%; $Z$ score 1.8) and 68 mm (coordinates: $X = -14$, $Y = 42$, $Z = 68$; rCBF increase 2.8%; $Z$ score 2.7) above the intercommissural (AC-PC) plane.

VS of the subject’s left pectoral region (ipsilateral to amputated arm, and source of referred phantom sensations) was associated with significant ($P < 0.05$, Bonferroni-corrected) activation of SII and thalamus bilaterally. In addition, significant activation was seen in the contralateral S1, primary motor cortex (M1), superior posterior parietal cortex (PPC), and anterior cingulate cortex (Brodmann area 32). The large focus of activation in S1 and M1 extended in the ventrodorsal ($Z$) dimension from +36 to +68 mm relative to the AC-PC plane.

The cortical areas activated at a conventional threshold of significance ($P < 0.05$, Bonferroni-corrected) in this subject during the two stimulation conditions are shown in quantitative form in Table 1 and in the form of an SPM in Fig. 3. **SUBJECT 2.** The results in subject 2 replicated those in subject 1, although, as already stated, the overall rCBF changes were less significant. VS of the subject’s left pectoral region (ipsilateral to intact arm) was associated with low-amplitude rCBF increases (significance threshold $P < 0.01$, non-Bonferroni-corrected) in the contralateral posterior insula and SII and the ipsilateral SII. However, there were no significant rCBF increases in the contralateral S1.

VS of the subject’s right pectoral region (ipsilateral to amputated arm, and source of referred phantom sensations) was associated with low-amplitude rCBF increases (significance threshold $P < 0.01$, non-Bonferroni-corrected) in SII bilaterally, the contralateral S1, and the ipsilateral posterior insula. The S1 rCBF increases extended in the ventrodorsal ($Z$) dimension from +44 to +64 mm relative to the AC-PC plane.

The cortical areas activated at a significance threshold of $P < 0.01$ (non-Bonferroni-corrected) in this subject during the two stimulation conditions are shown in quantitative form in Table 2 and in the form of an SPM in Fig. 4. **SUBJECT 3.** VS of the normal subject’s right fingers was associated with significant ($P < 0.05$, Bonferroni-corrected) activation of the contralateral S1 and M1, which extended in the ventrodorsal ($Z$) dimension from +36 to +64 mm relative to the AC-PC plane, and of the contralateral superior
the phantom fingers and forearm were elicited by PS in 2 small regions of the upper pectoral region ipsilateral to the amputation. B: the stump. RS in the phantom hand were elicited by both PS and VS of the stump. Talairach Coordinates Percent corrected ) were also present in the contralateral S1 at 56 R PPC 36 P

of the contralateral SII and adjacent mid-insular cortex, and R SII 42 P

Low-amplitude r CBF increases (significance threshold P < 0.05, non-Bonferroni-corrected) were also present in the contralateral posterior insular cortex (coordinates: X = −36, Y = −12, Z = 12; r CBF increase 2.5%; Z score 3.6) and SII (coordinates: X = −46, Y = −12, Z = 20; r CBF increase 2.1%; Z score 3.5).

VS of the subject’s right pectoral region was associated with significant (P < 0.05, Bonferroni-corrected) activation of the contralateral SII and adjacent mid-insular cortex, and the more ventral aspects of the ipsilateral anterior cingulate cortex (Brodmann area 24; Table 3). Low-amplitude r CBF increases (significance threshold P < 0.01, non-Bonferroni-corrected) were also present in the contralateral S1 at 56 mm (coordinates: X = −36, Y = −30, Z = 56; r CBF increase 2.8%; Z score 2.7) and 60 mm (coordinates: X = −16, Y = −26, Z = 60; r CBF increase 1.1%; Z score 2.9) relative to the AC-PC plane.

Thus two distinct patterns of cerebral activation were present in the amputees depending on which pectoral region was stimulated.

