

Six-months assessment of a hand prosthesis with intraneuronal tactile feedback

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Abstract

Objective: Hand amputation is a highly disabling event, which significantly affects quality of life. An effective hand replacement can be achieved if the user, in addition to motor functions, is provided with the sensations that are naturally perceived while grasping and moving. Intraneuronal peripheral electrodes have shown promising results toward the restoration of the sense of touch. However, the long-term usability and clinical relevance of intraneuronal sensory feedback have not yet been clearly demonstrated.

Methods: To this aim, we performed a six months clinical study with three trans-radial amputees who received implants of transverse intrafascicular multichannel electrodes (TIMEs) in their median and ulnar nerves. After calibration, electrical stimulation was delivered through the TIMEs connected to artificial sensors in the digits of a prosthesis to generate sensory feedback, which was then used by the subjects while performing different grasping tasks.

Results: All the subjects, notwithstanding their important clinical differences, reported stimulation-induced sensations from the phantom hand for the whole duration of the trial. They also successfully integrated the sensory feedback into their motor control strategies while performing experimental tests simulating tasks of real life (with and without the support of vision). Finally, they reported a decrement of their phantom limb pain and a general improvement in mood state.

Interpretation: The promising results achieved with all subjects show the feasibility of the use of intraneuronal stimulation in clinical settings.

Introduction

The loss of a hand affects persons's quality of life¹. From the first manufactured prostheses², more effective clinical solutions have been provided: commercially available hands have been developed³, enabling users to control the movement of up to six degrees of freedom. In parallel, a huge effort has been conducted in research to provide more dexterous artificial devices³, along with decoding methods allowing their control^{4,5}; and, as requested by the final users^{6,7}, to re-establish the sensory flow of information between the hand prosthesis and the central nervous system, through non-invasive and invasive approaches⁸⁻²¹.

Efforts have been dedicated to the use of implantable peripheral interfaces to elicit sensations¹⁴⁻²⁰. After the pioneering studies with LIFE¹⁴, three interfaces have been used with human subjects: epineural (cuff¹⁵⁻¹⁷ and FINE^{15,16}), penetrating (Utah array^{18,19}), and intraneuronal (TIME²⁰ and LIFE²¹) electrodes.

Tyler's group showed that FINEs can be implanted for more than 48 months^{15,16} in two upper-limb amputees allowing them to pull the stem from a cherry¹⁵. Notwithstanding preliminary encouraging results²², there is still no evidence that FINE-induced stimulation can be used to modulate some levels of the prosthesis grasping force, priority for amputees, and one of the reasons such devices are abandoned^{6,7,23}. Moreover, the quality of the elicited sensations was little differentiated (mostly paraesthesia was reported¹⁵).

Utah arrays^{18,19} have shown to evoke percepts of more varied quality (tingle, vibration, pressure) in upper-limb amputees but with poor reproducibility in time. In fact, about 80% of the sensations evoked in two subjects, changed location or type over a period of five weeks.

Finally, TIMEs²⁴ were transversally implanted into the residual nerves of an amputee for a period limited to 30 days by EU medical device regulations. During this experiment, TIMEs have been used to restore several features of the sense of touch including sophisticated ones such as the ability to control grasping force, to discriminate different textures, along with objects compliance and shape^{20,25}.

To date, TIMEs may represent an interesting trade-off between the other two solutions, reducing their specific limitations in terms of sensations quality (FINEs) and stability (Utah arrays), and allowing the restoration of features such as prosthesis grasping force control. However, the sole study investigating it in humans, was conducted on a single subject for only a month and with no detailed characterization of all the evoked sensations. Moreover, it is not clear²⁶ yet to what extent sensory feedback elicited by all these interfaces (not only TIMEs) can improve motor control performance in simulated ecological conditions²⁷ (e.g., handling fragile objects when users can exploit both visual and tactile feedback). Finally, even if anecdotally reported that direct nerve stimulation induces phantom limb pain relief^{15,21}, a more systematic characterization of the phenomenon has never been conducted.

In order to address these three issues, we conducted a six-months clinical study with three trans-radial amputees implanted with four TIMEs in the residual median and ulnar nerves. We intentionally recruited subjects with different clinical conditions (long or short time since the amputation, affected or not by phantom pain) to investigate the exploitability of our approach for a variegated population of trans-radial amputees.

After characterizing the subjects' response to the intraneuronal stimulation, we investigated the effects of sensory feedback on the performance of the subjects during different reaching and grasping tasks accomplished with the support of vision (in ecological conditions), and without it. Finally, we assessed the effect of intraneuronal stimulation on phantom limb pain as well as on mood states during the period of study.

Materials and Methods

Patient recruitment

Three left trans-radial amputees were included in the study (Table 1). The first subject (FNG) was a 37-year-old male, left-handed, who had a traumatic trans-radial amputation of the distal two-thirds of the

left forearm 2 years before the enrollment in the trial. At the time of the trial he was affected by strong phantom limb pain and very frequent non-painful phantom sensations. In addition to the amputation and the consequent phantom limb syndrome the clinical condition of the patient was unremarkable. The second subject (ALM) was a 48-year-old female, right-handed, with a traumatic transradial amputation of the distal third of the left forearm, occurred 23 years before the study. She was free of phantom pain and experienced very mild and infrequent spontaneous non-painful phantom sensations.

The third subject (LRP) was a right-handed, 53-year-old female trans-radial (distal two-thirds of the left forearm) amputee. The amputation occurred in December 2015, following a traumatic accident at work. She was affected by phantom pain.

