Basic Science

Functional Reorganization of the Primary Somatosensory Cortex of a Phantom Limb Pain Patient

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Functional reorganization of the somatosensory system was widely observed in phantom limb pain patients. Whereas some studies demonstrated that the primary somatosensory cortex (S1) of the amputated limb was engaged with the regions around it, others showed that phantom limb pain was associated with preserved structure and functional organization in the former brain region. However, according to the law of use and disuse, the sensitivity of S1 of the amputated limb to pain-related context should be enhanced due to the adaptation to the long-lasting phantom limb pain experience. Here, we collected neurophysiological data from a patient with 21-year phantom limb pain using electroencephalography (EEG) and functional magnetic resonance imaging (fMRI) techniques. EEG data showed that both laser-evoked potentials (LEPs) and tactile-evoked potentials (TEPs) were clearly presented only when radiant-heat laser pulses and electrical pulses were delivered to the shoulder of the healthy limb, but not of the amputated limb. This observation suggested the functional deficit of somatosensory pathways at the amputated side. FMRI data showed that significant larger brain activations by painful rather than non-painful stimuli in video clips were observed not only at visual-related brain areas and anterior/mid-cingulate cortex, but also at S1 contralateral to the amputated limb. This observation suggested the increased sensitivity of S1 of the amputated limb to the pain-related context. In addition, such increase of sensitivity was significantly larger if the context was associated with the amputated limb of the patient. In summary, our findings provided novel evidence for a possible neuroplasticity of S1 of the amputated limb: in an amputee with long-lasting phantom limb pain, the sensitivity of S1 to pain-related and amputated-limb-related context was greatly enhanced.

Key words: Phantom limb pain, primary somatosensory cortex (S1), functional reorganization, sensory deficit, neuroplasticity, functional sensitivity, electroencephalography (EEG), functional magnetic resonance imaging (fMRI)

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Phantom limb pain is a subjective experience where a person continues to feel pain with his/her amputated limb as if it were still attached to the body (1-2). A possible explanation for this phenomenon refers to the changes in neural pathways and synapses because of the bodily injury, i.e., neuroplasticity (3-6). Typically, after the limb has been amputated, the cortical maps of the removed limb in the postcentral gyrus (i.e., primary somatosensory cortex, S1) are believed to have been engaged with the area around them (7-9). This is evident from previous studies showing that touching different parts of the face led to the tactile sensations at different parts of the missing limb of amputees (4,10), and the perception of touch and pain were the perceptual correlate of cortical reorganization in the brain (7,11). However, a recent study indicated that phantom limb pain was associated with preserved structure and functional organization in the former

area (12), considering that neuroplasticity associated with phantom limb pain was driven by the chronic pain experience. Moreover, in response to the long-lasting pain experience, the function of some cortical areas (e.g., S1 of the amputated limb) could be enhanced in terms of sensitivity to pain-related context according to the law of use and disuse (13,14).

In the present case report, we (1) assessed the functional integrity of the nociceptive and non-nociceptive somatosensory pathways at both the healthy and amputated sides using laser-evoked potentials (LEPs) and tactile-evoked potentials (TEPs) respectively, and (2) studied the functional sensitivity of S1 of both healthy and amputated limbs to pain-related context (i.e., the observation of video clips showing painful or non-painful stimulation of the left or right hand).

Objectives

This case aimed to demonstrate that as compared to S1 of the healthy limb, the sensitivity of S1 of the amputated limb to pain-related context (i.e., the observation of video clips showing painful or non-painful stimulation of the left or right hand) was enhanced using electroencephalography (EEG) and functional magnetic resonance imaging (fMRI) experiments.

Case

A 52-year-old, left upper-limb amputee (male, right-handed), without psychiatric problems, presented with a 21-year history of phantom limb pain. The phantom limb pain is a constant pain (throbbing, aching, and pinching in nature) on the back of his left hand. The left upper-arm was amputated 21 years ago after a traffic accident. His pain was refractory to both pharmacological and non-pharmacological therapies, including oral opiates, acupuncture, and other physical therapies (e.g., massage). In daily life, the reported average pain was 6 (range = 2 – 8) on a 0 – 10 visual analogue scale (VAS), which seriously disrupted his sleep during the night (the patient was awakened frequently by the pain throughout the night). Significant exacerbation of his pain occurred when the patient was tired. No stump pain was observed and no trigger zone was detected. The patient gave his informed consent, and the local Ethics Committee approved the experimental procedures.