1) VS of the pectoral region ipsilateral to the intact arm: similar patterns of activation were observed in the two amputees during VS of the pectoral region ipsilateral to the intact arm (without RS) and in the normal control subject during VS of the right pectoral region. Activation of the contralateral SII was common to all three subjects, the SII activation foci lying at a similar ventrodorsal (Z) level relative to the AC-PC plane (compare Tables 1–3). Additional ipsilateral SII activation was present in both amputees. Differences in the patterns of activation included contralateral anterior thalamic activation in one amputee (subject 1) and ipsilateral anterior cingulate activation in the normal control subject. Low-amplitude r CBF increases were present in the dorsal S1 of one amputee (subject 1) and the normal control subject. These activation foci also lay at a similar ventrodorsal (Z) level, with one focus common to both subjects lying at +60 mm relative to the AC-PC plane. It is important to note that no S1 activation was present ventral to a level of +56 mm relative to the AC-PC plane in either of these subjects even at an uncorrected significance level of P < 0.01.

2) VS of the pectoral region ipsilateral to the amputated arm: the two amputees showed similar patterns of cerebral activation during VS of the pectoral region ipsilateral to the amputated arm (with RS). Bilateral activation of SII was again common to both subjects. One point of difference, however, was the presence of bilateral thalamic activation in subject 1. Activation of the contralateral S1 was also present in both subjects, but under this stimulation condition the S1 activation foci extended ventrally to levels of +36 and +44 mm (relative to the AC-PC) in subjects 1 and 2, respectively. Given that the most ventral of the S1 activation foci mapped in subject 1 and the normal control subject during VS of the pectoral region ipsilateral to the intact arm (without RS) lay at a level of +56 mm, it can be seen that

| Table 1. Cortical areas significantly activated during VS of the right and left pectoral regions in subject 1 |
|-----------------|----------------|----------------|----------------|
| Area            | Talairach Coordinates | Percent Change in r CBF | Z Score |
|                 | X               | Y     | Z     |                |                  |
| VS right pectoral region |                  |       |       |                |                  |
| L. thalamus     | −6              | −8    | 8     | 2.2            | 4.8              |
| R SII           | 42              | −26   | 12    | 3.5            | 4.8              |
| L SII           | −36             | −32   | 20    | 2.7            | 4.2              |
| VS left pectoral region with RS |                  |       |       |                |                  |
| R thalamus      | 4               | −22   | 4     | 3.3            | 4.5              |
| L. thalamus     | −4              | −14   | 8     | 3.8            | 5.3              |
| R SII           | 42              | −24   | 8     | 4.0            | 4.3              |
| L SII           | −48             | −24   | 20    | 3.5            | 4.4              |
| R anterior cingulate* | 10              | 32    | 28    | 2.9            | 6.3              |
| R SI            | 34              | −32   | 56    | 8.3            | 4.4              |
| R MI            | 32              | −22   | 60    | 9.8            | 4.4              |
| R PPC           | 36              | −38   | 60    | 6.4            | 5.1              |

P < 0.05, Bonferroni corrected. VS, vibrotactile stimulation; r CBF, regional cerebral blood flow; R, right; L, left; SII, 2nd somatic secondary cortex; RS, referred sensations in the phantom limb; SI, primary somatosensory cortex; MI, primary motor cortex; PPC, superior posterior parietal cortex (Brodmann area 5). * Brodmann area 32.
FIG. 3. Significant increases in regional cerebral blood flow (rCBF) in subject 1 during VS of the right and left pectoral regions. Sagittal, coronal, and transverse projections of the statistical parametric maps (SPMs) are shown. Voxels at which a significant increase in rCBF occurred are displayed in black within the standardized stereotactic grid system of Talairach and Tournoux (1988); nonsignificant voxels are displayed in white. VAC and VPC: vertical orientation lines transecting the anterior and posterior commissures. The significance threshold was set at $P < 0.05$ after application of a Bonferroni-like correction for the number of planes analyzed. The images were smoothed with a Gaussian filter to 8 mm full width at half maximum (FWHM) for display purposes. VS of the subject’s right pectoral region (ipsilateral to the intact arm) was associated with significant activation of the 2nd somatic sensory cortex (SII) bilaterally and of the contralateral anterior thalamus. VS of the subject’s left pectoral region (ipsilateral to the amputated arm, with RS in the phantom limb) was associated with significant activation of SII and thalamus bilaterally, and of the contralateral primary somatosensory cortex (S1) and primary motor cortex (M1; extending in the ventrodorsal (Z) dimension from +36 to +68 mm relative to the intercommissural (AC-PC) plane), superior posterior parietal cortex (PPC), and anterior cingulate cortex (Brodmann area 32). Thal, thalamus; Ant Cing, anterior cingulate cortex.

