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NEUROPROSTHETICS

Enhancing functional abilities and cognitive integration of the lower limb prosthesis

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Lower limb amputation (LLA) destroys the sensory communication between the brain and the external world during standing and walking. Current prostheses do not restore sensory feedback to amputees, who, relying on very limited haptic information from the stump-socket interaction, are forced to deal with serious issues: the risk of falls, decreased mobility, prosthesis being perceived as an external object (low embodiment), and increased cognitive burden. Poor mobility is one of the causes of eventual device abandonment. Restoring sensory feedback from the missing leg of above-knee (transfemoral) amputees and integrating the sensory feedback into the sensorimotor loop would markedly improve the life of patients. In this study, we developed a leg neuroprosthesis, which provided real-time tactile and emulated proprioceptive feedback to three transfemoral amputees through nerve stimulation. The feedback was exploited in active tasks, which proved that our approach promoted improved mobility, fall prevention, and agility. We also showed increased embodiment of the lower limb prosthesis (LLP), through phantom leg displacement perception and questionnaires, and ease of the cognitive effort during a dual-task paradigm, through electroencephalographic recordings. Our results demonstrate that induced sensory feedback can be integrated at supraspinal levels to restore functional abilities of the missing leg. This work paves the way for further investigations about how the brain interprets different artificial feedback strategies and for the development of fully implantable sensory-enhanced leg neuroprostheses, which could drastically ameliorate life quality in people with disability.

INTRODUCTION

Current prostheses do not restore sensory feedback to amputees, who, relying on very limited haptic information from the stump-socket interaction, are forced to deal with serious issues: the risk of falls (1), decreased mobility (2), the prosthesis being perceived as an external object (low embodiment) (3), and increased cognitive burden (4) during walking. Poor mobility is one of the causes of eventual device abandonment (5–7). Although considerable efforts have focused on developing (8) and controlling (9, 10) sophisticated lower limb prostheses (LLP), few trials have been conducted to restore sensory feedback (11–16). Surgery techniques (11) and noninvasive methods—such as continuous (12) or time-discrete (15) vibrotactile and electrocutaneous stimulation (13)—were tested to restore sensory feedback, although most of which

being in below-knee (transtibial) amputees. These noninvasive sensory feedback restoration devices showed only limited benefits such as improved symmetry between prosthetic and healthy legs during walking on even surfaces and the postural stability on a movable force platform (12, 15). Noninvasive systems have the drawback of not being homologous (they do not restore sensations from the missing leg) or selective (they evoke unrefined sensations); these drawbacks force the amputees to invest time in training, which only partially overcomes such limitation. A novel surgical procedure to restore proprioception in transtibial amputees has been developed (11). However, performance characterization of such approach in daily life activities was not shown yet, and this procedure might be difficult to transfer to higher-level amputations.

After an amputation, the neural pathways between the remaining periphery and the brain are still functional (17). Peripheral nerve electrical stimulation (PNES) (18) of the sensory fibers proximal to hand amputation can reactivate sensations from the missing extremity in the brain (19–22). The map of elicited sensations in transtibial amputees implanted with flat interface nerve electrodes has been reported (16), however, without prosthesis connection or functional assessment. The transtibial amputation is a much less disabling condition than the transfemoral one: transfemoral amputees have less mobility and gait symmetry, together with higher energy expenditure than transtibial ones, besides other issues (2, 23).

The purpose of this work was to demonstrate that homologous and somatotopic sensory feedback can be restored in transfemoral amputees and that it can be exploited by them to improve the use of the leg prosthesis during different ambulation tasks and to promote its integration in their body schema and image (24). To this aim, we designed a neuroprosthetic framework to restore sensory feedback referred on the phantom lower limb of transfemoral amputees and triggered from

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the bionic leg by stimulating the residual tibial branch of the sciatic nerve through implanted neural interfaces (Fig. 1, A and B). The stimulation of the tibial nerve was driven by the sensors, added to, or embedded in a commercial prosthetic leg. Together with the functional outcomes, we assessed the integration of the device into the body schema and image of the subjects (cognitive integration) through measurements of prosthesis embodiment and cognitive effort while using the artificial leg. Three volunteers participated in the study.