EEG Experiment

Radiant-heat laser stimuli were generated by an infrared neodymium yttrium aluminium perovskite (Nd:YAP) laser with a wavelength of 1.34 μm (Electronical Engineering, Italy). The laser pulse was transmitted via an optic fiber and focused by lenses to a spot with a diameter of ~7 mm (~38 mm^2). The duration for each laser pulse was 4 ms. After each stimulus, the laser beam target was shifted by ~10 mm in a random direction, to avoid nociceptor fatigue or sensitization. Transcutaneous electrical stimuli were constant current square-wave pulses (0.5 ms duration) delivered through a pair of surface electrodes (2 cm distance between electrodes) (15). Both laser and electrical pulses were delivered to predefined areas on left and right shoulders.

Prior to EEG data collection, detection thresholds of pain and tactile sensations were measured using laser and electrical stimuli (left shoulder: > 4.5 J for pain detection threshold, i.e., larger than the maximal limitation of the laser device, 10.4 mA for tactile detection threshold; right shoulder: 2.5 J for pain detection threshold, 9.1 mA for tactile detection threshold). During EEG data collection, we delivered 10 laser pulses at each of the 6 stimulus energies (from 1.75 J to 3 J, in step of 0.25 J) in pseudorandom order, for a total of 60 laser stimuli per shoulder. In addition, we delivered 100 electrical pulses at the stimulus intensity of 20 mA per shoulder.

EEG data were recorded using a 64-channel Brain Products system (Brain Products GmbH, Munich, Germany; pass band, 0.01 – 100 Hz; sampling rate, 1000 Hz). The nose was used as reference, and impedances of all electrodes were kept < 10 kΩ. Electro-oculographic (EOG) signals were simultaneously recorded using surface electrodes to monitor ocular movements and eye blinks.

EEG data were pre-processed and analyzed using EEGLAB (16) and in-house MATLAB scripts. Continuous EEG data were bandpass filtered between 1 and 30 Hz. EEG epochs were extracted using a window analysis time of 1500 ms (from -500 ms to 1000 ms) and baseline corrected using the prestimulus interval. Trials contaminated by eye-blinks and movements were corrected using an independent component analysis algorithm (16). A point-by-point independent-sample t-test was used to assess the differences between single-trial LEPs of the left and right shoulders, as well as between single-trial TEPs of the left and right shoulders.

As displayed in Fig. 1, clear LEP and TEP waveforms, which were characterized by a large N2-P2 complex and a large N1-P2 complex, respectively, were observed if laser and electrical pulses were delivered to the right shoulder. In contrast, both waveforms were not clearly presented if laser and electrical pulses were delivered