the S1 foci during VS of the pectoral region ipsilateral to the amputated arm (with RS) extended ventrally to distances of 20 and 12 mm beyond the normal S1 pectoral representation in subjects 1 and 2, respectively. The results in the normal control subject showed that this more ventrally situated area of S1 (extending from +36 to +56 mm relative to the AC-PC plane) was activated exclusively by VS of the contralateral fingers. This suggests that VS of the pectoral region ipsilateral to the amputated arm was associated with activation of not only the contralateral S1 pectoral representation (dorsal to +56 mm relative to the AC-PC plane) but also the contralateral S1 finger (or hand/arm) representation (ventral to +56 mm relative to the AC-PC plane). Furthermore, it appears that the entire S1 finger representation was activated during this stimulation condition in subject 1. Considering that both these subjects reported RS in the phantom limb during this stimulation condition, it is probable that the S1 hand/arm activation is the cortical correlate of these RS.

### TABLE 2. Cortical areas significantly activated during VS of the right and left pectoral regions in subject 2

<table>
<thead>
<tr>
<th>Area</th>
<th>Talairach Coordinates</th>
<th>Percent Change in rCBF</th>
<th>Z Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>VS left pectoral region</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R SII/posterior insula</td>
<td>34 -18 16</td>
<td>3.1</td>
<td>3.1</td>
</tr>
<tr>
<td>L SII</td>
<td>-36 -2 -4</td>
<td>3.1</td>
<td>3.0</td>
</tr>
<tr>
<td>VS right pectoral region with RS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L SII</td>
<td>-42 -24 20</td>
<td>3.3</td>
<td>3.0</td>
</tr>
<tr>
<td>L SI</td>
<td>-30 -34 64</td>
<td>3.7</td>
<td>3.0</td>
</tr>
<tr>
<td>R SII/posterior insula</td>
<td>28 -18 16</td>
<td>3.6</td>
<td>3.9</td>
</tr>
</tbody>
</table>

$P < 0.01$, non-Bonferroni corrected. For abbreviations, see Table 1.

### Connectivity study

**SUBJECT 1.** The Talairach coordinates used to study the connectivity of the normally afferented and deafferented S1 hand/arm representations were derived from the PET activation study. The voxel of maximal significance in the contralateral S1 during VS of the subject’s left pectoral region (with referred phantom sensations) was assumed to lie within the deafferented hand/arm representation. This assumption was supported by the results obtained in the normal subject showing that VS of the fingers was associated with activation of this same area of S1 (compare Tables 1 and
ABNORMAL ACCESS OF VIBROTACTILE INPUT TO DEAFFERENTED S1 IN AMPUTEES

**FIG. 4.** Significant increases in rCBF in subject 2 during VS of the left and right pectoral regions. Conventions of SPMs as in Fig. 3. Because of a lower overall magnitude of rCBF response in this subject, the significance threshold was set at $P < 0.01$ without a correction for the number of planes analyzed. The images were smoothed with a Gaussian filter to 8 mm FWHM for display purposes. VS of the subject’s left pectoral region (ipsilateral to the intact arm) was associated with significant activation of the contralateral posterior insular cortex and SII and of the ipsilateral SII. VS of the subject’s right pectoral region (ipsilateral to the amputated arm, with RS in the phantom limb) was associated with significant activation of SII bilaterally, the contralateral S1 [extending in the ventrodorsal ($Z$) dimension from $+44$ to $+64$ mm relative to the AC-PC plane], and the ipsilateral posterior insula. Post insula, posterior insular cortex.

The intrinsic connectivity of the subject’s normally afferented left S1 hand/arm representation (coordinates: $X = -34, Y = -32, Z = 56$) extended from 44 to 60 mm above the AC-PC plane. The intrinsic connectivity of the subject’s deafferented right S1 hand/arm representation (coordinates: $X = 34, Y = -32, Z = 56$) extended from 40 to 68 mm above the AC-PC plane. The reference voxel in the deafferented representation was functionally connected with $\sim 3$ times as many S1 voxels as the reference voxel in the normally afferented representation (725 compared with 250 voxels) (Fig. 6). This abnormal connectivity extended in both the rostrocaudal and ventrodorsal dimensions. The subject’s deafferented right S1 hand/arm area also showed abnormal connectivity with the ipsilateral ventral S1 between 20 and 32 mm (face area).