RESULTS

Subjects and procedures

The volunteers (S1 to S3) had suffered a transfemoral amputation, as a consequence of traumatic events, and received an implant of four transversal intraneural electrodes (25) in the distal section of the tibial nerve for more than 90 days each (Fig. 1B).

Sensation characterization

We characterized the nerve stimulation during the first month after the implant of the electrodes. For this purpose, each channel of each electrode was connected to an electrical stimulator. The stimulator injected electrical current pulse trains with variable intensity, duration, and frequency. Subjects had to describe the sensation in terms of type, location, extent, and intensity, and their report was recorded through a dedicated graphical user interface.

Physiologically plausible or natural sensations, intended as feelings that could be associated with the ones of the healthy extremity—touch, pressure, vibration, or muscle contraction—were elicited in more than 20 positions over the phantom foot sole and lower limb [42% (27 of 48) for S1, 68% (36 of 53) for S2, 57% (27 of 48) for S3] (Fig. 2, A to C, and table S1). All these positions were covered through multiple active sites, making the induced sensations redundant and consequently resistant to potential failures of active sites. We found that there is a positive proportional relationship between the amplitude of the injected stimulation current and the intensity of the corresponding evoked sensation (fig. S1, A to C). This is consistent with what was observed in upper limb amputees implanted with the same nerve interface (21, 26, 27) and found in modeling works (28).

Prosthesis

To develop the neuroprosthetic leg, we built a fully portable real-time platform for recording sensors and stimulating nerves in humans. The following essential implementation steps were defined (Fig. 3, A to C) with the aim to optimize the neuroprosthetic device.

A custom-made transfemoral prosthesis (composed of commercially available prosthetic components: RHEO KNEE XC, Pro-Flex XC foot, transfemoral flexible brim socket, and Iceross Seal-In X5 TF silicone liner, Össur hf.), where the microprocessor-controlled knee has an integrated knee encoder communicating the knee angle with 1° of resolution via Bluetooth at 50 Hz, was equipped with a custom-made
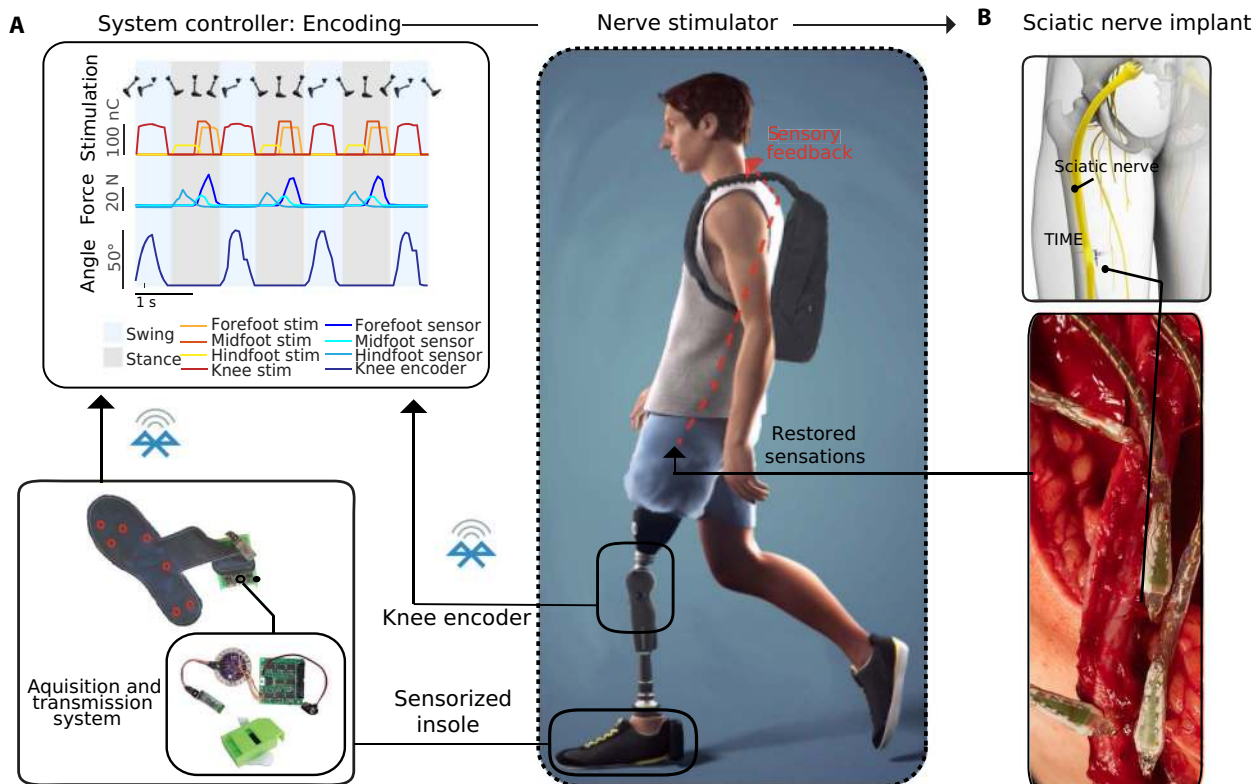

