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Relieving phantom limb pain with multimodal sensory-motor training

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Abstract

Objective. The causes for the disabling condition of phantom limb pain (PLP), affecting 85% of amputees, are so far unknown, with few effective treatments available. Sensory feedback based strategies to normalize the motor commands to control the phantom limb offer important targets for new effective treatments as the correlation between phantom limb motor control and sensory feedback from the motor intention has been identified as a possible mechanism for PLP development. *Approach.* Ten upper-limb amputees, suffering from chronic PLP, underwent 16 days of intensive training on phantom-limb movement control. Visual and tactile feedback, driven by muscular activity at the stump, was provided with the aim of reducing PLP intensity. *Main results.* A 32.1% reduction of PLP intensity was obtained at the follow-up (6 weeks after the end of the training, with an initial 21.6% reduction immediately at the end of the training) reaching clinical effectiveness for chronic pain reduction. Multimodal sensory-motor training on phantom-limb movements with visual and tactile feedback is a new method for PLP reduction. *Significance.* The study results revealed a substantial reduction in phantom limb pain intensity, obtained with a new training protocol focused on improving phantom limb motor output using visual and tactile feedback from the stump muscular activity executed to move the phantom limb.

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Keywords: phantom limb pain, sensory feedback, motor training

S Supplementary material for this article is available online

(Some figures may appear in colour only in the online journal)

Introduction

Phantom limb pain (PLP) is pain felt in the missing limb in amputees and represents an extremely challenging pain condition to treat. Regardless of the reason for amputation, the prevalence of PLP is reported to be as high as 85% [1, 2]. Previous studies have suggested that the primary sensorimotor cortex (SM1) undergoes a reorganisation involving both structural [3–5] and functional [6–11] changes contralateral to the amputation. These changes are believed to result from the loss of afferent input, allowing for invasion of neighbouring cortical regions into the former limb representation area in the SM1.

Another aspect that has been extensively investigated is sensory-motor incongruence [12–14], implying that pain, in the absence of ongoing tissue damage, might be caused by incongruence between motor intention and proprioceptive feedback [14, 15]. According to Ramachandran [12], when a limb is intact, motor commands to move a limb are usually damped by sensory error feedback, such as vision and proprioception [16, 17]. With a phantom limb, this damping effect is not present and the motor output may become amplified and experienced as painful [18, 19]. Training with visual feedback on the phantom movements driven by the motor activity of the stump may possibly dampen the painful and uncontrolled motor output, decreasing phantom-limb pain [20, 21]. Therefore, mirror therapy, graded motor imagery, tactile training or sensory discrimination may correct cortical body maps by removing the incongruence between motor commands and sensory feedback [20].

The motor capacities of the phantom limb are of interest for PLP treatment because there is some evidence of a relation between the ability to control movements of the phantom limb and the severity of PLP [22–25]. This is supported by the clinical observation that many amputees feel that their phantom limb is fixed in one position and report cramping sensations as one of the main characteristics of their phantom pain [22, 24]. Moreover, activation of remnant muscles in the stump is impaired in patients with pain relative to amputees who are pain free [22], where PLP participants show slower cyclic phantom movements with a higher degree of muscular modulation compared to the pain-free amputees [22]. The interaction between central motor commands and sensory feedback in the perception of phantom movement was further demonstrated by Reilly and colleagues [26]. In an amputee with a frozen phantom limb, they observed that the stump-muscle activity did not vary when attempting to perform different movements of the phantom limb. This behaviour could also be induced in amputees able to differentiate muscle activity of their stump following ischemic nerve block. Therefore, there is an established link between PLP and the motor control of phantom movements showed by the positive correlation between the amount of electromyography (EMG) activity acquired in remnant stump muscles during phantom limb movements and the intensity of the phantom pain [22]. This connection supports the idea of training phantom limb motor control with direct feedback of the different muscular effort of the residual limb muscles.

However, few effective treatments are available for PLP [10]. Some are invasive, such as local anaesthesia, sympathectomy and rhizotomy, and therefore not always accepted by patients. Pharmacological interventions, such as anticonvulsants, neuroleptics and muscle relaxants, may lead to side effects that negatively impact on quality of life [27]. Current non-pharmacological/non-invasive approaches, such as mirror therapy [28, 29], provide visual feedback in order to counteract sensorimotor incongruence but show hindered efficacy in case of limited integration between sensory feedback and the kinaesthetic sensation of the phantom (e.g. shrunk in case of telescoping) [30]. Virtual visual feedback during phantom movement to alleviate phantom pain has been used in previous studies [24, 31, 32] and the results are encouraging. However, controlled studies with larger samples are needed to determine which patients are most likely to benefit from such virtual feedback therapy [33, 34]. Somatosensory feedback provided by electro-tactile stimulation has been proposed to reduce PLP in a very homogeneous patient group of transradial myoelectric prosthesis users [35]. Sensory discrimination training programmes, based on electrical or mechanical tactile stimuli have demonstrated a significant reduction in PLP (upper and lower limb amputees) [36, 37]. Moreover, in a recent clinical trial [38], augmented reality was used to decrease the PLP intensity, using visual data as the only source of sensory feedback.

There are no reports exploring multimodal sensory feedback substitution associated with phantom motor execution for PLP relief. In the current study, we devised and tested a new phantom-limb movement training protocol focused on providing visual and tactile feedback substitution in upper-limb amputees suffering from chronic PLP with the main aim of reducing PLP intensity. Volitional control of the phantom limb was used as the principal component of the proposed treatment, sustained by visual and tactile feedback of the EMG activity generated by the remnant muscles of the residual limb during the execution of the phantom movements. The participants were asked to train the execution of specific wrist, hand and finger movements with their phantom and to learn to control the muscular effort to accomplish such movements. Participants were able to modulate the motor output using visual and tactile feedback from the EMG activity of the

muscles involved in the execution of the phantom movement itself.

The participants underwent a 16-day treatment which commenced two weeks after enrolment into the study. Pain evaluation, quantitative sensory testing (QST), and functional magnetic resonance imaging (fMRI) were performed before and after training. Pain evaluation was again carried out at a follow-up six weeks after the end of the training. A detailed daily pain diary was used to assess intensity and fluctuation of pain during the entire duration of the study, which lasted 76 days in total.

It was anticipated that training the residual limb muscles, that are meant to move the phantom limb, might represent an effective approach to improve motor control over the phantom, as concurrent information about the intensity and distribution of the muscular activity meant to control the actual movement is returned. The phantom-limb motor commands were fed back as visual and tactile information substitution with the intent of providing a concurrent feedback of the volitional motor intention over the phantom limb movement control. The administered multimodal (visual and tactile) feedback were used as an alternative sensory information over the missing visual and proprioceptive information. As phantom movements involve motor execution and a specific effort in contracting the residual limb muscles, in this study visualisation and tactile sensations of such effort are meant to improve phantom motor control and provide synchronised feedback of putative phantom movement. The multimodal visual and tactile feedback served even as reinforcement of the sensory afferences already available at the stump level (proprioceptive afferences from muscular activity). Functional magnetic resonance imaging (fMRI) mapping the tactile lip, lower arm, and (phantom) hand representation was performed contra- and ipsilaterally before and after the three-week training, in order to assess whether the training induced somatosensory cortical plasticity, ideally reducing potential maladaptive plasticity.

Materials and methods

The present study aimed to explore the treatment of PLP using a novel paradigm of training phantom-limb movement control using stump muscular activity as a source of visual and tactile feedback. The reduction of PLP intensity across the time of the treatment, at the end of it and at the follow up period of six weeks represented the main outcome of the study.

Participants

A group of ten participants (five women, aged 57.7 ± 12.4 years, range: 28–75 years) were selected from an initial screening interview. Inclusion criteria were: (i) major unilateral upper-limb amputation, (ii) PLP at least twice a week, with an average peak intensity of 3 on a VAS scale (Visual Analog Scale anchored with 2 points: 0 = no pain—10 = the worst pain ever felt), (iii) amputation executed more than two years ago from the enrolment, to rule out acute PLP. The average time since amputation was 17.7 ± 16.0 years (range: 6–52 years). All participants reported feeling a phantom limb.