Thalamic connectivity was not explored in this subject, despite significant thalamic activation, because it was not possible to specify the location of the main sensory nucleus of the thalamus.

**TABLE 3.** Cortical areas significantly activated during VS of the right fingers and the right pectoral region in the normal subject (subject 3).

<table>
<thead>
<tr>
<th>Area</th>
<th>Talairach Coordinates</th>
<th>Percent Change in rCBF</th>
<th>Z Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>VS right fingers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L SI</td>
<td>$-38$ $-30$ $56$</td>
<td>7.9</td>
<td>6.5</td>
</tr>
<tr>
<td>L PPC</td>
<td>$-2$ $-46$ $64$</td>
<td>3.6</td>
<td>3.8</td>
</tr>
<tr>
<td>VS right pectoral region</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L SII</td>
<td>$-40$ $-8$ $16$</td>
<td>3.2</td>
<td>3.9</td>
</tr>
<tr>
<td>L mid-insula</td>
<td>$-38$ $-8$ $12$</td>
<td>2.5</td>
<td>4.9</td>
</tr>
<tr>
<td>R anterior cingulate*</td>
<td>$8$ $0$ $28$</td>
<td>2.5</td>
<td>5.1</td>
</tr>
</tbody>
</table>

$P < 0.05$, Bonferroni corrected. For abbreviations, see Table 1. * Brodmann area 24.
also show that the most significant rCBF response within the S1 finger representation lies at a dorsal (Z) level of 48 mm above the AC-PC plane. These findings replicate those of Fox et al. (1987) and Meyer et al. (1991), who each used a vibrotactile stimulus similar to that employed in the present study to show that the finger area in S1 was located at a mean vertical (dorsal) distance above the AC-PC plane of 47 mm.

S1 blood flow responses during VS of the pectoral region were of lower magnitude and significance in comparison with those mapped during VS of the fingers. Two observations from the present study indicate that the S1 pectoral or trunk representation is situated dorsal to the finger representation. First, in the normal subject low-amplitude rCBF increases to VS of the pectoral region were present in the contralateral S1 at a level of 56 ± 60 mm above the AC-PC plane. Second, in one amputee (subject 1), similar blood flow responses to VS of the contralateral pectoral region were present in the normally afferented S1 at levels of 60 and 68 mm above the AC-PC plane. The evidence therefore suggests that the pectoral, or trunk, representation in S1 is located at a dorsal level of ~60 mm above the AC-PC plane, whereas the center of the finger representation is located more ventrally at a level of 48 mm. The finding of a more dorsal location of the trunk representation in S1 is consistent with the findings of Penfield and Rasmussen (1950) and of Woolsey et al. (1979) in humans.

**Activation of SII by VS**

The role of SII in vibratory processing is well established. McIntyre et al. (1967) were the first to show that evoked potentials from Pacinian corpuscles could be recorded in SII of the cat. Single-cell recordings have confirmed that neurons with Pacinian-like responses to high-frequency vibration (>128 Hz) are relatively common in SII of primates (Robinson and Burton 1980) and cats (Bennett et al. 1980). In fact, in the cat, there appears to be a greater proportion of neurons responsive to Pacinian input in SII than in S1 (Bennett et al. 1980; Fisher et al. 1983). It has also been shown that SII neurons display phase-locking of impulse activity to the vibratory stimulus waveform when the frequency of cutaneous vibration is in the range of 100–300 Hz (Ferrington and Rowe 1980), although the majority of SII neurons activated by tactile stimuli appear to have a bandwidth of 10–100 Hz (Bennett et al. 1980; Robinson and Burton 1980).

Coactivation of the contralateral S1 and SII was present during VS of the fingers and pectoral region in the normal subject. The vibratory stimulus frequency of 100 Hz used in the studies lies toward the lower end of the bandwidth for Pacinian neurons in both S1 (Mountcastle et al. 1969) and SII (Ferrington and Rowe 1980). It is, therefore, not surprising that such a stimulus should coactivate these two somatosensory areas. Indeed, similar coactivation of S1 and SII was reported by Fox et al. (1987) in normal human subjects during vibration of the fingers at a frequency of 130 Hz.