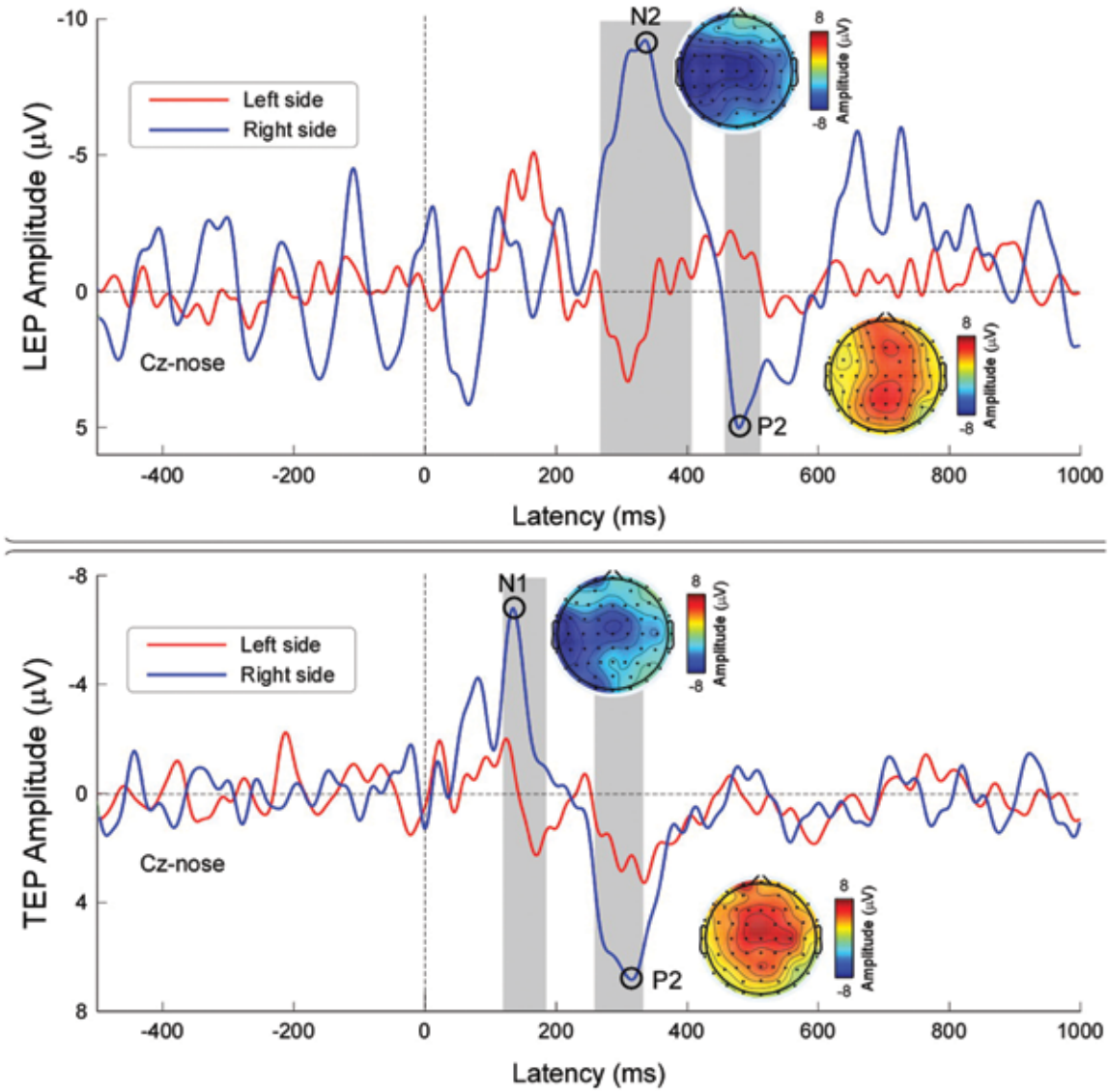

Fig. 1. *Comparison of laser-evoked potentials (LEPs) and tactile-evoked potentials (TEPs).*
Displayed signals were group-level LEP (top) and TEP (bottom) waveforms elicited by left (amputated side; marked in red) and right (healthy side; marked in blue) shoulder stimuli (Cz-nose). Clear LEP and TEP waveforms, characterized by a N2-P2 complex and a N1-P2 complex, respectively (as well as their scalp topographies), were elicited by right shoulder stimuli, but not by left shoulder stimuli. As marked using gray rectangle, single-trial LEPs elicited by left and right shoulder stimuli were significantly different at 268 – 408 ms and at 460 – 510 ms, and single-trial TEPs elicited by left and right shoulder stimuli were significantly different at 121 – 184 ms and at 259 – 334 ms.

to the left shoulder. Note that significant differences between left and right shoulders were detected at 268 – 408 ms (N2 wave) and at 460 – 510 ms (P2 wave) for single-trial LEPs, and at 121 – 184 ms (N1 wave) and at 259 – 334 ms (P2 wave) for single-trial TEPs (Fig. 1). These findings indicated that nociceptive and non-nociceptive somatosensory pathways by the amputated side were functionally deficient, while both pathways of the healthy side were functionally intact.

fMRI Experiment

The fMRI experiment was conducted with a 2 (stimulated hand: left and right hands in video clips) × 2 (stimulation modality: painful or non-painful stimuli in video clips) within-subject design, comprising of watching 4 types of video clips extracted from the samples in Avenanti et al (17). The video clips included (1) "left pain" video clips showing a needle penetrating the first dorsal interosseous muscle at the surface of the left hand (between the thumb and index finger); (2) "right pain" video clips showing a needle penetrating the first dorsal interosseous muscle at the surface of the right hand; (3) "left touch" video clips showing a Q-tip slightly touching the left hand; (4) "right touch" video clips showing a Q-tip slightly touching the right hand.

FMRI data collection was composed of 2 sessions, and 20 video clips for each type were presented in a pseudorandom order. For each trial, a video clip was presented for 3 seconds, which was followed by a black screen for 7 – 8 seconds. Following, participants were instructed to rate the subjective intensity of pain elicited by the video clips using an electronic 0 – 10 VAS ranging from 0 (no pain) to 10 (worst pain imaginable).

FMRI data were acquired using a Siemens 3.0 Tesla Trio scanner with a standard head coil at the Key Laboratory of Cognition and Personality (Ministry of Education) of the Southwest University (China). We used a whole-brain gradient-echo, echo-planar-imaging sequence for functional scanning and its repetition time was 2000 ms (30 ms echo time, 32 contiguous 3.0 mm thick slices, 3 × 3 mm in-plane resolution, field of view 192 × 192 mm, matrix 64 × 64; flip angle = 90º). A high-resolution, T1-weighted structural image (1 mm3 isotropic voxel MPRAGE) was acquired after functional imaging for registration.