Table 1 provides basic information about the participants, while table S1 (supplementary material (stacks.iop.org/JNE/15/066022/mmedia)) provides detailed information, including cause of amputation, pain medication and PLP frequency. Participants were instructed to refrain from new pain therapies across the entire duration of the study. The study was approved by the ethical committee of the University Medical Center Göttingen, Germany and performed in accordance with the Declaration of Helsinki. Informed written and oral consent was obtained from all participants prior to the intervention.

Temporal succession

After a preliminary telephone interview to evaluate the participants' eligibility according to the study inclusion criteria, the participants were enrolled. From two weeks prior to the start of the training (*T*0, table 2) until the follow-up evaluation six weeks after the end of the treatment (*T*3) they were asked to complete a daily pain diary (table 2), which consisted of an evaluation of PLP and stump pain intensity reported on a VAS scale and had to be completed four times per day. Also the characteristics of the pain and the pharmacological regimen were self-reported. Additionally, the PLP and stump pain domains were assessed in three sessions (pre, post, and follow-up evaluation at *T*1, *T*2, and *T*3, respectively) as reported in table 2.

Between *T*1 and *T*2, each participant trained, twice per day, five days per week for a total of 12 effective training days (within 16 days), with the protocol described below.

Phantom-limb treatment protocol

The muscular activation at the stump level was recorded by eight superficial bipolar electrodes and differential electromyography (EMG) amplifiers with wireless data transmission (Myo™ Armband, Thalmic Labs Inc., Canada—figure 1(A)), evenly embedded in an elastic plastic band. The EMG band position was selected to obtain the best muscular activation (visually evaluated) across the eight acquisition locations. Sensory feedback was implemented by eight micro-vibrators (C3 Tactor, Engineering Acoustics Inc., USA) for tactile stimulation and an intuitive visual representation as visual feedback on a screen. Both of the feedback modalities conveyed the information of the eight EMG electrodes. The micro-vibrators were aligned and evenly positioned around the stump with an elastic band as each EMG amplifier provided the control signal for the corresponding vibrator. The position of the EMG amplifiers and micro-vibrators were marked with a medical skin marker to assure consistent positioning along the entire training period. Visually, the analysed EMG data were displayed using a radial plot arrangement to be congruent with the anatomical position of the acquired muscles (see figure 1(A)).

Table 1. Participant details. 'Telescoping' refers to a phenomenon in which the phantom limb of an amputee is not perceived at the location previously occupied by the intact limb but retracted inside the stump.

Participants	1	2	3	4	5	6	7	8	9	10	Mean (SD)
Gender	M	F	F	M	F	M	F	M	F	M	5M, 5F
Age (years)	59	56	59	28	60	64	64	75	63	49	57.7 (12.4)
Amputation side	L	R	L	L	L	R	L	L	L	L	2R, 8L
Amputation level	TR	TR	TR	TH	TR	TR	TR	TH	TH	TH	6TR, 4TH
Time since amputation (years)	6.2	14.7	5.7	13.8	6.2	7.2	8.2	52.9	26.2	36.7	17.7 (16)
Telescoping	N	N	N	N	N	Y	Y	N	Y	Y	4Y,6N
Myoprosthesis user	Y	Y	Y	N	Y	Y	Y	Cos	N	Y	7Y, 3N
Pain intensity @ *T*1 (VAS)	6.5	8.5	3	4.3	3.5	6	4.1	7	4	4	5.1 (1.8)
Stump pain	N	Y	N	Y	N	N	N	N	N	N	2Y, 8N

Further abbreviations: TR = Transradial, TH = Transhumeral, Cos = Cosmetic Prosthesis, VAS = Visual analogue scale.

The EMG data were sampled at 200 Hz, acquired through a Bluetooth communication protocol, rectified, and smoothed via RMS (moving windows of 250 ms), displayed on the polar plot (figure 1(B)) and used to command, via USB port, the controller of the micro-vibrators. The training software, composed of a participant interface and an operator interface, was developed in Matlab (MathWorks, USA). The motor activity was based on a game-like exercise to train the execution of a selection of phantom movements among a list of ten movements, including five wrist movements (wrist flexion/extension and pronation/supination, ulnar deviation), three hand movements (hand open, key grip, fine pinch) and two finger movements (index-finger extension, ring-finger flexion).

Each training session started with calibration of the training software and lasted around 1 h. The operator selected a series of phantom movements to train during the calibration phase, taking into account the actual current control ability of the participant. The calibration consisted of a slow maximal voluntary contraction (MVC) of the phantom movements to be trained, played in a sequential way. The contractions were supposed to be performed by following the visual cues provided by a 3D hand model on the participant interface (figure 1(A)—'visual cue'). This procedure was necessary to normalise the visualisation of the EMG data and to store the polar plot shape of the MVC EMG amplitude for each calibrated movement (see figure 1(B)—'Reference Polar Plot'). Scaling the EMG individually, based on the participants' specific MVC values, provided a clear visual feedback in all the trained phantom movements as the EMG values moved within the 0%–100% MVC range.

Only those movements selected during the calibration phase were trained during the following training phase. During each required phantom-movement run, the name of the movement to be realised (e.g. wrist supination) was indicated. The participants executed the prescribed phantom-limb movement starting from and returning to a neutral position of the limb (elbow 90° flexed, wrist in neutral position), following the motions of the 3D hand model. The 3D hand model informed the participants about the type, way and speed of phantom movement to be executed in each trial. Each prescribed movement therefore implied a 'contraction phase' in which an increasing EMG amplitude was registered and a 'release phase' where the EMG amplitude decreased until the resting level. The 3D hand model motion was synchronised with the contraction and release phase prompted by the Reference Polar Plot, which expanded and collapsed showing the participants the amount (in % of MVC) of EMG activity to be generated during the prescribed phantom movement to train (see a snapshot of this activity in figure 1(B)). While the hand model and the actual polar plots (figure 1(B)—'EMG Polar Plot' and 'EMG activity') gave the participant visual feedback about how to properly control the stump muscular activity, the vibrators tactilely reinforced the mechanoreceptive feedback of the activated muscles. They were proportionally controlled modulating the vibration amplitude using, in real time, the amplitude of the analysed EMG data (figure 1(B)—'EMG activity'). Each vibrator delivered a stimulus intensity proportionally modulated with the intensity of the normalized EMG amplitude expressed at the corresponding amplifier, with a vibration frequency of 100 Hz.

During the training runs, the participants were additionally guided in controlling the EMG amplitude via a reference plot created using the MVC values registered in the previous calibration phase (figure 1(A)—'visual myo feedback and gaming activity' and figure 1(B)—'Reference Polar Plot'). The gaming activity provided a further reinforcement to improve the control of the phantom movement. A moving circle was shown on the reference polar plot as a target for the participants, which they were asked to follow using the EMG polar plot. The circle moved to and from the most active EMG channel recorded during the corresponding calibration phase (figure 1(B)—'Moving circle') locked on the reference polar plot. To be successful on a run, they had to stay inside the circle for a certain amount of time, depending on the determined difficulty of the training session. A scoring system, based on three different remunerations (a silver coin, a gold coin, and a diamond), was devised to engage the participants and avoid frustration (figure 1(A)—'motivation'). As further motor reinforcement, the participants were asked to mirror the phantom movement with their intact side. If a participant could e.g. only marginally open the phantom hand, then he/she should also only marginally open the intact hand. As

Table 2. Study trial timeline. Phases and time-points characterizing the progression of the study which lasted 76 days in total.

Time-point	*T*0		*T*1		*T*2		*T*3
Duration	1 day	2 weeks	1 day	16 days	1 day	6 weeks	1 day
Phase	Enrollment		Pre evaluation	Training	Post evaluation		Follow-up evaluation
Tasks		Daily pain diary completion	Baseline and pain questionnaires, QST, fMRI	Training 1 h, twice per day, 5 days/week, daily pain diary completion	Pain and satisfaction questionnaires, QST, fMRI	Daily pain diary completion	Pain questionnaires

Baseline questionnaires = MPI-D, PCS, DASS-21, SF-36, PainDETECT, CEQ. Pain questionnaires = VAS, SES, QST = Quantitative sensory tests.

soon as the selected movements were rather well executed, another new phantom movement was added to the training.

The developed training software gave the operator the possibility of tailoring the training activity for each of the patients, as every parameter was adjustable, e.g. control over the selected movements, the movement speed, which had a smooth sinusoidal profile, its amplitude in terms of EMG range of activity (as a percentage of MVC), the amplitude of the target circle, and the 'polar plot in-circle' time duration for scoring was provided. These parameters were selected and changed according to the single participant's abilities and improvements to obtain challenging and still manageable training sessions.