**Reorganization of S1 following deafferentation**

The results obtained in subject 1 leave little doubt that VS of the subject’s ectopic phantom limb representation in
ABNORMAL ACCESS OF VIBROTACTILE INPUT TO DEAFFERENTED S1 IN AMPUTEES

FIG. 6. Pattern of intrinsic and extrinsic connectivity of the normally afferented left S1 hand/arm area (Talairach coordinates: X = −34, Y = −32, Z = 56) and the deafferented right S1 hand/arm area (Talairach coordinates: X = 34, Y = −32, Z = 56) of subject 1. Voxels showing functional connectivity (voxel-to-voxel r CBF correlation across scans of R ≥ 0.823; significance P ≤ 0.001 uncorrected at 10 degrees of freedom) with the reference voxel are displayed in black within the standardized stereotactic grid system of Talairach and Tournoux (1988); voxels showing no functional connectivity are displayed in white. Conventions of SPMs otherwise as in Fig. 3. Reference voxel in the deafferented right S1 hand/arm representation was intrinsically connected with ~3 times as many S1 voxels as the reference voxel in the normally afferented representation (725 compared with 250 voxels). The subject’s deafferented S1 hand/arm area also showed abnormal connectivity with the ipsilateral ventral S1 (face area), the ipsilateral insular cortex, the anterior cingulate cortex (areas 24 and 32) bilaterally, and the contralateral ventral premotor cortex (area 6). The subject’s normally afferented left S1 hand/arm area showed extrinsic connectivity that was limited to a small number of voxels in lateral visual association (VA) cortex (Brodmann area 19) bilaterally. Premotor, ventral premotor cortex.

The results observed in subject 2, although less striking than those in subject 1, are again consistent with abnormal access of axial vibratory input to the deafferented S1. Several factors may have contributed to the variability between the two subjects with amputations. The overall rCBF changes in subject 2, which were less pronounced than those in subject 1, may have occurred as a result of individual differences in the extent of radioactive tracer uptake. In addition, it is possible that the different magnitudes of rCBF response were influenced by differences in stimulus presentation. In subject 1, a moving vibratory stimulus was employed, whereas in subject 2, the vibratory stimulus was stationary. It is likely that a moving stimulus is a more optimal source of activation. Finally, intrinsic within-subject variability needs to be considered. Because of variability between individuals, most PET activation studies employ averaged data gathered from a number of subjects. Group averaging is used to improve the statistical significance of the activation in such studies. However, this approach was not possible in this study because amputees reporting ectopic phantom representations are rare and the two subjects in our study had amputations on different sides. Under such circumstances, it is not possible to perform intersubject averaging. Variability in control subjects is an important issue for future studies. However, the activations observed in subject 3 are in keeping with previous normative studies of the relative neurophysiological localization of the stimulated areas (Fox et al. 1987; Meyer et al. 1991). There is, furthermore, considerable similarity between the overall patterns of activation in the two amputees and that in the control subject during VS of the pectoral region ipsilateral to the intact arm.

By contrast, the large-scale changes observed during VS of the pectoral region ipsilateral to the amputated arm (involving shifts of the S1 trunk representation into the deafferented S1 hand/arm areas spanning 20 and 12 mm in the 2 subjects, respectively) are consistent with the results of previous magnetic source imaging studies in amputees. In those studies, shifts of the S1 facial dipole into the deafferented S1 hand/arm representation of between 15 and 30 mm have been recorded (Elbert et al. 1994; Yang et al. 1994).
FIG. 7. Pattern of intrinsic and extrinsic connectivity of the normally afferented right S1 hand/arm area (Talairach coordinates: \( X = 30, Y = -34, Z = 64 \)) and the deafferented left S1 hand/arm area (Talairach coordinates: \( X = -30, Y = -34, Z = 64 \)) of subject 2. Conventions of SPMs as in Fig. 6. Because of abnormal confluent connectivity between the deafferented S1 and other cortical areas, quantification of the intrinsic connectivity of the deafferented hand/arm representation in S1 was not possible in this subject. Nevertheless, the intrinsic connectivity of the deafferented S1 hand/arm representation could be seen to be much more extensive than that of its normally afferented counterpart. The subject’s deafferented left S1 hand/arm representation also showed abnormal connectivity with the ipsilateral ventral S1 (face area), paracentral lobule (medial area 5), inferior parietal lobule (area 40), and dorsal premotor cortex (area 6). The subject’s normally afferented right S1 hand/arm representation showed extrinsic connectivity that was mainly confined to a small number of voxels in the ipsilateral mesial and lateral visual association cortex (Brodmann areas 18 and 19). PCL, paracentral lobule; IPL, inferior parietal lobule.