Ethical approval was obtained from the Institutional Ethics Committees of Policlinic A. Gemelli-IRCCS at the Catholic University, Rome, Italy, where the surgery was performed. The protocol was also approved by the Italian Ministry of Health, division for experimental devices. All the subjects signed the informed consent. During the entire duration of our study, all experiments were conducted in accordance with EU guidelines and regulations.

Surgical procedures

Four TIMEs (14 active sites each) were implanted in the median and ulnar nerves of FNG on November 14th 2015, of ALM on June 24th 2016, and of LRP on June 24th 2017 (Fig. 1A and Supp. Video 1). The explant of the electrodes was executed on June 11th 2016, on December 17th 2016, and on December 16th 2017, for FNG, ALM, and LRP respectively.

The surgical approach used to implant TIMEs is extensively reported elsewhere²⁰. Briefly, during general anesthesia, through a 15 cm-long skin incision on the left arm, the median and ulnar nerves were exposed to implant a proximal and a distal TIME in each nerve (Fig. 1A). The microelectrodes and a segment of their cables were drawn through four small skin incisions into proximity with the exposed nerve, two laterally and two medially to the center of the main surgical cut. The cable segments were located in subcutaneous pockets, externalized (and secured with sutures and subcutaneous strain release loops) in order to be available for the transcutaneous connection with a neural stimulator. Then, using an operating microscope (Zeiss, Pentero), the single microelectrode was implanted transversally within the nerve fascicles. Appropriate microsutures secured the nerve implant. This implantation procedure lasted 11 hours for FND, 8 hours for ALM, and 11 hours for LRP. The impedance of the electrodes active sites was monitored intraoperatively, to verify the integrity and functionality of the implant. After 180 days, the microelectrodes were removed under an operating microscope in accordance with the protocol and the obtained permissions.

A clinical and electrophysiological follow up was executed at four months for ALM, ten months for FNG, and three months for LRP.

Perceived sensations characterization

Regarding the subjects' response to the intraneuronal stimulation (sensation characterization or mapping procedure), the prosthesis was not worn, and the patient was connected only to the neurostimulator using the transcutaneous cables of the implanted TIMEs. The stimulation was triggered by the experimenter using a custom-made software (Matlab, R2016b, The Mathworks, US). The patient was blind to the stimulation configuration delivered. We injected trains of biphasic charge-balanced cathodic-first pulses of increasing amplitude from 10 µA to 980 µA (steps of 10 µA), and fixed pulse width (chosen in the range 10 µs to 120 µs depending on the active site) and frequency (50 Hz as in our previous study²⁰). Pulse trains of 2 seconds duration were delivered with an interval between trains of 2 seconds. The same set of stimulation parameters was delivered three times per active site. Hence, for example, per each active site the sensory threshold was determined as average of the three minimum values of charge (one per repetition, i.e. 2-s train of current pulses) at which the subject reported a percept.

In each of these trains of increasing amplitude, patients had to press a button to stop the stimulation ramp when and whether they perceived a sensation. Then, they had to report using a custom-made

graphical user interface (Matlab, R2016b, The Mathworks, US) the sensation properties (location, type, quality and intensity). The patients could select a word from a list (similar to the one prepared by Kim and colleagues²⁸) to describe the evoked sensation, but they could also add a new word if needed.

At the end of this procedure, a map of the sensations type, location, extent and intensity (including the sensory threshold) referred to the correspondent active sites was obtained and used for the calibration of the sensory feedback restoration system.

The sensation characterization was performed once per week or every two weeks, according to the availability of the subjects (if they were spending the whole week at the clinical site for performing the tests or only part of it). In particular, FNG and ALM did it weekly, while LRP every two weeks.

Finally, we assessed the stability of the elicited sensation on the phantom hand over time as change of location. In particular, we considered nine location clusters on the phantom hand (five fingers and four palm areas). A sensation was considered stable when, from one week to the other, an elicited sensation was perceived in the same cluster. We did not consider stump percepts in this analysis, because their stability was not of interest for the study. Conversely, we studied the occurrence of stump sensations over time.

Electrode characterization

For the characterization of the electrode-nerve impedance and the subjects' response to intraneuronal stimulation we delivered trains of cathodic-first, biphasic and symmetric square-shaped, current-controlled stimulation pulses of variable intensity, duration, and frequency, through a dedicated software controlling an external electrical neural stimulator. The impedance was computed as the ratio between the potential difference between the selected active site and the reference electrode (both, left and right one of the electrode) at the end of the cathodic pulse phase. The potential resulted from the average of 5 pulses with an amplitude of 20 μ A and a width of 300 μ s, repeated at a frequency of 1 Hz. Electrodes were considered still working if their impedance was below 100 kOhm.

Prosthesis movement control

The subjects controlled a research hand prosthesis (IH2 Azzurra, Prensilia) using the residual muscle activity of their forearm residuum. In total two pairs of disposable surface electrodes (Ag/AgCl, 24mm diameter, 20 mm inter-electrode distance) were placed on the digit flexor and the extensor extrinsic muscles. The signals were sampled at 2 kHz and digitally filtered using a 4th order pass-band (15-375 Hz) Butterworth IIR filter, and a notch filter to remove the 50 Hz power line interference (through Grapevine system, Ripple, LLC, USA). A robust three-state (open=-1, close=1, rest=0) k-Nearest Neighbor (kNN, k=3) classifier with a decision-based velocity ramp²⁹ was used to control the hand. The classifier was fed with the waveform length (WL) of each EMG channel, computed over a window of 100 ms, and allowed a gated-ramp control³⁰ of the speed of the robotic hand movements, as in the following relation:

$$V_{\text{hand}} \leftarrow V_{\text{hand}} + A * \text{Class}_{\text{output}}; \quad \text{when } \text{Class}_{\text{output}} = -1, 1; \quad (1)$$

$$V_{\text{hand}} \leftarrow 0; \quad \text{when } \text{Class}_{\text{output}} = 0; \quad (2)$$

where A is a proportional factor that can be controlled by the experimenter, $\text{Class}_{\text{output}}$ is the output of the classifier, and V_{hand} is a number in the range between 0 and 511, that controls the velocity of the motors of the robotic hand. When $V_{\text{hand}} = 1$ the angular velocity of the robotic hand is 0.297 deg/sec, when $V_{\text{hand}} = 511$ the angular velocity of the robotic hand is $511 * 0.297$ deg/sec = 151.76 deg/sec.