Baseline and pain questionnaires

Clinical characteristics, exploration of painful and non-painful phantom phenomena and stump sensations were obtained with the questionnaire developed by Kern and colleagues [39] at the pre-evaluation session (*T*1, table 2). During the same session, the participant's treatment expectancy and rationale credibility were measured with the CEQ [40] as factors possibly representing non-specific treatment effects [41]. PLP and stump pain domains were evaluated using the following questionnaires: (i) the west haven-yale multidimensional pain inventory (MPI-D) [42, 43], a reliable and valid measure of physical functioning of the pain domain; (ii) the pain perception scale ('Schmerzempfindungsskala', SES) to measure the affective and sensory characterization [44]; (iii) the PainDETECT screening questionnaire to identify neuropathic pain components [45]; (iv) the pain catastrophizing scale (PCS) as a measure of catastrophic thinking related to pain [46] as it can be a risk factor for chronicity [47, 48]; (v) the Depression, Anxiety, Stress Scales (DASS-21), as a self-rating measure of depression, anxiety, and stress [49]; and (vi) the 36-Item short form health survey (SF-36), as a measure of the general health status of the participants [50]. Additionally, at *T*2 a satisfaction scale anchored with two points (0 = completely dissatisfied, 100 = completely satisfied) was used to rate the participants' satisfaction with the received training.

Quantitative sensory tests (QST) and two-point discrimination test

During the pre-evaluation (*T*1, table 2), a stump mapping procedure was performed. Systematic touch was applied to the distal portion of the stump in order to determine any points giving rise to referred sensations in specific parts of the phantom hand or fingers. The point triggering the strongest referred sensation of a finger was then marked on the stump and used as one of the location for the QST and the two-point discrimination test (see QST supplementary material). In the few participants where several finger sensations could be elicited, one of the most sensitive spots at the stump was used as the 'stump trigger point'.

Table 3. Sequence of (f)MRI measurements. Sequence of acquisitions comprising anatomical MRI and fMRI with different stimulation sites.

Run 0	Anatomical image of the brain
Run 1	Lips (air-puff) & 'digit' (stump location with digit sensation, brush)
Run 2	Arm (stump location without digit sensation, brush)
Run 3	Lips (air-puff) & intact-site digit (corresponding to Run 1, brush)
Run 4	Arm (location on intact side corresponding to stump location of Run 2, brush)

Two-point discrimination test was administered to evaluate the participants' somatosensory acuity [51]. The two-point discrimination test measures the participants' ability to perceive two stimuli simultaneously presented at varying distances from each other as distinct. The minimal distance where the stimulations are separated is given in mm. It was measured using two rounded tips of a sliding calliper, applying just the weight of the calliper. Starting at 2 cm, the sliding-tips distance was reduced by 1 mm each trial, or increased by 1 mm over the location with lower sensibility (on the stump and the shoulder), until the participant could no longer feel a separation between the two points, or start feeling the two tips as separated stimuli. The two-point discrimination test was repeated three times, data were presented as mean value [52]. The evaluators administering the baseline interview, the pain questionnaires, the QST and the two-point discrimination test did not take part in the training process to avoid a possible source of bias.

fMRI protocol

We assessed the possible cortical reorganisation with fMRI. Functional and anatomical magnetic resonance imaging was performed using a 3 T Tim Trio scanner (Siemens, Germany) and

standard imaging sequences at time points $T1$ and $T2$ (table 2). Standard anatomical ($1 \times 1 \times 1\,mm^3$) and functional measurements ($2 \times 2 \times 2\,mm^3$, 10% gap, repetition time $= 0.8\,s$) were performed, as described in table 3. During the functional measurements, the skin areas to be explored were stimulated using a traditional brush or an air-puff stimulator developed for utilisation in the scanner environment (pneumatic device), in order to evoke cortical activation in the primary sensory motor cortex.

In the first run (Run 1, table 3), the lips were stimulated via air-puff stimulation, and a stump spot was brushed by the experimenter (using a block paradigm with two conditions, alternating with rest periods), with the stump location being the location with the strongest referred phantom finger sensation (selected during the QST, see corresponding Methods section). In the second run, a slightly more proximal location (at the forearm/upper arm in transradial/transhumeral amputees, respectively) was brushed in a rest/stimulation block design. In the third run, the lips and the intact digit corresponding to the amputated one in Run 1 (commonly the thumb or index finger) were stimulated, while the fourth run served for mapping the location at the intact forearm/upper arm that corresponded to the arm position in Run 2.

Training data

The number of trained phantom movements, number of repetitions, single movement time duration and tracking error were acquired for each daily session through the entire training period. The tracking error was calculated as the sum of the absolute difference between the actual RMS EMG activity (figure 1(B)) and the reference polar plot (figure 1(B)) created using the MVC values registered during the calibration phase. The mean value of the tracking error was calculated for each executed trial.

Data analysis and statistics

The acquired EMG data were exported and processed offline in Matlab (MathWorks) and the data and scores from the pain-domain evaluation and QST tests were noted on Excel spreadsheets (Microsoft). All data were reported as mean values ± standard deviation (SD), or standard error (SE) of the mean when indicated. The training data were clustered in three groups composed of four consecutive training days, from day 1 to day 4 as the initial training period, from day 5 to 8 as mid training and 9 to 12 as the last training period. Average and standard deviation of the three training periods were analysed. Where data distributions were not Gaussian (according to Shapiro–Wilk tests), statistical evaluations were performed using the Wilcoxon Signed Rank test for paired data or the Mann–Whitney U test for unpaired data. Repeated measures ANOVA and Student's t test for independent samples were used for normally-distributed data. Bonferroni correction was applied for multiple comparisons. Significance was considered when $p < 0.05$. Pearson r (ESr) were used to estimate the effect size for normally distributed data. ESr less than 0.19

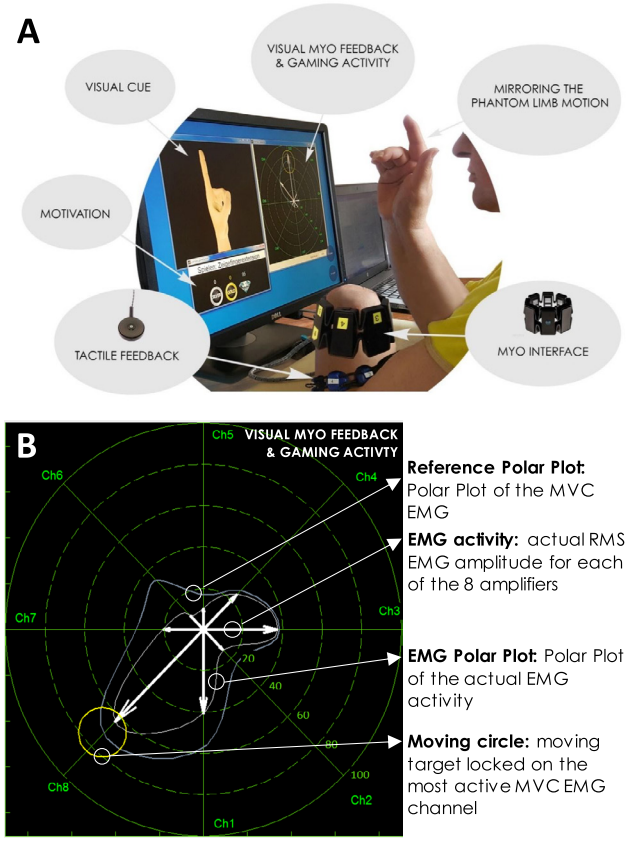

Figure 1. Experimental set-up of the multimodal sensory-motor training. (A) All the main components of the system are reported. The visual patient interface is based on several windows which are used as a reference on the movement to be executed with the phantom limb: 3D-hand model: 'Visual Cue'; window where scores are reported: 'Motivation'; polar plot where visual feedback of myoelectric stump activity is reported: 'Visual Myo Feedback'. The 'Myo Interface' is represented by the Myo™ Armband. The 'Tactile Feedback' is generated using an elastic band with eight embedded micro-vibrators. (B). Snapshot of the Visual Myo Feedback interface and Gaming activity. The polar space contains the axis to show the acquired EMG amplitude in % of the MVC (from Ch1 to Ch8). The patient interface shows in real-time (i) the actual EMG activity acquired at each of the eight amplifiers (eight white arrows: 'EMG activity'); (ii) the interpolated plot ('EMG Polar Plot') across the eight RMS EMG values; (iii) the interpolated polar plot acquired during the calibration phase and used as a reference for the participants ('Reference Polar Plot'); (iv) the 'Moving circle' used as a target for the gaming activity. The Moving circle is locked on the most active channel (Ch8 in this example) of the corresponding MVC calibration acquisition and it moves along this channel promoting a contraction and release of the stump muscles as the participant has to keep the EMG activity inside it and close to the Reference Polar Plot to score (see text for further details).

was classified as 'very weak', 0.2–0.39 as 'weak', 0.4–0.59 as 'moderate', 0.6–0.79 as 'strong', 0.8–1 as 'very strong' [53]. The 95% confidence interval (CI) for the mean difference was calculated. Statistical analysis was performed with the SPSS Statistics software (IBM, Version 22).