In conjunction with the psychophysical results, it is difficult to escape the conclusion that this abnormal S1 activation reflects abnormal access of axial (pectoral) vibratory input to the deafferented S1 finger and hand/arm areas.

The findings above raise two additional questions. What is the route via which the reorganized pectoral input is allowed access to the deafferented cortex? What mechanism mediates this reorganization? There are several possible routes by which this reorganized input may gain access to the deafferented cortex, including reorganized thalamocortical and reorganized corticocortical pathways. A number of animal (Garraghty et al. 1991; Gilbert and Wiesel 1992; Jones 1993) and human (Brasil-Neto et al. 1993) studies have concluded that deafferentation-induced reorganization of this kind may take place at a cortical rather than a thalamic or spinal level. For example, although arborizations of individual thalamocortical neurons can be quite extensive, on the order of 2–3 mm (Jones 1993), it has been suggested that their unmasking is probably not sufficient to account for the large-scale cortical reorganization found in some studies (Pons et al. 1991). In addition, if alterations in thalamocortical input were to explain the reorganization of the cortex, changes in \( \gamma \)-aminobutyric acid (GABA) immunoreactivity (see below) should remain confined to those cortical layers (specifically layer IV) that receive this input, and this has been shown not to be the case; in fact, all layers of S1 show reduced staining for GABA after peripheral deafferentation, suggesting that this affects the intrinsic inhibitory circuitry of the cortex (Garraghty et al. 1991). Furthermore, the observation that discrete retinal lesions in primates result in an initial unresponsive zone in the corresponding striate cortex that rapidly regains responsiveness to visual input from the perilesion retina, whereas a large permanent unresponsive zone remains in the corresponding lateral geniculate nucleus, provides further evidence of reorganization at a cortical rather than a thalamic level following deafferentation, at least in the visual system (Gilbert and Wiesel 1992).

The results of neurophysiological studies in human subjects have been in agreement with these findings in animals. Temporary forearm deafferentation by ischemic nerve block has been shown to be associated with increases in the size of motor evoked potentials elicited from muscles immediately proximal to the block by transcranial magnetic stimulation, but not by transcranial electrical stimulation or spinal electrical stimulation (Brasil-Neto et al. 1993). Because transcranial magnetic stimulation is thought to excite M1 pyramidal neurons presynaptically, whereas transcranial electrical stimulation excites these neurons directly, this finding is likewise consistent with a modulation of inhibitory neuronal networks within the cortex following deafferentation. Having said this,
recent studies in primates have clearly demonstrated that reorganization following peripheral nerve injury may be equally extensive at thalamic and cortical levels (Garraghty and Kaas 1991). There is also evidence that reorganization may occur at an even earlier stage in the sensory processing hierarchy, for example at the level of the dorsal column cuneate nucleus (Pettit and Schwark 1993).

The analysis of functional connectivity in subjects 1 and 2 provides evidence that changes in intracortical connectivity may be important for the somatotopic reorganization of S1 in humans following amputation. In both subjects, the intrinsic connectivity of the deafferented S1 hand/arm cortex was expanded in volume (3-fold in subject 1) in comparison with the normally afferented S1, and this abnormal connectivity encompassed the dorsal aspects of S1 that we assume to be the S1 pectoral representation. This finding is consistent with the hypothesis that the pectoral vibrotactile input may have gained access to the deafferented S1 hand/arm area via abnormal intracortical connections that had become unmasked as a result of the deafferentation. The findings do not, however, exclude the possibility of reorganization of thalamocortical afferent projections. Our experience, which is similar to that of others (Fox et al. 1987), is that VS of the fingers in normal subjects is not accompanied by activation of the thalamus. Therefore the observation that thalamic activation was prominent in subject 1, particularly during VS of the ectopic phantom representation, could be cited as evidence that thalamocortical reorganization had taken place. As stated above, there is good experimental evidence that thalamocortical reorganization does occur after this type of injury in primates (Garraghty and Kaas 1991). Indeed, there is no reason to suppose that reorganization should not occur simultaneously at thalamic and cortical levels in the human brain.