The classifier required a training session every time the surface EMG electrodes were newly placed. During the training the user was asked to relax (one time for 2s), then open (one time for 2s) and finally close (one time for 2s) the phantom hand. The resulting EMG activity was recorded and manually separated into three classes basing on the WL.

Prosthesis Sensory Feedback

Two force sensors embedded in the little and index fingers of the prosthesis were used as control

inputs for the intraneuronal stimulation of two active sites in the sensory innervation territories of the median and ulnar nerves. In particular, the amplitude of biphasic, symmetric, cathodic-first and rectangular charge-balanced pulses was modulated according to the following linear relationship:

$$c = (C_{max} - C_{min}) * (s - s_{15}) / (s_{75} - s_{15}) + C_{min}, \quad (3)$$

$$c = 0, \quad \text{when } s < s_{15}; \quad (4)$$

$$c = C_{max}, \quad \text{when } s > s_{75}; \quad (5)$$

where:

c is the amplitude of stimulation current, s is the sensor readout, s_{15} and s_{75} represent 15% and 75%, respectively, of the maximum range of the sensor readout, which characterize, respectively, the contact threshold of the robotic hand and a value tuned to exploit the full range of sensations for all objects. C_{min} and C_{max} are the stimulation current amplitudes that elicited, respectively, the minimum and maximum (i.e., below pain threshold) touch sensations as reported by the subject according to the last sensation characterization procedure. The frequency of the stimulation is 50 Hz²⁰. The overall control scheme is provided in Fig. 1A.

We used two active sites for the development of the bidirectional prosthesis, one in the sensory innervation territories of the median nerve and one of the ulnar nerve, eliciting sensations respectively in an area of the phantom hand that was the closest to the prosthesis index finger and in an area closest to the little finger. This was chosen to maximize the somatotopy of the phantom sensations during prosthesis exploitation.

Neurostimulators

Three neural stimulators were used to inject currents into the nerves of the two subjects by means of TIME electrodes: STIMEP, EARNEST and Grapevine Neural Interface System.

STIMEP (University of Montpellier and Axonic) is a wearable neural stimulator based on a generic neural stimulation architecture^{31,32}. It allows the control of 64 poles of implanted electrodes, divided in 4 ports of 16 poles each. Therefore, up to 4 TIME electrodes can be driven in real-time (pulse-width, intensity, frequency). Each port addressing each TIME electrode includes 14 capacitively coupled active outputs (channels) and 2 non-capacitively coupled references. STIMEP further embeds safety procedures (charge limit) and impedance measurements. This stimulator was used for all the tests of FNG.

EARNEST (University of Cagliari) is a prototypical, wearable, complete embedded platform for neural prosthetic applications³³. It allows to simultaneously control and use up to 4 implantable multi-site (16 channels) electrodes, placed on the same subject, for a total of 64 stimulation/recording channels. The device allows recording of neural signals and, at the same time, provides electrical stimulation fully programmable in terms of amplitude, duration, shape and frequency. It embeds also a recording module for electromyographic (EMG) signals from 4 differential surface electrodes. This stimulator was used on ALM for Sensation characterization, Force Control Test and Blindfolded Sensory Blocks Test. The Grapevine Neural Interface System (Ripple, LLC) is a commercial device that can be used for the recording of neurophysiological data and for delivering current-controlled stimulation through up to 32 high-impedance microelectrodes. This system was used on ALM for the Sensation characterization, Force Control Test, Virtual Eggs Test, and for the whole trial with LRP.

Validation Tasks

In order to verify whether sensory feedback could allow subjects to improve performance in the prosthesis movement control, three functional tasks were used: Virtual Eggs Test (VET³⁴), Blindfolded Sensory Blocks Test (BSBT¹²), Force Control Test (FCT²⁰).

First of all, in order to assess whether intraneuronal stimulation in addition to vision could improve motor control we asked the subjects to perform a test resembling the realistic task of transferring fragile objects (such as eggs), namely the Virtual Eggs Test (VET). The VET is a recently proposed test for sensorimotor assessment³⁴. In brief, it is a modification of the well-known box and blocks test³⁵ except

that fragile/breakable blocks (resembling virtual eggs) are used instead of the standard wooden ones. During the VET, the subjects, wearing the prosthesis, are instructed to transfer the fragile blocks presented in front of them from one side to the other over a 15 cm tall wall as fast as possible and without breaking them. The performance is measured as a percentage, by the number of transferred blocks over the total number of blocks (transferred plus broken) during 1-minute trials, similar to the standard box and blocks test. In this work, the virtual eggs (40x40x40 mm³ blocks, ~80 g) exploited a magnetic fuse mechanism, which would collapse/break when grasped with a grip force larger than a specified threshold. The latter was calibrated at a force value that was roughly 50% larger than the grip force required by the artificial hand to lift it (1.23±0,02 N). This value was validated during the tests with FNG (four trials feedback-on and four trials feedback-off). The test is schematized in Fig. 2A and shown in Supp. Video 2. The VET was performed by FNG at month 4 after the surgery, by ALM at months 2 and 3, and LRP at months 2 and 3.