Using the PLP intensity between $T1$ and $T2$ (figure 2), an post-hoc analysis of the achieved power of the study was executed with the Software G∗Power (version 3.1.9.2) [54] retrieving a power, $1 - \beta$ error probability, of 0.99

($N = 10$, type I error probability $\alpha = 0.05$, Cohen's dz effect size = 1.73 [55])

fMRI analysis was performed in Brain Voyager (Brain Innovation, Maastricht, The Netherlands). Using the general linear model, cortical representation areas (statistical maps) for each of the stimulated sites could be statistically defined. For each individual, the cortical mesh was reconstructed for each hemisphere, and the statistical maps were projected onto it. As such, the statistically most significant vertices (peak vertices) and their 3D coordinates could be determined for each stimulated site. Differences in these peak locations between the representations ipsi- and contralateral to the amputation could be assessed. As this procedure was performed both pre- and post-training, possible shifts after training would be recognisable. Three Wilcoxon Signed-Rank tests were performed for each of the three 3D axes, in which the respective coordinates of the average-lips peak-activations were compared (1) prior to the training between hemispheres and pre-to-post-training within the hemisphere (2) contra- and (3) ipsilateral to the amputation.

Results

Baseline pain characteristics

The patients' baseline pain characteristics, assessed with the Pain Inventory domains (MPI-D), were compared to normative values derived from a population of 185 patients with chronic pain [43]. Participants reported a significantly lower amount of interference that pain had on their life (1.78 ± 1.11 (mean $\pm$ SD), $t(193) = 2.38$, $p = 0.0179$, $0.27 \leqslant \mathrm{CI} \leqslant 1.69$, normative − participants' mean), a lower level of distress caused by pain (2.29 ± 0.85, $t(193) = 3.19$, $p = 0.0016$, $0.7 \leqslant \mathrm{CI} \leqslant 1.82$), a higher perception of life-control (4.65 ± 0.81, $t(193) = -2.17$, $p = 0.0308$, $-1.38 \leqslant \mathrm{CI} \leqslant -0.31$), a higher performance of household chores (3.91 ± 1.5, $t(193) = -4.99$, $p < 0.0001$, $-3.1 \leqslant \mathrm{CI} \leqslant -1.19$), considered as one of the common everyday life activities, and a higher performance on the general activity subscale (sum of all the subscales of the activity domain, 3.52 ± 0.61, $t(193) = -3.07$, $p = 0.0024$, $-1.31 \leqslant \mathrm{CI} \leqslant -0.5$).

The entire group of participants, with one exception, reported a catastrophizing score less than 30, which is the cut-off value for clinical relevance [56–59]. The PCS score of 38 obtained for one patient is equivalent to the 90th percentile of the normative clinical distribution. The same participant showed an emotional state of depression at a moderate level (Depression, Anxiety, Stress Scales (DASS-21), Depression Subscale = 16) while two participants reported a state of mild anxiety (DASS-21, Anxiety Subscale = 8) [49]. The average physical functioning score of the 36-Item Short Form Health Survey (SF-36) was 77.5 ± 29.2 (mean $\pm$ SD), revealing no significant difference ($t(2479) = -0.79$, $p = 0.428$, $-25.07 \leqslant \mathrm{CI} \leqslant 11.29$) to normative healthy-participant data ($N = 2741$, 70.6 ± 27.4) [60–62]. The bodily pain score of the SF-36 showed a significant difference compared to normative data (53.75 ± 20.21, $t(2479) = 2.11$, $p = 0.0349$, $4.44 \leqslant \mathrm{CI} \leqslant 29.59$).

The PainDETECT screening questionnaire score revealed an unlikeliness of a neuropathic component of PLP in six of the participants since their score was lower than 12, being the cut-off point for neuropathic pain. No clear indications could be determined [45, 63] for the other four participants since their PainDETECT scores fell between 12 and 19. For these participants the screening results were ambiguous, a neuropathic pain component could be present.

Treatment effect and retention on PLP intensity

PLP intensity was obtained by averaging the scores from the two VAS scales (Visual Analog Scale anchored with two points: 0 = no pain − 10 = the worst pain ever felt) used to rate the intensity of the actual pain and the average pain over the last week. A significant reduction in pain intensity was observed from *T*1 to *T*2 (from 5.08 ± 1.79 to 4.02 ± 1.7, mean $\pm$ SD, $F(2, 8) = 19.36$, $p = 0.001\,14$, $0.49 \leqslant \mathrm{CI} \leqslant 1.62$, $\mathrm{ESr} = 0.94$, $\mathrm{SS(W)} = 12.29$, sum of squares within subjects) and *T*1 to *T*3 (3.55 ± 1.8, $p = 0.001\,01$, $0.7 \leqslant \mathrm{CI} \leqslant 2.3$, $\mathrm{ESr} = 0.89$), reaching the clinically significant reduction threshold of more than 30% (32.1%, with six participants showing a decrease of more than 30% in PLP intensity and five, an approximate decrease of two points on the VAS scale) at *T*3 [64]. No significant difference was found between *T*2 and *T*3 ($p = 0.317$, $-0.2 \leqslant \mathrm{CI} \leqslant 1.23$, $\mathrm{ESr} = 0.89$) (figure 2). The multimodal sensory-motor training was therefore clinically effective in determining a reduction of PLP intensity which lasted for at least six weeks.

As a secondary outcome, the affective and sensory characterization of pain was obtained using the pain perception scale ('Schmerzempfindungsskala', SES) [44]. SES revealed that the affective characterization of PLP reduced significantly after the training period, from 27.8 ± 9.8 (mean $\pm$ SD) at *T*1 to 21.9 ± 8.5 at *T*2 ($F(2, 8) = 6.01$, $p = 0.033$, $0.44 \leqslant \mathrm{CI} \leqslant 11.35$, $\mathrm{ESr} = 0.8$, $\mathrm{SS(W)} = 182.86$), but was not significantly different from *T*1 at *T*3 (23.6 ± 6.3, $p = 0.204$, $-1.7 \leqslant \mathrm{CI} \leqslant 9.9$, $\mathrm{ESr} = 0.78$). No statistically significant difference was found for the same subscale score between *T*2 and *T*3 ($p = 1$, $-9.4 \leqslant \mathrm{CI} \leqslant 5.8$, $\mathrm{ESr} = 0.41$), showing that the improvement in the affective characterization of PLP lasted just after the end of the treatment. No differences were reported for the sensory characterization of PLP, a second subscale score of the SES. Hence, the training reduced the significance of the pain (seen as less cruel, horrible or violent) but not its characteristics regarding sensory perception (temperature or rhythmicity). At *T*2, a questionnaire rating the patient's satisfaction with the received intervention showed a high treatment satisfaction ($94.1\% \pm 8\%$, mean $\pm$ SD). Table 4 visually displays the data reported above on the VAS to measure the PLP intensity and the SES results across the evaluation phases of the study.