As well as more extensive activation of the thalamus, VS of the ectopic phantom representation in subject 1 was associated with significant activation of the anterior cingulate cortex (area 32). This area plays a role in attentional processing (Pardo et al. 1990), and it is possible that its activation reflected the direction of the subject’s attention toward the abnormal perception elicited by the stimulus.

Because the representation of the trunk in S1 is located dorsomedially, but immediately adjacent to the representations of the hand and arm (Penfield and Rasmussen 1950; Woolsey et al. 1979), the results support the notion that areas of cortex adjacent to a deafferented zone are most likely to provide it with reorganized input, as suggested by others (Aglioti et al. 1994a,b; Ramachandran et al. 1992a,b). Why the deafferented hand/arm area should be invaded from different directions in different subjects, i.e., from the trunk representation in the present subjects, but from the facial representation in the subjects studied by others (Elbert et al. 1994; Halligan et al. 1993; Knecht et al. 1996; Ramachandran et al. 1992a,b; Yang et al. 1994), is unclear. Nonetheless, it is of interest to note that the present study did demonstrate abnormal functional connections between the deafferented S1 hand/arm area and the adjacent S1 face area, although there were no perceptual correlates of this abnormal connectivity on psychophysical testing.

The mechanism whereby abnormal intracortical connections are unmasked cannot be determined from the results of the present study. However, we and others have previously provided evidence that limb amputations acquired by human subjects in adult life are accompanied by marked alterations in the excitability of the deafferented M1 to transcranial magnetic stimulation (Cohen et al. 1991; Kew et al. 1994), and that similar increased excitability of the deafferented S1 and PPC may be important in the genesis of phantom limb perceptions (Kew et al. 1994). A plausible substrate for these changes is a modulation of GABAergic inhibition within the deafferented cortex (Kew et al. 1994). Although such modulation has yet to be demonstrated directly in humans, it has been confirmed clearly in animal studies. Functional deafferentation of monkey visual cortex by unilateral enucleation, eyelid suture, or intraocular injection of tetrodotoxin has been shown to result in a 50% reduction in the number of neurons immunoreactive for GABA and its synthesizing enzyme glutamic acid decarboxylase in ocular dominance columns subserving the deprived eye (Hendry and Jones 1986, 1988; Jones 1990). Similar reductions in glutamic acid decarboxylase immunoreactivity have been observed in the S1 barrel cortex of rodents after whisker removal (Welker et al. 1989), and in primates, deafferentation of an area of S1 by peripheral nerve section leads to a dramatic (75%) local reduction in the number of GABA-immunoreactive neurons (Garraghty et al. 1991). It has been suggested that this deafferentation-induced localized loss of GABAergic inhibitory neurons may allow functional access of normally suppressed, subthreshold inputs to the deafferented cortex (Garraghty et al. 1991; Jones 1993). The presence of such inputs has been inferred from studies of pharmacological manipulation of cortical GABAergic inhibition in both S1 (Dykes et al. 1984) and M1 (Jacobs and Donoghue 1991). These inputs are likely to be intracortical and may originate from long-range horizontal collaterals of pyramidal neurons situated in regions of primary cortex adjacent to the deafferented zone (DeFelipe et al. 1986; Huntley and Jones 1991; Jones 1993). Such horizontal collaterals are known to extend for $\approx 6$ mm (Jones 1993).

The present results could be interpreted in a similar way: vibrotactile input gaining access to the S1 pectoral representation via thalamocortical afferents would then be relayed into the deafferented S1 hand/arm representation via long-range horizontal collaterals unmasked by local deafferentation-induced modulation of GABAergic inhibition. This hypothesis is currently tenable and is amenable to testing by further PET studies with the use of the benzodiazepine antagonist $^{11}$C-flumazenil to measure levels of cortical GABA_A binding.

We thank the volunteers for participation; Dr. Sellaiha Sooriakumaran for help in recruiting the subjects; Dr. Karl Friston for statistical guidance; colleagues in the Medical Research Council Cyclotron Unit Radiochemistry and PET methods sections; and G. Lewington, A. Williams, and A. Blyth for assistance with the PET scanning.

This work was supported by the Medical Research Council.

Address for reprint requests: P. Halligan, Rivermead Rehabilitation Centre, Abingdon Rd., Oxford OX1 4XD, UK.

Received 6 March 1996; accepted in final form 9 January 1997.

REFERENCES