Fig. 1. Neuroprosthetic leg. The lower limb amputees wear a custom-made prosthesis with commercially available knee and ankle components equipped with a system for restoring sensory feedback. An encoder is embedded in the prosthetic knee (**A**) indicating the amount of flexion of the device and a sensorized insole is placed under the prosthetic foot. The readout from these sensors is transmitted via Bluetooth as input to an external controller, which translates it into the language of the nerve, meaning the parameters of stimulation. These instructions drive the activity of an external stimulator, which is connected to four transversal intrafascicular multichannel electrodes (TIMEs), previously implanted into the tibial portion of the sciatic nerve (**B**). The neural interfaces are placed transversally in the nerve, inside the fascicles.
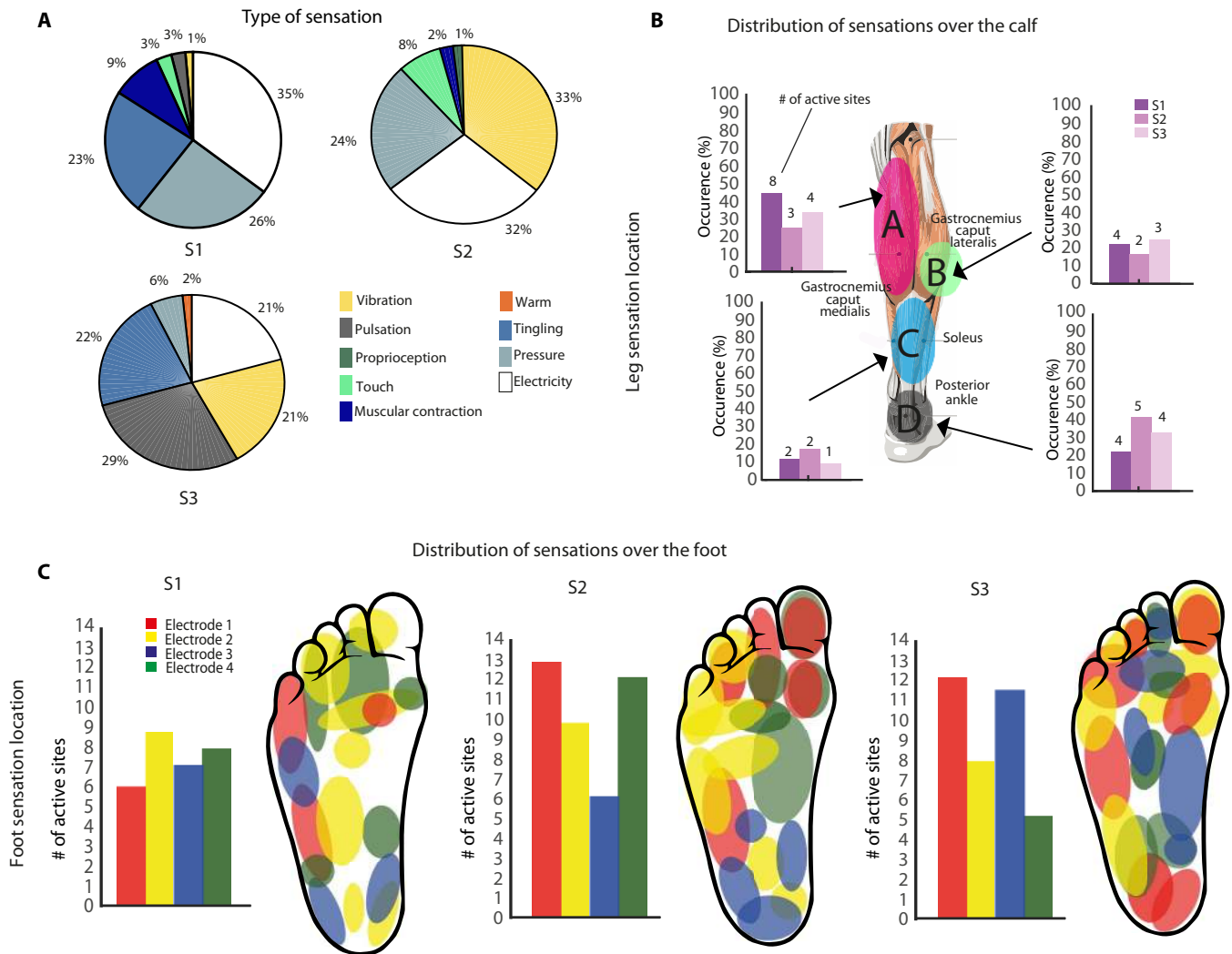
Fig. 2. Characterization of the evoked sensations. (**A**) The distribution of the sensation type for each subject according to all the phantom sensations evoked in the first month by every active site. (**B**) Distribution of sensations over the lower limb. The lower limb is divided into four areas representing the muscles innervated by the tibial nerve (A, gastrocnemius caput medialis; B, gastrocnemius caput lateralis; C, soleus; and D, posterior ankle). The bar plots show the percentage and number of active sites eliciting sensations on the lower limb for each subject. (C) The maps of foot sensations and the bar plots with the number of active sites eliciting sensations for each subject are reported. Each colored area indicates the 75th percentile of the phantom sensation evoked for an active site (the most frequently reported). The maps are related to the first month of each implant.