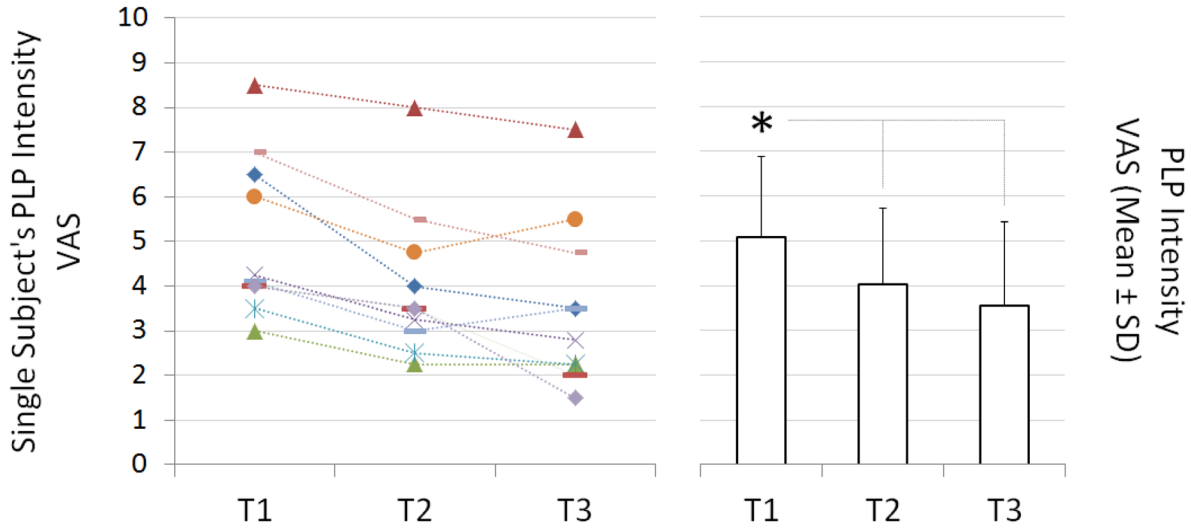

Figure 2. Pain intensity: treatment and retention according to the assessments at *T*1, *T*2, and *T*3. The graph on the left reports the individual evaluation of PLP intensity as the average of the actual pain and average pain over the last week. The graph on the right reports the mean and standard deviation of pain intensity scores for the entire group showing a statistically significant reduction ($p < 0.01$, reported with an asterisk) of PLP intensity between the evaluation executed before treatment (*T*1) to immediately after (*T*2) and six weeks after (*T*3) treatment.

Table 4. Treatment effect on PLP. Averaged VAS values (visual analog scale anchored with two points: 0 = no pain—10 = the worst pain ever felt) to rate the intensity of the PLP across the three evaluation phases (at *T*1, *T*2 and *T*3). SES Affective and Sensory characterization is reported for the same phases. The statistics is referred to the comparison between the values at *T*1 and *T*2, or *T*3.

Time-point	*T*1	*T*2	*T*3
Phase	Pre evaluation	Post evaluation	Follow-up evaluation
PLP intensity VAS (mean ± SD)	5.08 ± 1.79	4.02 ± 1.7[b]	3.55 ± 1.8[b]
SES affective characterization (mean ± SD)	27.8 ± 9.8	21.9 ± 8.5[a]	23.6 ± 6.3
SES sensory characterization (mean ± SD)	17.1 ± 5.1	16.0 ± 5.0	16.6 ± 5.3

SES = Schmerzempfindungsskala.
[a] $p < 0.05$.
[b] $p < 0.01$.

Treatment effect and retention according to the pain diary

Each participant was instructed to complete a daily pain diary where the PLP intensity was reported four times per day: in the early morning, at midday/lunchtime, in the afternoon, and at night/before sleeping. The participants were instructed to rate their PLP intensity using a VAS scale taking into account also the intensity and duration of pain attacks (if any). The completion of the pain diary commenced two weeks before treatment (*T*0) and ended at the follow-up (*T*3).

The PLP intensity was extracted from the pain diaries until six weeks post-training to assess the retention of the induced positive effects. We did not include *T*1 and *T*2 in the evaluation, since during these two days the participants underwent long and possibly fatiguing evaluations (QST and functional Magnetic Resonance Imaging) which could have affected their pain [65]. The PLP intensity reported by the participants for one week before entering the intervention were averaged and used as a reference. From *T*1 to *T*3 (excluding the day of *T*2) the data from all the participants were pooled in groups of two consecutive days and compared to the reference. The resulting two-day pooled PLP intensity data showed statistically significant differences ($p < 0.047$) compared with the reference value (black diamond, figure 3), at 5th and 8th comparison (last two days of treatment) until the 23rd comparison, with the exclusion of the 17th and 20th (see figure 3). Thus the effects of the treatment lasted for 30 days.

To analyse the daily variability of PLP intensity, the standard deviation of the four daily scores of each patient was used following the same pooling approach reported above for the average PLP intensity. Significant reductions of the daily variability of PLP just at the 9th, 10th and 12th comparisons ($p < 0.029$) and after the 20th with a sparse occurrence were found (see figure 3). Hence, an effect of reduction in daily PLP variability was obtained, which lasted until eight days after the end of the intervention, but with a less continuous characteristic than the mean PLP intensity reduction. The reduction in daily PLP variability demonstrates that the oscillatory characteristics of PLP were significantly reduced.

Muscular activity of the residual limb and correlation with PLP intensity

Across the 12 effective days of training (16 in total, considering the weekends when the participants received no training sessions) no differences were registered regarding the number

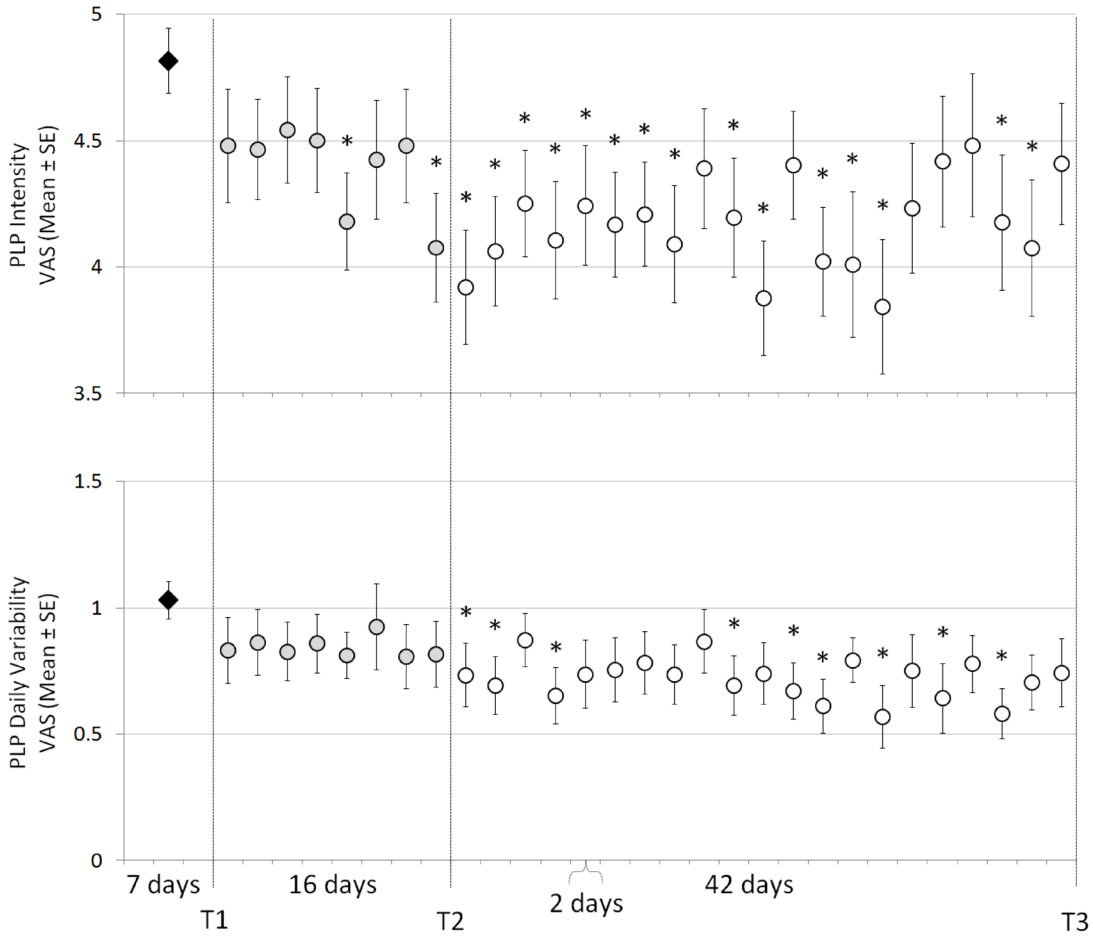

Figure 3. Treatment and retention according to the pain diary. The upper graph reports the average PLP intensity of the two-day pooled data from all participants for their daily PLP evaluation for seven days before starting the treatment (black diamond before *T*1), each two consecutive days during treatment (grey circles between *T*1 and *T*2), and each two consecutive days after treatment until follow-up (white circles between *T*2 and *T*3). The lower graph reports the average standard deviation of the pooled data for the daily PLP evaluation from all participants during the same time frames as reported above. Statistics were calculated comparing to the reference value (black diamond before *T*1) with each single following average (grey and white circles from *T*1 to *T*3), significant differences are marked by an asterisk ($p < 0.047$).