FMRI data were preprocessed and analyzed using Statistical Parametric Mapping software SPM8 (Wellcome Trust Center for Neuroimaging, London, UK). The first 5 volumes were discarded to allow for signal equilibration. Images were slice-time corrected, motion corrected, normalized to the Montreal Neurological Institute (MNI) space, and spatially-smoothed using a Gaussian kernel of 8 mm full width at half maximum (18). The pre-processed images were analyzed on a voxel-by-voxel basis using a general linear model (GLM) approach (19). Significant differences between brain activations to painful and non-painful stimuli in video clips were assessed using paired-sample t-test, as implemented in SPM8 (significant threshold: $P_{FWE} <$ 0.05 at cluster level) (20). In addition, signal changes (%) of blood-oxygen-level dependent (BOLD) responses within 2 region-of-interests (ROIs) (i.e., bilateral hand S1) were extracted (21), and compared using a 2-way repeated-measures analysis of variance (ANOVA), with stimulated hand and stimulation modality as within-subject factors. When the main effects or interaction was significant, post-hoc pairwise comparisons were performed. Note that ROIs of bilateral hand S1 were defined using MarsBar toolbox (http://marsbar.sourceforge.net).

The top panel of Fig. 2 shows that significant larger activations by painful rather than non-painful stimuli in video clips were observed not only at visual-related brain areas and anterior/mid-cingulate cortex, but also at right S1. ROI analysis indicated that left S1 was not significantly modulated by the stimulated hand ($F = 1.282$, $P = 0.273$), stimulation modality ($F = 1.950$, $P = 0.181$), and their interaction ($F = 0.595$, $P = 0.451$; Fig. 2, bottom panel). In contrast, right S1 was significantly modulated by stimulation modality ($F = 5.590$, $P = 0.030$), but not by the stimulated hand ($F = 0.066$, $P = 0.801$), and their interaction ($F = 0.003$, $P = 0.961$; Fig. 2, bottom panel). Post-hoc pairwise comparisons revealed that the activation of right S1 was significantly larger to "left pain" video clips than to "left touch" video clips ($P = 0.046$), but not significantly different between "right pain" and "right touch" video clips ($P = 0.092$). These findings indicated that right S1 (contralateral to the amputated limb) was sensitive to the pain-related context (Fig. 2, top panel), especially for the context that was associated with the amputated limb (i.e, painful stimuli of the left hand in video clips; Fig. 2, bottom right panel).

Discussion

In the present case report, we investigated the relationship between phantom limb pain and somatosensory reorganization using EEG and fMRI experiments in a patient with a 21-year history of phantom limb pain. EEG findings showed that whereas nociceptive and non-nociceptive somatosensory pathways at the healthy side were functionally intact, both pathways at the amputated side were functional deficient. FMRI findings indicated that the activation of contralateral S1 to the amputated limb was significantly enhanced to pain-related context than to non-pain-related context, and such enhancement was significantly greater if the pain-related context was associated with the amputat-