sensorized insole (SensArs Neuroprosthetics), giving pressure information from seven positions of the foot sole (see Materials and Methods for more details). The readouts of three of these sensors and of the encoder were used to linearly drive the stimulation of neural electrode active sites, that elicited touch (or pressure or vibration) under the foot sole (forefoot, midfoot, and hindfoot positions) and contraction of the gastrocnemius (for S1 and S2), or pressure on the gastrocnemius (for S3), which were intuitively interpreted by subjects as knee flexion (or specific angle positioning and its variation) within physiological range, emulating a proprioceptive feeling (fig. S2, A to C). The subjects, after having been instructed on the functioning of the neuroprosthesis, did not need any training to associate the restored sensation with the flexion/extension of the knee (subjective reports from the experimenters and volunteers). We engineered wireless communication modules to control the parameters of PNES with a latency of less than 50 ms.

Passive tasks
First, we assessed the ability of the subjects to exploit the sensations restored through neural stimulation to feel and recognize contact on the prosthesis insole and the angular position of the prosthetic knee and its variations. We thus implemented three passive tasks (touch, proprioception and combined), in which the participants were blindfolded and acoustically isolated, and the prosthesis was disconnected from the user. In the touch test (Fig. 4A and movie S1), the users were asked to discern the location of the pressure executed by an experimenter over different foot sole positions. The participants achieved a performance of around 90% (S1, 92.6%; S2, 94.8%; and S3, 89.5%; Fig. 4A and fig. S3). During the proprioceptive trials, the subjects were asked to recognize the level of flexion of the knee from four different angles. In this case, the performance was 77.7% for S1, 83.7% for S2, and 69.2% for S3 (Fig. 4B, movie S1, and fig. S3). Last, in the combined test, the subjects were asked to simultaneously recognize proprioceptive
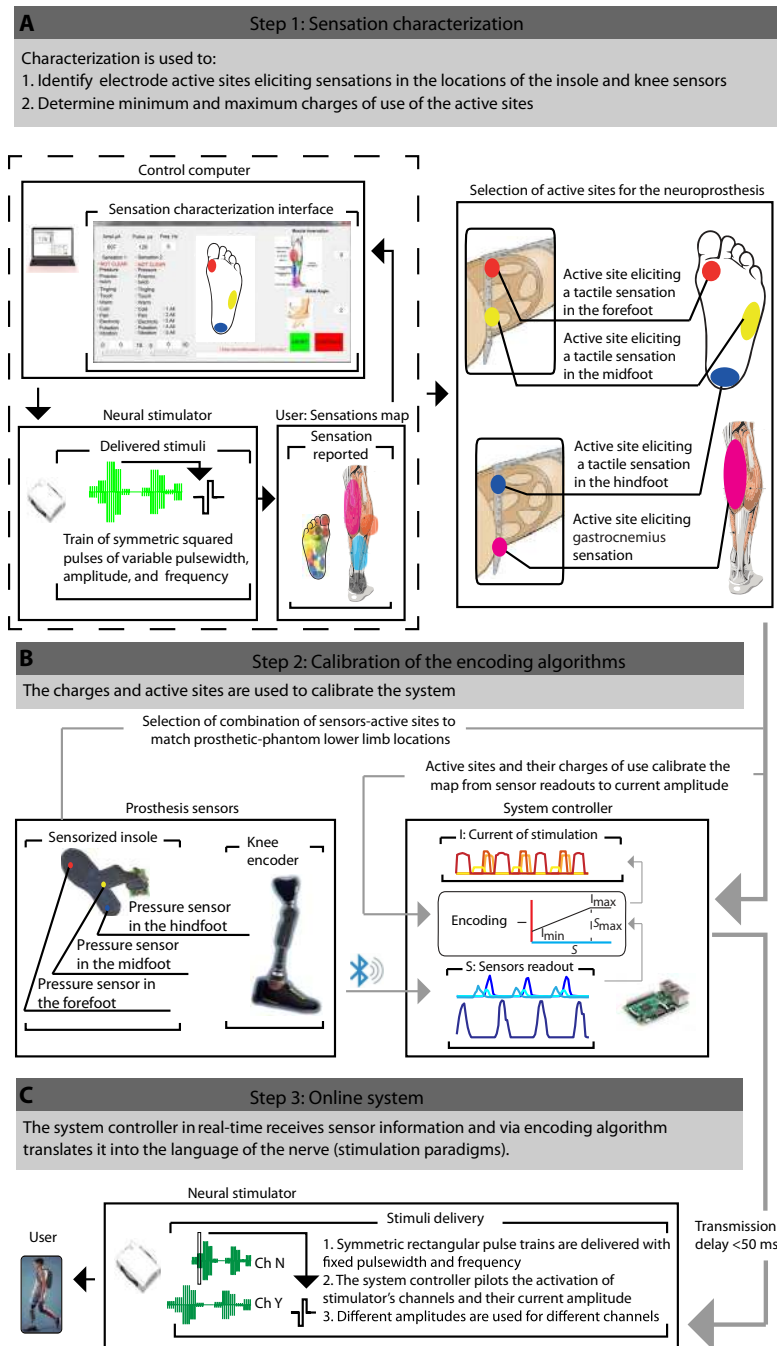
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A   Step 1: Sensation characterization