Once we assessed the benefit of intraneuronal sensory feedback in addition to vision during tasks requiring sensibility, we further tested the efficacy of our approach during two additional experiments, in which the volunteers were blindfolded and acoustically isolated: the Blindfold Sensory Blocks Test and the Force Control Test.

In the BSBT subjects, wearing the prosthesis, transferred wooden and unbreakable blocks, during 240 s sessions, from one side of a table to the other (Fig. 2D and Supp. Video 2). In particular, an operator presented or not objects to the prosthesis, and the subjects had to decide whether he/she had successfully grasped them, and then had to transfer them to the other side of the table. If the participant felt that no object was gripped, he/she was instructed to re-open the hand for the next repetition. The performance was rated through a score: one point was attributed for every successfully transferred block or when the subjects correctly identified that there was not an object in their robotic hand; in any other condition, no points were assigned, and errors were counted. Since the same score was given to the recognition of a grasped object and of a missing object, the number of transfers (hence, the overall score) depended on the time the subjects spent to take a decision about a grasp. The number of object presentation events was balanced with the case in which no object was placed in the prosthesis. This design was put in place to avoid performance discrepancies between trials due to objects seeking. The BSBT was performed by FNG at months 2,3 after the surgery, by ALM at month 2, and LRP at months 2.

In the FCT (Fig. 3A), as in Raspopovic et al.²⁰, the subjects acoustically shielded and blindfolded were asked to apply 3 levels of grasping force (low, medium and high) on a dynamometer, using (but not wearing) the robotic hand. The prosthesis and the dynamometer were constrained by two presses at a constant position, to guarantee the repeatability of the grasps. The participants were instructed to rely on the sensory information to judge how much force they were applying and to stop when they considered they had reached the desired level (low, medium or high). They had to decide the amount of force required to apply its minimum, medium and maximum level. The patients performed a short familiarization session (approx. 5 minutes), during which they could squeeze the dynamometer with the bidirectional prosthesis exploring the sensory feedback. Then, the experiment was performed. The experimenter asked the requested force level touching the patients' shoulder one (low), two (medium) or three (high) times. Patients reproduced the desired force level, maintaining the level for 2s, and then they returned to the initial hand position (fully open hand) ready for the next level. The velocity of the hand movement execution was randomly modified (3 velocities, by setting A=1, A=2, A=3 in (1)) without informing the participants, in order to prevent that they could rely on learned closing times to execute the task. The FCT was performed by FNG at months 2 and 5 after the surgery, by ALM at months 2 and 5, and LRP at months 2 and 5.

Phantom limb pain and mood states assessment

FNG and LRP were asked to fill the Neuropathic Pain Symptom Inventory (NPSI³⁶), the Visual analogue scale (VAS^{37,38}), and the Profile of Mood States³⁹ questionnaires, during 6 weeks before the implant, and each day they received the intraneuronal stimulation (i.e. every week). ALM, not having any phantom limb syndrome did not fill the questionnaires.

Statistical analysis

All data were extracted and processed in Matlab (R2016b, The Mathworks, US). The normality of the data was first checked (one-sample Kolmogorov-Smirnov test). Since none of the data was normally distributed, for the analyses in the paper a two-tailed Friedman's test was used to analyze data and, when needed, a Tukey-Kramer correction for multi-group comparison was applied. Only for performance comparisons over time, i.e. first and last session of VET (Fig. 2), BSBT (Fig. 2), and FCT (Fig. 3), a Fisher's exact test was computed. For the comparison of different levels of intensity of sensations, a Kruskal-Wallis test was executed.

Additionally, for the analysis of the force levels (Fig. 3), for each trial, the duration was normalized, and an average force value was computed over a fixed interval (interval from 60% to 90% of trial completion). To compute the “performance” score (given as a percentage of correct trials), we first obtained the average force value for each force level, using the method outlined above. Then, we assigned each repetition to the nearest force level. Finally, we computed the performance score as the percentage of repetitions correctly assigned to the right force level.

The number of repetitions per sessions and the number of sessions per test are reported in the figures captions.

Results

Surgical results

No side effect related to the surgical procedures was observed. Complications related to the median and ulnar nerves secondary to surgical injury, infection, bleeding or relocation of the electrodes were not observed in FNG and LRP during the trial. ALM had an infection from three of the implants (*Staphylococcus Aureus*, confirmed by wound swab culture) two weeks after the surgery. During this period one electrode fell out (after seven weeks), while the patient was sleeping. The infection was treated and eradicated with six weeks of combined antibiotic therapy. Nevertheless, also in the case of ALM there were no complications related to the median and ulnar nerve injury at the end of the trial. The follow up excluded long-term complications or loss of function with respect to pre-surgery conditions. The results presented in the rest of the manuscript take into consideration three electrodes for FNG and ALM, and four for LRP. Indeed, as mentioned, ALM lost an electrode after 7 weeks, while for FNG the external connector of one implant completely broke at week 20.

Perceived sensation

Throughout the whole trial, all the subjects reported sensations elicited by intraneuronal stimulation on the phantom hand (Fig. 1B). In terms of location, FNG reported sensations in all his phantom digits, while subject ALM felt phantom sensations more on the palm and LRP more on the ulnar territories of innervation of the hand (Fig. 1B). Interestingly, at the beginning of the trial ALM and LRP reported sensations mainly localized at the residuum, while over time most of these perceptions progressively moved to the phantom hand (Fig. 1C).