of trained phantom movements (hand and wrist movements). The participants were able to execute and train on 7.40 ± 2.57 (mean $\pm$ SD) types of movements across the first four days to end with 8.00 ± 1.98 different movements' types during the last four days of training. No significant differences ($p > 0.1$) were found across the three clustered groups of data (days 1–4 as the initial training period, 5–8 mid training and 9–12 last training period). On the contrary, the number of phantom movements' repetitions executed on average ($\pm$SD) across the three training periods showed a significant increase ($p < 0.001$) moving from 105.43 ± 45.22, during the initial training period (days 1–4) to the last training period (days 9–12) when the participants were able to repeat 176.97 ± 69.00 the prescribed phantom movements (148.77 ± 52.02 average repetitions during the mid training period, days 5–8). If averaged across each single day, the number of phantom movements' repetitions negatively correlate with the reduction in PLP intensity acquired with the pain diary, with Pearson's $r = -0.68$ ($p = 0.013$). The tracking error, as percentage of the EMG MVC value registered during the calibration, indicates the ability to control the effort of the residual limb muscles to move the phantom limb. The tracking error represents the distance between the actual EMG activity to execute the prescribed phantom movement to train (see figure 1(B), EMG Polar Plot) and the reference EMG polar plot to follow (figure 1(B), Reference Polar Plot). The tracking error showed a significant reduction from the initial compared to the last training period ($p < 0.001$, from 126.91 ± 77.90%MVC to 108.21 ± 69.58%MVC, respectively). The average $\pm$ SD of the tracking error during the mid training period was equal to 115.83 ± 80.97%MVC. Interestingly, the daily average of the tracking error values, across the 12 days of effective training, was positively correlated with the reduced PLP intensity with Pearson's $r = 0.65$ ($p = 0.022$). A significant reduction between the first and last training period was achieved for the single trial duration time (s), and therefore, a consistent increase in the speed of the executed phantom limb movements ($p < 0.001$, from 22.01 ± 4.38 s to 16.36 ± 8.14 s, respectively; 17.94 ± 7.29 s for the mid training period). A positive correlation was reached between the daily average of the single trial duration time and the PLP intensity, with Pearson's r = 0.68 ($p = 0.014$).

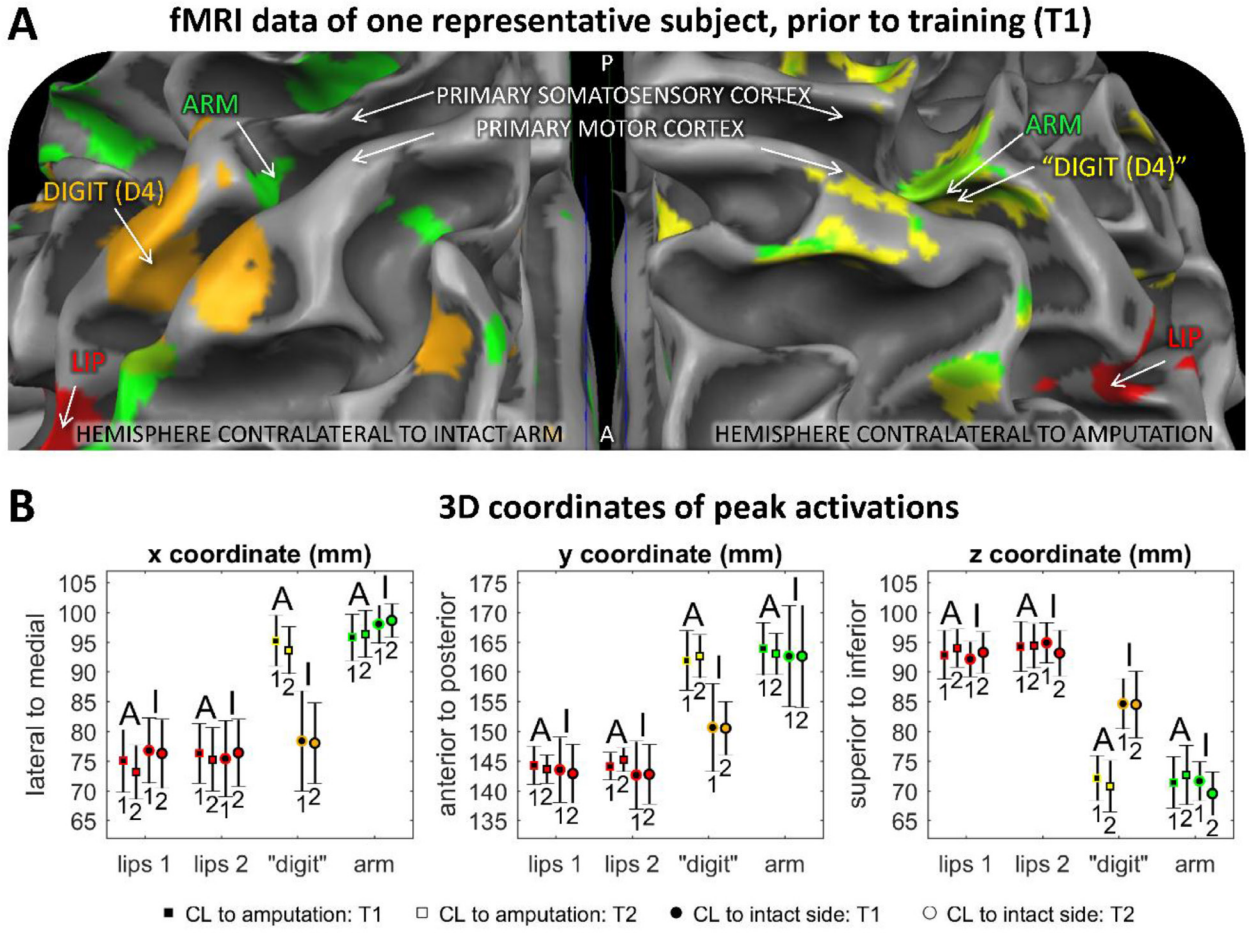

Figure 4. fMRI data. (A) fMRI data of one representative participant, prior to training, shown on the reconstructed white-to-grey-matter surface. The sensory-evoked activations of the lips (red), intact digit (here the ring finger, D4, orange), phantom 'digit' (perceived D4, yellow), and lower arm or stump (green) are marked. A = anterior, P = posterior. (B) 3D coordinates of fMRI activations across participants. For each axis, the mean and standard deviation of the fMRI representations contralateral (CL) to the amputated (squares, also marked with large (A) and intact (circles, also marked with large (I) side, obtained before (black-filled, also marked with 1 below) and after training (black-framed, also marked with 2 below), are given for the (twice-measured) lips 1 and lips 2 representations (red) as well as for the digit (orange) or phantom 'digit' (yellow) and arm (green) representation.

Placebo effect and expectancy

Expectation, hope, motivation, therapeutic relationships, conditioned responses and other psychological processes of the patient can contribute to a placebo effect [66]. Among them, expectancy is one of the most influential components. The role of expectancy, where the placebo effect generates from the anticipation that a treatment will end in a specific outcome, has been verified by a systematic review of 85 studies [67]. The credibility and expectancy questionnaire (CEQ) was administered in this study to report the level of bias regarding the efficacy of the treatment induced by expectation as a placebo effect [40]. The results obtained from the CEQ, divided in 'credibility' and 'expectancy' scores (49.6% ± 31.9%, 35.1% ± 19.4%, respectively, mean ± SD), showed no significant correlation with the reduction of PLP intensity (figure 2) between *T*1 and *T*2 (expressed as percentage of the value reported at *T*1), with Pearson's $r = 0.408$ ($p = 0.242$) for credibility and 0.266 ($p = 0.458$) for expectancy. These results indicate that the PLP-treatment effects are unlikely to be attributed to a placebo effect.