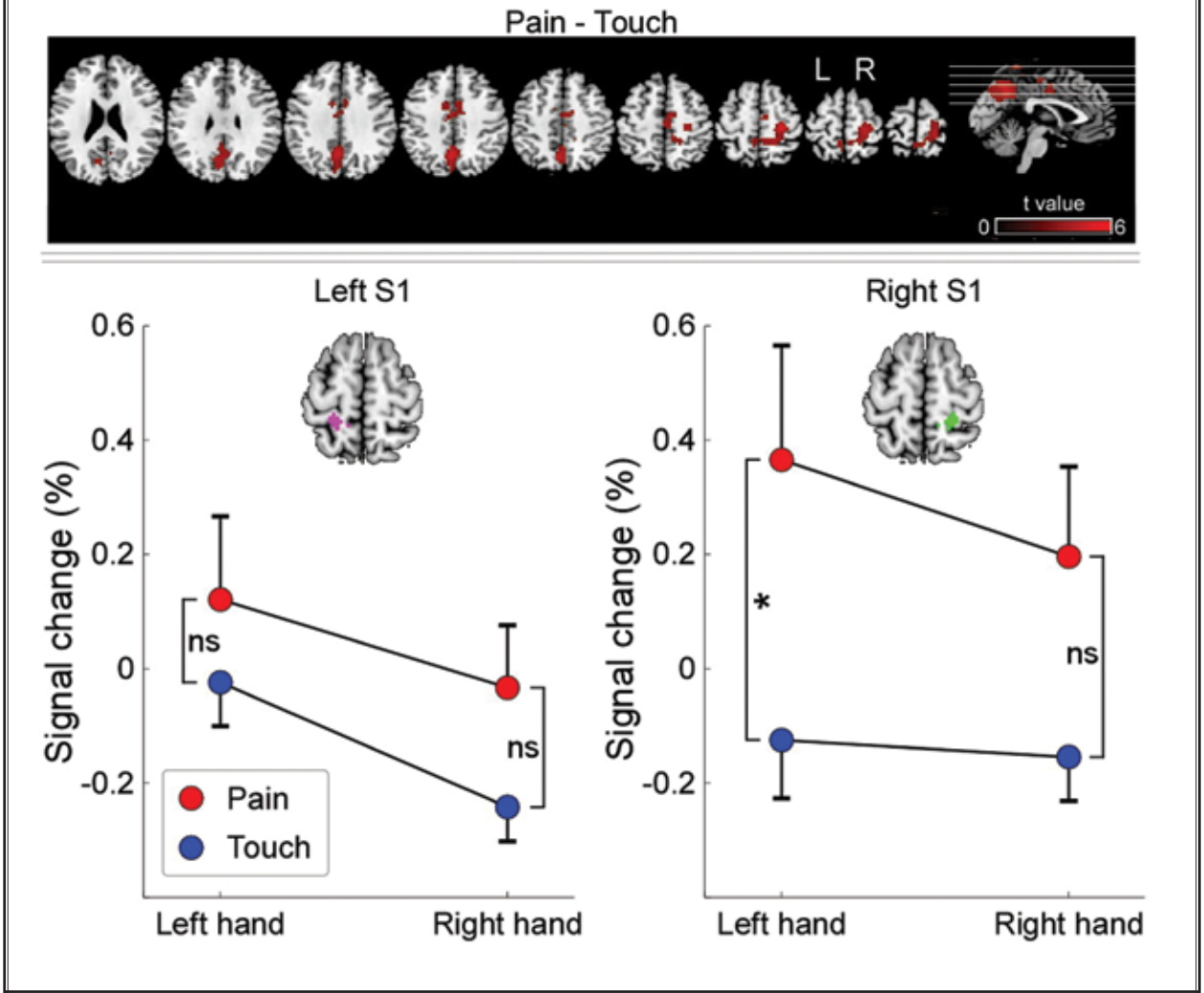

Fig. 2. *Comparisons of brain activations in different conditions.*
Top panel: Significantly larger activations to painful than to non-painful stimuli in video clips were observed at visual-related brain areas, anterior/mid-cingulate cortex, and right S1 (contralateral to the amputated limb; cluster level PFWE < 0.05). L: left, R: right. Bottom panel: Signal changes (%) of BOLD responses in left S1 were not significantly modulated by stimulated hand, stimulation modality, and their interaction. In contrast, signal changes of BOLD responses in right S1 were significantly modulated by stimulation modality, but not by stimulated hand, and their interaction. Specifically, signal changes of BOLD responses in right S1 was significantly larger to "left pain" video clips than to "left touch" video clips, but not significantly different between "right pain" and "right touch" video clips.

ed limb rather than the healthy limb. In contrast, the activation of contralateral S1 to the healthy limb was not sensitive to the pain-related or amputated-limb-related context. These results showed enhanced neuroplasticity in the primary somatosensory cortex, in terms of sensitivity to pain-related and amputated-limb-related context of an amputee with long-lasting phantom limb pain. Such sensitivity enhancement, which was in line with the law of use and disuse, may serve as a novel neural signature for long-lasting phantom limb pain, and may be used as a new neural basis for the diagnosis and treatment of phantom limb pain (2,22-27). Considering that neuroplasticity could be observed rapidly after bodily injury (28), we speculated our ob-

servation, i.e., the enhanced sensitivity of the primary somatosensory cortex to pain-related and amputated-limb-related context, could be observed in patients with phantom limb pain lasting shorter than 21 years, especially for younger patients. Indeed, the reliability and validity of our observation should be tested using neurophysiological data from more phantom limb pain patients. Even our strategy could be used to assess the level of neuroplasticity of phantom limb pain patients in the future, the diagnosis and treatment of phantom limb pain should also consider the chronic pain related comorbidities, e.g., psychological disorders and sleep disturbance (29).

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