Along with the trial, the perceptions became very stable: almost 100% of the active sites for ALM, FNG, and LRP elicited a sensation that maintained the exact same phantom hand location (Fig 1D). ALM and LRP reached very soon this level of stability, while FNG, which reached about 80% stability at the 4th month, needed all the study period.

A wide range of sensation types (11 different ones) was elicited by the intraneuronal stimulation (Fig. 1E): as a note, FNG and LRP experienced a feeling of electricity in the phantom hand less than about 10% of the times, while in ALM such percept was reported in three cases out of four.

Analogously to the location of the elicited sensations, also the minimum charge (threshold) required to elicit a percept reached a plateau at around 20 nC for FNG, 40 nC for ALM, and 60 nC for LRP (Fig. 1F,G,H), respectively. It is interesting to notice that while for the first two subjects, the plateau was reached after a period of adaptation (4 and 7 weeks, for FNG and ALM, respectively, p<0.05, Fig. 1F,G),

for LRP the threshold recorded the first week of experiments, was the same until the explant (Fig. 1H). All these thresholds were far below the tolerated maximum safe charge injection capacity of the TIMEs, determined to be 120 nC²⁴.

Finally, all the subjects reported a direct proportional correlation between the charge of the current injected in the electrodes active sites and the intensity of the evoked sensations (Fig. 4). Conversely, a raise of the injected charge, even if producing a very limited increase of elicited sensations extension, did not affect their location cluster (intended as defined before).

Electrode characterization

The impedance of the active sites was stable for FNG and LRP at 80 kΩ and 70 kΩ, respectively, since the beginning of the study, while for ALM changed after the implant in the residual nerves, to stabilize after 3 weeks around a plateau of 40 kΩ ($p>0.05$, Fig. 5A,B,C, top). Equally important, we noticed a reduction of around 45% of usable channels (impedance higher than 100 kΩ) from the three studied electrodes towards the end of the study for FNG and ALM (Fig. 5A,B, bottom). Further investigation, though, of the explanted electrodes allowed us to relate this event exclusively to a loss of electrical continuity between the extracorporeal connectors and the transcutaneous cables (Fig. 5D) due to repeated mechanical stress during connection and disconnection of the TIMEs from the stimulator. Indeed, neither the active sites nor the two segmented counter electrodes per TIME showed signs of corrosion due to electrical stimulation or delamination from the substrate and the insulation (Fig. 5E). No alterations of the assembling structures of the ceramic carrier with the thin-film structure, the precision mechanics cables or the cables themselves were observed during optical inspection (Fig. 5E). Wire breakage at the connector site was actually the only failure mode that occurred.

In order to avoid this breakdown, the portion of the electrodes cables of LRP that was solicited because of the continuous connection and disconnection with the external stimulator, was reinforced with an additional layer of silicon. Thanks to this expedient, the four electrodes of LRP had about 90% of active sites functioning until the end of the study (Fig. 5C, bottom).

Validation tasks

In the VET, all the subjects transferred a number of unbroken virtual eggs significantly larger when the intraneuronal feedback was provided with respect to the no-feedback condition (57% vs. 72% for FNG, 18% vs. 28% for ALM, 45% vs 70% for LRP, $p<0.05$) (Fig. 2B). Interestingly, all the patients increased the percentage of unbroken and transferred blocks (manual accuracy) when using the sensory feedback condition, maintaining the number of transferred blocks in total (gross manual dexterity).

No significant improvement over time in the number of transferred virtual eggs (overall number or successfully displaced items) was observed (Fig. 2C).

The BSBT was successfully executed by FNG, ALM, and LRP. Indeed, the objects displacement score for both of them was higher than the errors count (12 versus 4, 24 versus 0, and 13 versus 1, $p<0.05$, respectively for FNG, ALM, and LRP, Fig. 2E). For ALM, remarkably, we did not observe any mistaken trial. As in the VET, even if a positive trend in score was observed over the course of the study, this was not statistically significant (Fig. 2F).

Finally, during the FCT, the subjects were able to modulate their grip force at the three different levels at the end of the study ($p<0.05$ for FNG and $p<0.01$ for ALM and LRP, Fig. 3B,C,D,E). ALM and LRP managed since the beginning to complete the task with a performance of about 83% and 88% respectively (Fig. 3C,D,F), which was maintained until the last session of tests (22nd week for ALM and 13th week for LRP, $p>0.05$, Fig. 3F). FNG instead began with a score just above chance level (41%, Fig. 3F), but improved by more than 50%, achieving a score of about 65% in the last trial (23rd week for FNG, $p<0.05$, Fig. 3B,F).

Phantom limb pain and mood states assessment

Remarkably, during the whole period of provision of the intraneuronal stimulation, both FNG and LRP experienced a reduction of pain, according to all the rating scales that were provided to them. Indeed, FNG reported a significant decrease of 36% in the NPSI and 57% in the VAS ($p<0.01$, Fig. 6A,B). Similarly,

the NPSI of LRP decreased from 18 to 5 ($p<0.05$, Fig. 6D), and her VAS from 6 to 3.2 ($p<0.01$, Fig. 6C). Finally, we observed that the mood states of the subjects benefited of the use of the bidirectional prosthesis: e.g., the subjects felt less nervous or miserable at the end of the study (Fig. 6E).