QST and two-point discrimination test

A complete series of QST were executed at *T*1 and *T*2 to report any training effects on the peripheral components of the aetiology of PLP (e.g. impaired sensory perception). Following the recommendations of Rolke *et al* [68], log-transformation with base 10 to achieve normal distribution was executed for the following QST variables: cold and warm detection thresholds (CDT, WDT), thermal sensory limen (TSL), mechanical detection threshold (MDT), mechanical pain threshold (MPT), vibration detection threshold (VDT) and pressure pain thresholds (PPT) (see QST supplementary material for further indications). The QST was performed at four locations: (i) at the stump trigger point for a phantom thumb/finger, (ii) at the corresponding thumb/finger of the intact side, (iii) on the lateral aspect of the shoulder on the amputated side, (iv) at the equivalent location on the intact side (see the corresponding Methods section for further details). Statistical comparisons were executed between *T*1 and *T*2 for the same location. For the Cold and Heat Pain Threshold (CPT and HPT, $N = 8$, two participants were removed from the analysis as they reached

the limit of the Thermal Analyser without reporting pain), a statistically significant difference was obtained between $T1$ and $T2$ for the stimulation at the stump trigger point (i), with a threshold change from 12.5 °C ± 10.2 °C to 16.2 °C ± 8.2 °C (mean ± SD, $p = 0.007\,39$, $1.34 \leqslant \mathrm{CI} \leqslant 6.03$, $\mathrm{ESr} = 0.97$) for CPT and from 44.8 °C ± 3.6 °C to 42 °C ± 3.9 °C ($p < 0.0001$, $1.91 \leqslant \mathrm{CI} \leqslant 3.52$, $\mathrm{ESr} = 0.97$) for HPT. A similar change was observed on the lateral aspect of the shoulder on the amputated side (iii) (CPT from 13.6 °C ± 9.3 °C to 19.1 °C ± 8.4 °C°, $p = 0.008\,51$, $1.91 \leqslant \mathrm{CI} \leqslant 9.12$, $\mathrm{ESr} = 0.88$, HPT from 45.3 °C ± 3.5 °C to 41.7 °C ± 2.9 °C, $p = 0.001\,18$, $2.25 \leqslant \mathrm{CI} \leqslant 4.83$, $\mathrm{ESr} = 0.89$).

For the two-point discrimination test a significant difference from $T1$ to $T2$ was found only at the stump trigger point (i), indicating an improvement in tactile discrimination ability at the stump level (from 45.3 ± 16.5 mm at $T1$ to 28.4 ± 19.4 mm at $T2$, $p = 0.012\,09$, $4.99 \leqslant \mathrm{CI} \leqslant 28.75$, $\mathrm{ESr} = 0.69$, $N = 8$, same participants used as to report CPT and HPT). No other statistically significant differences were observed.

fMRI data analysis

During fMRI, somatosensory stimulation was performed at a phantom digit and another stump location, at the respective finger and arm position on the intact side as well as at the lips. Significant cortical activation could be observed upon that stimulation for each of these skin locations and each participant (except for participant 7, who refused to undergo fMRI, and participant 4, who did not show any significant activation in the primary sensorimotor cortex for some stimulated spots, making a between-spots analysis impossible, and therefore was excluded) in the hemispheres ipsi- and contralateral to the amputation. As the average Euclidean distance between the peak lip activations of the two runs within a session was only 3.8 ± 1.2 mm (less than two voxels), rather reproducible statistical maps were obtained, indicating a high imaging quality. The activation pattern can be seen in a representative participant in figure 4(A) and in the (D) peak-activation coordinates given in figure 4(B). Prior to training, contralateral to the intact side the lips were represented most lateral, anterior, and inferior, followed by the digit and then the arm representation, as expected from the sensory homunculus by Penfield and Rasmussen [69–71]. While the lips-arm arrangement was the same contralateral to the amputation, the phantom 'digit', effectively being a stump area, was found more medial, posterior, and superior compared to the respective digit representation of the intact side, not-differentiable from the slightly more proximal arm area (not statistically tested). In contrast to our expectation from previous literature [27, 36, 72, 73], the lip representations did not vary in position between the intact and the amputated side pre training (p-values along all three coordinates >0.5). Hence, the participants did not seem to have undergone cortical plasticity, such that a training effect was very unlikely to be found.

Nevertheless looking for a training effect, the average lips 3D coordinates pre- and post-training were compared within both hemispheres. While no significant training-induced changes were found for either of the two hemispheres (figure 4), a trend with a large effect size ($p_{x\text{-axis}} = 0.06$) for a small lateral lips-representation shift (1.4 ± 1.7 mm on average) was observed along the x-axis contralateral to the amputation, in accordance with a reversal of maladaptive plasticity.

Discussion

The multimodal sensory-motor training employed in this study led to a significant PLP-intensity reduction which was greater than 30%. The treatment was well accepted by the participants since on average they rated it with ~95% satisfactory. These significant treatment effects have been obtained irrespective of the large variability in patient characteristics, regarding the level of amputation (e.g. transhumeral and transradial), stump pain, telescoping, and stump mapping. Mirror therapy is one of the most useful non-pharmacological approaches for PLP treatment [20]. In fact, a previous RCT study reported a reduction in PLP intensity from approximately 30/100 initially to 5/100 after four weeks of mirror therapy [20, 28], (relative PLP reduction of ~83.3%), with a further PLP reduction to approximately 3/100 after a further four weeks of treatment (8 weeks of treatment in total, total PLP reduction of ~90%). The administered therapy lasted 15 minutes per day suggesting that mirror therapy is an effective approach in terms of therapy time. Even if more effective, mirror therapy may be less effective when a telescoped phantom is perceived [30] and, contrary to the proposed approach, cannot be applied to treat PLP in bilateral amputees. Whereas, the proposed multimodal sensory-motor training tested in this study led to a significant PLP reduction both for the four participants with and six participants without a telescoped phantom. Because of the high incidence of telescoping (around one-third of amputees), the results of this study are promising for a broad clinical applicability of the approach. The training also reduced stump pain from 2.25 ± 1.7 to 0.87 ± 1.2 (mean ± SD) in the two participants who suffered from such pain.

The multimodal sensory-motor training adopted in this study represents a new approach for PLP treatment, addressing phantom motor execution enriched with sensory feedback of the moving phantom arm. Among the several strategies currently available for PLP treatment (34 commonly employed and 13 new approaches specifically addressing maladaptive plasticity [27]), which have limited clinical effectiveness [27, 64, 74], the proposed multimodal sensory-motor training represents the first effective treatment using multimodal sensory feedback (visual + tactile) of phantom motor execution. The presence of a multimodal sensory feedback is fundamental, as vision alone has only led to a significant increase of phantom awareness or control [74]. The inclusion of additional sensory feedback differentiates our approach from another recently proposed method [38] that provided a visual, EMG-controlled representation of the phantom limb movement by exploiting machine learning. In the treatment proposed in the current study, tactile stimulation patterns proportional to the stump muscles' level of activity provided a functional perceptual input aimed at improving the volitional

control over the motor output during phantom movements. The tactile stimulus during phantom motor execution could have had an effect on (i) providing a further sensory feedback substitution (more than just the visual input), (ii) potentially improving sensory discrimination on the stump as suggested by the results of the two-point discrimination test. However, even if approaches aimed at improving sensory discrimination at the stump have been shown to be effective methods for PLP reduction [35–37], the main aim of the tactile stimulus, provided during our study, was to provide an additional feedback to the volitional motor control of the phantom limb with no direct intention of improving sensory discrimination over the residual limb as the two objectives can have different working mechanisms.