Discussion

Long-term usability of intraneuronal electrodes

We proved for the first time that intraneuronal interfaces are stable (in terms of elicited sensations and from materials science viewpoint) and functional during chronic implantation in humans to deliver tactile sensory feedback. After the clinical trial, the location and the minimum charge needed to elicit a sensation were stable as well as the impedance of the working active sites, confirming our previous results with animal models⁴⁰. During the whole study period, all the subjects reported sensations from the phantom hand, when intraneuronal stimulation was provided. These (above all the stability of the location of the sensations) are the most important requirements to guarantee the clinical exploitation of the intraneuronal sensory feedback restoration device. A slight oscillation of the number of active sites with impedance lower than 100 kΩ was observed from week to week in FNG, ALM and LRP, probably due to the impedance measuring setup. Also, for subject LRP we observed an increase of the number of active sites with a sensation threshold lower than 120 nC. This was maybe due to a minor increase over time of sensitivity of the subject to stimulation.

A great variety of sensation qualities has been elicited both intra- and inter-subjects. Many of these sensations (~60%, ~15%, ~50%, respectively for FNG, ALM, LRP, Fig. 1E) can be associated with the ones that can be perceived with the healthy limb when interacting with objects (e.g. pressure or vibration, which we consider “natural” in this manuscript, i.e. similar to the ones that are perceived with the intact limb). It is worth mentioning that in FNG and LRP we also demonstrated that the intraneuronal stimuli were able to elicit brain responses in the sensory areas (Somatosensory Evoked Potential) with a shape similar to the one evoked when stimulating sensory fibers in intact nerve⁴¹. Similar sensation qualities were reported in studies on amputees implanted with penetrating electrodes, however the stability of the percepts obtained with them being low, compared to our results^{18,19}. Mostly unnatural percepts (paresthesia) were instead reported with epineurial electrodes¹⁵. This observation makes us hypothesize that the quality of elicited sensations is primarily coded by the types of nerve fibers recruited through neural stimulation. This suggests that electrodes able to target a circumscribed population of fibers (likely of similar type^{42,43}) may be more suitable to elicit natural sensations: indeed, the simultaneous activation of fibers of different types is not physiologic, since they have their own specific firing rates⁴⁴.

We need also to note that some of the sensations reported by the three subjects were unnatural (e.g. electricity). We believe that new encoding strategies must be identified in the future to elicit more natural sensory feedback. We think that, being sensory feedback one of the requirements of amputees for future prostheses and one of the reasons of abandonment of presently available ones⁷, the quality of the restored sensations will play an important role on device acceptance^{45,46}. Neural stimulation should elicit sensory feedback being at the same time efficient (i.e. usable to increase motor control performance) and highly natural, as the naturalness of the feedback plays a pivotal role in prostheses acceptance²². More investigations are necessary to test whether a more natural sensory feedback could improve besides acceptance, other factors such as prosthesis control, dexterity or embodiment in amputees.

An interesting phenomenon was reported by subjects ALM and LRP during the study. In their case many sensations moved from the residuum to the phantom hand throughout the weeks. We hypothesize that both subjects had a deeply reorganized connectivity between the central nervous system and the peripheral nerves of the stump, with respect to FNG. In LRP, indeed, the nerve ultrasound, performed before the electrodes implant, showed a reduced cross-sectional area (between 4 and 5 mm²) of the median nerve in the tract between the elbow and the proximal third of the arm, compared to the physiological values for median⁴⁷ and ulnar nerves⁴⁸. The surgery confirmed the reduced size of the median nerve, compared to the ulnar one (Fig. 7B). In ALM, the long interval

between the amputation and the enrollment in the study (more than 20 years) may have induced great and stabilized plastic changes in the paths between the brain and the peripheral nerves⁴⁹. It is known⁴⁹ that in such condition the de-afferented areas of the brain can be “invaded” by the adjacent ones (the forearm and the face, in the case of hand amputees), and that the passive electrical activation of fibers originally directed to the lost body part can generate a sensory perception on these areas and not in the phantom⁴⁹. The six-months stimulation of these fibers, combined with the use of a prosthetic hand, may have induced a partial regression of the abovementioned plastic changes. The other option - not excluding the previous one - is that the continuous stimulation of peripheral nerves (generating a peculiar pattern of sensation) combined with the intensive use of the prosthetic hand may have promoted a process of embodiment of the device (taking advantage of brain plasticity), with a progressive association of the peculiar pattern of sensations with the prosthetic hand itself⁵⁰.

Since the clinical features of the two subjects were very different, we hypothesize that this phenomenon could interest a larger population of amputees, with no restriction. Further studies on this matter are needed.

Also, this phenomenon could be exploited in the future to develop novel therapies of neurorehabilitation, which could involve the combination of neurostimulation with other techniques such as virtual reality^{37,51}.

As a side but important note, the results from FNG and ALM clearly confirm that the use of a transcutaneous device makes the overall approach more fragile and prone to stress-induced mechanical failures while connecting and disconnecting electrodes and stimulators. These sites of the connectors have undergone redesign on LRP to increase strength and deliver higher strain relieve during every day handling. This solution provided a great step towards immunity to active sites failure until the end of the study. For future clinical practices, though, the solution may be represented by a fully implantable system, which will avoid any daily connection and disconnection between the electrodes cables and the neural stimulator.

Intraneural stimulation improves prosthesis control performance and mood states, while it reduces phantom limb pain

Results from BSBT strengthen the evidences, about the benefit of using sensory feedback to complete functional tasks, without any visual or acoustic clue, that were previously shown through a trial with 2 subjects implanted with FINE electrodes^{52,53}. In the very same trials, the outcome of the clinical tests executed by the two amputees, seemed to display that very low or no improvement is obtained when sensory feedback is provided when also vision is available.

In this work, notably, with the VET, we demonstrated that the control of the prosthesis in ecological conditions is improved by the sensory feedback. Finally, with the FCT, we reinforced previous findings²⁰ of the capability, given by intraneural stimulation, to modulate the grasping force exerted through the prosthesis, which is one of the main users' requirements. The ability of the subjects to modulate the prosthesis grasping force comes probably from the combination of: i) the proportional relation between the charge of the intraneural stimulation and the intensity of the elicited sensation, ii) the independency of the cluster location of the evoked perception on the charge of the intraneural stimulation. These properties come probably from the unique selectivity of intrafascicular electrodes, which allow fibers recruitment in a confined area of a fascicle²⁵. To date, the capability of controlling several different levels of prosthesis force through the sensory feedback induced by intraneural stimulation has not been shown with other implantable electrodes. As expected, after the results of the sensation characterization proving the stability of intraneural stimulation over time, the subjects were able to execute the different functional tasks throughout the whole study.

Also, we demonstrated that combining sensory feedback to the control of a myoelectric prosthesis results in significant decrease of phantom limb pain and amelioration of the user's mood. This is a great advantage for users since it would avoid them to undergo additional therapies for pain treatment.

Intraneural stimulation is usable with patients with different clinical conditions

The participants involved in the study presented very different clinical conditions. In more detail, FNG

had a phantom limb syndrome, which moderately affected his daily activities. He had painful and painless phantom sensations (Fig. 6A,C,E-7A).

Only painful phantom sensations were reported by LRP, but these did not affect her activities of daily leaving. Both LRP and FNG underwent the amputation less than 3 years before the enrollment in the study. ALM did not report any phantom pain and lost the hand more than 20 years before the start of the pilot trial.

The clinical differences of FNG, ALM and LRP could justify some discrepancies in terms of elicited sensations and tasks performance. In fact, FNG, reported a difficulty in clearly identifying percepts evoked by intraneuronal stimulation because of the continuous presence of spontaneous sensations from the phantom hand. This could explain the reduced performance of FNG compared to ALM and LRP, in the FCT.

Conversely, intraneuronal stimulation evoked in FNG and LRP sensations that could be associated to the ones perceived by the healthy hand, mostly located over the fingers, while in ALM evoked mostly perceptions of electricity, which rarely extended to the digits. In this case, the long interval from the amputation, and the consequent plastic changes in the central nervous system pathways and relays connected to the stump nerves, could have been the reason of the less natural quality of the sensations and their location perceived by ALM. In the case of LRP, even at the end of the study, the stimulation of the median nerve evoked no sensations on the hand. We hypothesize that the Wallerian degeneration of a consistent contingent of fibers innervating the hand (Fig. 7B) after the amputation was to a point that did not allow to elicit sensations from correspondent areas of the phantom limb (i.e. the phantom fingers).

Outstandingly, independently on the peculiarities of each subject, FNG, ALM, and LRP felt sensations on the phantom hand induced by intraneuronal stimulation for the whole duration of the trial and exploited these sensations to improve the control of the prosthesis. This suggests that the bidirectional prostheses can be used by subjects of very different clinical conditions, which would allow medical doctors to adopt loose inclusion criteria for the selection of users.

Conclusions

We believe that our findings support the hypothesis that intraneuronal stimulation represents a valuable clinical solution to provide sensory feedback to trans-radial amputees and that this approach would be ready to be tested with a larger cohort of patients during long-term clinical trials outside the controlled hospital environment, having established already that in the case of TIMEs the device cable/connector portion has to be replaced by a fully implantable solution. Indeed, we showed that intraneuronal stimulation represents a solution to amputees' requirements for future prostheses, such as the control of the force grip; improves the control of myoelectric prostheses for the completion of tasks executed with or without the assistance of vision; reduces phantom limb pain; and ameliorates patients' mood state.

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Author contribution:

F.M.P., G.G, M.C, C.Ci, E.F., T.S., D.G., J.L.D., M.B., L.R., S.R, P.M.R., and S.M contributed to the conception and design of the study. F.M.P., S.R., I.S, G.V, E.D.A, J.C, G.G, R.D.I, F.I, F.C, P.C., M.M., D.A.,

A.H., L.B. and C.Ca. contributed to the acquisition and analysis of data, S.M, F.M.P., G.V, G.G, and I.S contributed to drafting the text and preparing the figures.

Potential Conflicts of Interest:

S.R., F.M.P., and S.M. hold shares of "Sensars Neuroprosthetics Sarl", a start-up company dealing with commercialization of neurocontrolled artificial limbs. C.Ci., M.C. and F.C. hold shares of "Prensilia", a start-up company commercializing robotic hands and assessment tools. D.G. is author of the WO2006027473 A1 patent. The other authors do not have anything to disclose.

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Figures captions

Fig. 1. Intranodal stimulation stabilized over time. (A) The subjects, after the implantation of four TIMEs in the median and ulnar nerves, periodically received sessions of stimulation in combination with or without the prosthesis. For the bidirectional control, the sensors readout was used to drive the intranodal stimulation. The relationship between these parameters was linear. The robotic hand was controlled through the EMG of the forearm residual muscles. The stimulation, when delivered without the prosthesis, was driven by a custom-made software through which the parameters of current were set, and the subjects' reports were recorded. The results of this characterization are shown in (B) as 75th percentile of the phantom sensations locations evoked during the six-month clinical trial. EL1 is the electrode #1 implanted in the proximal part of the median nerve; EL2 is electrode #2 implanted in the distal part of the median nerve; EL3 is the one (#3) implanted in the proximal part of the ulnar nerve; EL4 is the electrode (#4) implanted in the distal part of the ulnar nerve. AS means active sites. Also, the number of sensations reported by ALM and LRP on the stump (the same value from FNG is negligible) over time is plotted (C), along with the stability of the sensations elicited from the phantom hand (and not the stump) of FNG, ALM, and LRP (D). The stability was evaluated in terms of location of the percept. The characterization results were used to calibrate the sensory feedback restoration system of the bidirectional prosthesis, whose motion was instead controlled by means of the electrical activity of the residual muscles of the amputated limb. (E) The types of sensations reported during the whole trial. The minimum charge needed to evoke a sensation in FNG, ALM and LRP are in (F), (G) and (H). The boxplots are computed from the sensory thresholds of 3, 3 and 4 electrodes respectively for FNG, ALM, and LRP. The averages are obtained from the sensory thresholds of the active sites, which were lower than 120 nC. Per each active site the threshold was determined as average of three repetitions. The fitting performance (R^2 and p) of the saturating exponential function (dashed line) is reported. The function is defined as $-D+C/(B+\exp(-x/A))$. Two-tailed Friedman's test with Tukey-Kramer correction for multiple groups of data was performed.