The positive effects of the treatment lasted at least until the end of the study. The PLP intensity was significantly lower at $T2$ compared to prior to training ($T1$) and remained significantly lower six weeks after the end of the treatment (follow-up evaluation at $T3$). The more detailed assessment of PLP intensity and its daily fluctuations, obtained from the pain diary, showed that the reduction of PLP lasted for 30 days after the end of the treatment. This difference can be explained by taking into account the different time frames used to rate PLP. The PLP intensity accounts for actual pain and average pain over the previous week, whereas the evaluation executed using the pain diary data is based on a day-by-day multiple rating. The pain diary also allowed precise documentation of daily PLP fluctuations, which could indirectly estimate the troublesomeness of the daily pain attacks [75]. A significant simultaneous reduction of both the PLP intensity and the daily PLP fluctuations and pain attacks has the potential to lead to an improved quality of life for the participants [76]. The results reported in figure 3 show a linear decrease in the average PLP intensity with the progression of the treatment (data between $T1$ and $T2$, Spearman's rho $= -0.669$, $p = 0.0485$), being significant at the last two days of the treatment. It would be expected that placebo-biased participants would have shown a more rapid pain reduction during the initial days of treatment [77]. Moreover, the results of the CEQ, rating the expectation and credibility biasing effects on the treatment to be received [40, 78, 79], makes it unlikely that the effects obtained on PLP were determined only by a placebo effect. While the proposed method successfully reduced PLP, no significant cortical shifts were observed between $T1$ and $T2$, expect for a trend for a small lateral lips-representation shift. However, as no shift in the lips representations was found between the intact and affected hemisphere in the included amputees even prior to training (at $T1$), in contrast to several earlier studies [27, 36, 72, 73], no significant maladaptive plasticity was observed in the present study anyway, making a change from $T1$ to $T2$ rather unlikely.

As described in the methods section, the lip was stimulated via air puffs, while a brush was used for stimulation of the (phantom) digit and stump. The reason for this heterogeneous stimulation was the following: while manual brush stimulation proved to be difficult to control and uncomfortable at the lips, pilot studies revealed a too weak activation at the stump upon air-puff stimulation, making the use of a brush necessary at that site. Nevertheless, we are confident that this heterogeneity has not prevented us from observing cortical plasticity, as the comparison between locations stimulated with different methods was not clearly necessary to test for plasticity: If significant plasticity had been present, the representation location within a body part, in particular the lips, which was consistently stimulated with the same method, should have changed from pre- to post-training; that was not the case. Also, the cortical distance between the phantom digit and the stimulated stump location was not altered from pre to post training, although both locations were stimulated with the same method.

The reported approach to effectively decrease PLP intensity, and daily fluctuations is simple to use and can be applied in people with PLP with different clinical characteristics. It has been developed to specifically provide substituting multimodal sensory stimulation (tactile and visual) to feedback volitional motor control of the phantom limb. As the sensory stimuli are correlated with the residual limb muscular activity, which is meant to control phantom movements, they provide a rather physiological way for improving the modulation and motor control of the residual limb muscles during phantom movement execution. The technology used can be set-up quickly and configured in a completely portable way. All the devices were wireless except for a cable running from the vibrating band to its battery-powered controller. Therefore, using a small form factor PC and video glasses, the entire experimental set-up can be fully portable. The patient software interface was simple and intuitive, and no participant reported difficulty in understanding and executing the prescribed exercises. This approach is also individualized as the operator interface contains several adjustable parameters which can be easily tuned to match the participant's ability, resulting in a challenging but not frustrating training experience. The entire hardware and software set-up was approved by the local ethics committee to be used in a clinical setting as well as at the patient's home with indirect telephone supervision of an operator. Therefore, this new treatment approach to reduce PLP can be exploited in a clinical setting, as described in the current study, or used at home for chronic pain management.

Training the volitional motor output of the residual limb muscles

The multimodal sensory-motor training was effective in reducing PLP intensity while improving the motor capacity of the trained muscles. The approach tested in this study addressed the remnant muscles of the residual limb activated during the execution of different phantom movements (indifferently from the ability to feel or move the phantom) as the participants trained using a visual and tactile feedback of the muscular effort to execute these phantom movements. The motor control ability over the muscles of the residual limb improved across the 12 effective training days as the participants were able to significantly improve the amount of repetitions of the trained phantom limb movements of 167.85% on average from the first to the last four days of training. On the other hand the trained phantom limb movements, across the

daily sessions, were continuously increasing in speed as their time duration reduced of a 25.67% from the first to the last four training days. Interestingly, considering the amount of accomplished movements and their duration in time, a quick calculation of the overall trained time per session can be extracted to show the participants were able to train ~10 min more (from 38.67 to 48.26 min per hour) during the final training period compared to the first four days of training as a consequent possible reduced fatigability in moving their phantom. Significantly improved muscular effort and motor output control over the residual limb muscles controlling a phantom movement can be drawn from the data on the tracking error which reduced of 14.73% on average from the first to the last four training days.

Effects on participants showing a blocked or telescoped phantom and with no referred fingers sensation

A further analysis was conducted to study the effect of the training approach for the single participant who showed no finger sensation during the initial assessment (subject 4, table S1). The average PLP intensity, acquired for the four days before starting the training ($T1$), and used as a reference value for PLP intensity was compared to the average of the four days before ending the training ($T2$). A significant reduction ($p < 0.001$) was reached as the initial PLP intensity of 4.9 ± 1.2 (mean $\pm$ SD) decreased to 3.3 ± 0.4. Interestingly, the multimodal sensory-motor training significantly reduced the PLP intensity in this amputee who referred no finger sensations even if part of his training was based on executing finger movements and an intact 3D hand model was followed as reference regarding the type and timing of the movement to train (making unlikely the embodiment of the 3D hand model [80, 81]). Possibly, in this case, the success in reducing PLP depends on training the modulation of phantom limb motor execution and effort benefitting from visual and tactile feedback originating from the motor activity meant to control the phantom limb [22]. More surprisingly, similar results were obtained for subjects 5, 6 and 9 (table S1) who showed no movement abilities for the phantom limb ($p < 0.019$, from 3.1 ± 0.8 to 2.3 ± 0.4 in subject 5; from 5.6 ± 0.5 to 4.9 ± 0.4 in subject 6; from 5.6 ± 2 and 3.9 ± 1.5 in subject 9). Multimodal sensory-motor training was effective in participants with an inability to move their phantom but who were still able to modulate the motor output of the residual limb muscles. The approach tested in this study used the actual residual limb muscular effort as feedback signal and the MVC EMG as reference polar plot (figure 1(B)). Even in the absence of an activity that involved the residual limb muscles, as it possibly happens for amputees with a frozen phantom [26], the tested training led to improved motor control over these muscles, as reported for the significant reduction in the tracking error and a positively correlated reduction in PLP intensity.

Study limitations

Besides the significant positive results achieved by the approach proposed in this study, some limitations and drawbacks should be taken into consideration. The small number of trained participants and the lack of a control group are limitations, both due to the high effort per subject (two training-hours per day) and in particular the availability of amputees with PLP who choose to take part in a three-week out-of-home study. On the other hand the entire treatment set-up is based on easily controllable, low-cost commercially available devices (PC and low-cost EMG-wrist band). The eight electromagnetic micro-vibrators used for the tactile stimulation, which represent a more expensive piece of equipment, can be easily replaced with cheap micro electrical motors rotating an eccentric mass on the shaft (the same devices which provide vibrating function in mobile phones). Therefore, in the future patients could train at home at the frequency that they wish.

Conclusion

Using a multimodal sensory-motor training on phantom-limb movements with visual and tactile feedback, we report a significant reduction in PLP intensity, which lasted for at least 30 days after the end of the treatment. Although preliminary, this is the first study applying multimodal sensory feedback approach (visual and tactile) to train the motor output of phantom movements leading to an average PLP intensity reduction of 32.1%.

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Conflict of interest disclosure

The authors have declared that no competing financial interests exist.

Author contributions

AMD, Conception and design, Acquisition of data, Analysis and interpretation of data, Drafting and revising the manuscript; MAS, Design, Acquisition of data, Analysis and interpretation of data, Revising the manuscript; NG, Development of training tool, Acquisition of data, Analysis of data; DF, Conception and design, Revising the manuscript; JH, Development of training tool, Acquisition of data, Revising the manuscript; KG, Acquisition of data, Analysis and interpretation of data; FP, Conception and design, Revising the manuscript; MS, Acquisition of data, Analysis of data; PD, Design, Revising the manuscript; TW, Interpretation of data,

Revising the manuscript; HF, Interpretation of data, Revising the manuscript; BG, Design, Interpretation of data, Revising the manuscript; OCA, Interpretation of data, Revising the manuscript; DF, Conception and design, Interpretation of data, Revising the manuscript. All authors have read and given permission to the final draft of the manuscript.

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