Fig. 2. The Virtual Eggs and the Blindfolded Sensory Blocks Tests. (A) During the VET, the subjects are instructed to transfer the fragile blocks presented in front of them from one side to the other over a 15 cm tall wall as fast as possible and without breaking them. (B) Performance for FND (N=4x2 repetitions), ALM (N=27x2 repetitions) and LRP (N=5x2 repetitions). The percentage of transferred blocks over the total number of blocks (transferred plus broken) with and without sensory feedback is indicated on the left. On the right, the total number of transferred blocks is displayed. Data are shown as mean values \pm SD. Statistical evaluations results in (B) were obtained using the Friedman's test. (C) Performance and total number of transferred blocks over time. The data related to the first and the last sessions, with and without sensory feedback for both subjects is reported. Statistical evaluations results were obtained using the Fisher's exact test. (D) Schematic of the BSBT experiments. (E) The performance in the BSBT for FND (N=9), ALM (N=3) and LRP (N=3) are shown. The score and errors were indicated, and the data are reported as mean values \pm SD. Statistical evaluations results in (E) were obtained using the two-tailed Friedman's test. (F) The BSBT performance over time is shown. The data related to the first and the last sessions, for both subjects are shown. Statistical evaluation results were obtained using the Fisher's exact test.

Fig. 3. Force Control Test. (A) Schematic of the FCT experiments. (B) The performance in the last executed FCT for FND (N=90), (C) ALM (N=90) and (D) LRP (N=90) are shown. The confusion matrices for both subjects are indicated. For FND, ALM and LRP (E) the sensor readings in the three force levels are reported as mean values \pm SD. Statistical evaluations (results in the figure) were performed using the two-tailed Friedman's test. (F) The FCT performance over time for all subjects (N=180 for FND, N=180 for ALM, N=180 for LRP) is shown. Statistical evaluation results in (E) were obtained using the Fisher's exact test.

Fig. 4. Perceived sensation levels and locations. The ranges of injected charge for each pressure level perceived by FND (A), ALM (B) and LRP (C) related to 4 active sites per subject used for the implementation of the bidirectional prosthesis are reported. Data in the figure is represented as median and 25th-75th percentiles. * means p < 0.05. Two-tailed Kruskal-Wallis test with Tukey-Kramer correction for multiple groups of data was performed. Sensation locations for maximum and minimum intensities reported by each patient during the modulation of charge of the stimulation train are reported (top). In the maps of hand sensations, each colored area indicates the 75th percentile of the phantom sensation evoked for four active sites for each patient (two on median and two on the ulnar nerve). The number of statistically different pressure levels perceived by the subjects is shown in (D) as mean ± SD.

Fig. 5. Intraneuronal electrodes are stable over time. The impedance of the electrodes active sites after an initial increase reached a plateau for both FNG and ALM (respectively (A) (B), top). For LRP the impedances were stable since the first week (C). The number of active sites, with impedance lower than 100 kΩ, used to compute the average impedance is displayed for FNG, ALM and LRP (respectively (A), (B) and (C), top). Per each active site a single measure of impedance was executed. Two-tailed Friedman's test with Tukey-Kramer correction for multiple groups of data was performed. The loss of active sites (impedance higher than 100 kΩ) is due to the mechanical stress related to the connection and disconnection of the electrodes cables with the external stimulator. Indeed, microscopic inspection (C and D) indicates damages only at the extracorporeal transition between cables and plugs and not at the implanted transition between cables, ceramic portion and polymer-thin-film structure. The deposited thin-film electrodes adhered well on the polymer substrate and showed no signs of corrosion nor deterioration.

Fig. 6. Phantom Pain Evaluation. The NPSI, VAS and POMS-SF were recorded before the implant and during each session of neural stimulation before the explant. The NPSI evolution throughout the weeks is displayed for FND (A) and LRP (B). The VAS scores are displayed in (C) for FND and in (D) for LRP. The data are reported as mean values ± SD. Statistical evaluations were performed between first and last session using the two-tailed Friedman's test. (E-F) The POMS-SF results related to the first (left) and the last (right) session are shown for FNG (E) and LRP (F). * indicates a reduction from the first to the last session.

Fig. 7. FNG spontaneous phantom sensations and LRP median and ulnar nerves. (A) On the left, the localization of the not painful spontaneous phantom sensations of FNG is depicted. On the right, respectively from top to bottom, there are impact to life, and frequency occurrence evaluation questionnaires of phantom sensations filled out by FNG. (B) The picture, taken from the intraoperative microscope, shows the small size and the altered aspect of median nerve (in comparison with the adjacent ulnar nerve) of LRP.

Supp. Video 1. TIMEs implants.

Supp. Video 2. VET and BSBT tests.