Characterization is used to:
1. Identify electrode active sites eliciting sensations in the locations of the insole and knee sensors
2. Determine minimum and maximum charges of use of the active sites

B   Step 2: Calibration of the encoding algorithms

The charges and active sites are used to calibrate the system

C   Step 3: Online system

The system controller in real-time receives sensor information and via encoding algorithm translates it into the language of the nerve (stimulation paradigms).

Fig. 3. Sensory feedback restoration system calibration and real-time use. (**A**) Step 1: A custom-made software was developed to control the stimuli injected through the stimulators into the TIME electrodes and to record the reports corresponding to the evoked sensations. The stimuli are balanced, symmetric cathodic first trains of rectangular pulses. Desired amplitude, pulsewidth, and frequency were inserted by the operator. The active sites, whose stimulation elicited a tactile percept in the locations matching the areas of placements of the sensorized insole, were selected. In addition, an active site evoking a muscle contraction of the calf was saved for further use in the sensory feedback restoration device. Along with these values, the minimum charge to elicit a sensation and the threshold to pain were saved. (**B**) Step 2: The parameters of stimulation identified are used to calibrate the encoding algorithm, running on the external controller. The encoder transduces the data from the sensorized insole and the knee encoder (Bluetooth communication) into execution instructions for the external stimulator. The function transfer between these two sets of values is linear. To calibrate it, the subgroup of channels to read from the sensorized insole and knee encoder, the minimum and maximum values of their readout, the minimum and maximum active sites charges, and the corresponding stimulator channels to activate are needed. (**C**) Step 3: the instructions of stimulation are delivered in real-time. The stimulator injects into the TIMEs balanced, symmetric cathodic first trains of rectangular pulses of fixed frequency and pulse width and amplitude variable with respect to the real-time sensors' readouts.

and tactile information (Fig. 4C, movie S1, and fig. S3). All the volunteers were able to accomplish this task with S2 and S3 recognizing two insole positions and knee flexion combinations with a score of 67.4 and 76.2%, respectively, whereas S1 recognized one tactile sensation and knee flexions with an accuracy of 89.2% (Fig. 4C and fig. S3). Overall, the participants performed more than 1224 recognition trials.

To rule out that recognition of leg touch or movement could be achieved through the stump-socket interaction, the users were connected to the prosthesis and were asked to identify touch or flexion events without stimulation. The performance in each passive task dropped to around chance level (fig. S4, A to F), showing that the participants were unable to accomplish these tasks without neural stimulation (fig. S4, G and H).

Functional tasks

The artificially induced sensations of foot contact and knee motion (flexion-extension) were integrated by the users walking with the prosthesis, without prior gait training. We connected the map of sensations to the prosthesis and explained the experiments to the user (that touch would have been restored from the insole and that sensation of knee angle and its variation would have been restored from the knee, in a similar fashion as the "passive experiments"). From the first steps, the subjects referred to perceive sensations coming from the phantom foot and leg accordingly with the walking. They found it easy to understand that the sensations were due to the interaction of the prosthetic foot with the ground and to the movement of the prosthetic knee. To verify whether the use of the neuroprosthesis could boost walking capacity, the participants performed three functional tests: climbing and descending stairs, completing an obstacle course, and walking over a straight line. The first two tests were carried out in four different conditions: without neural feedback (NF), with only foot sole tactile feedback (T), with emulated proprioceptive feedback from the phantom gastrocnemius muscle (P), or a combination of the latter two (PT), whereas the last test was performed in NF and PT conditions (see Materials and Methods).

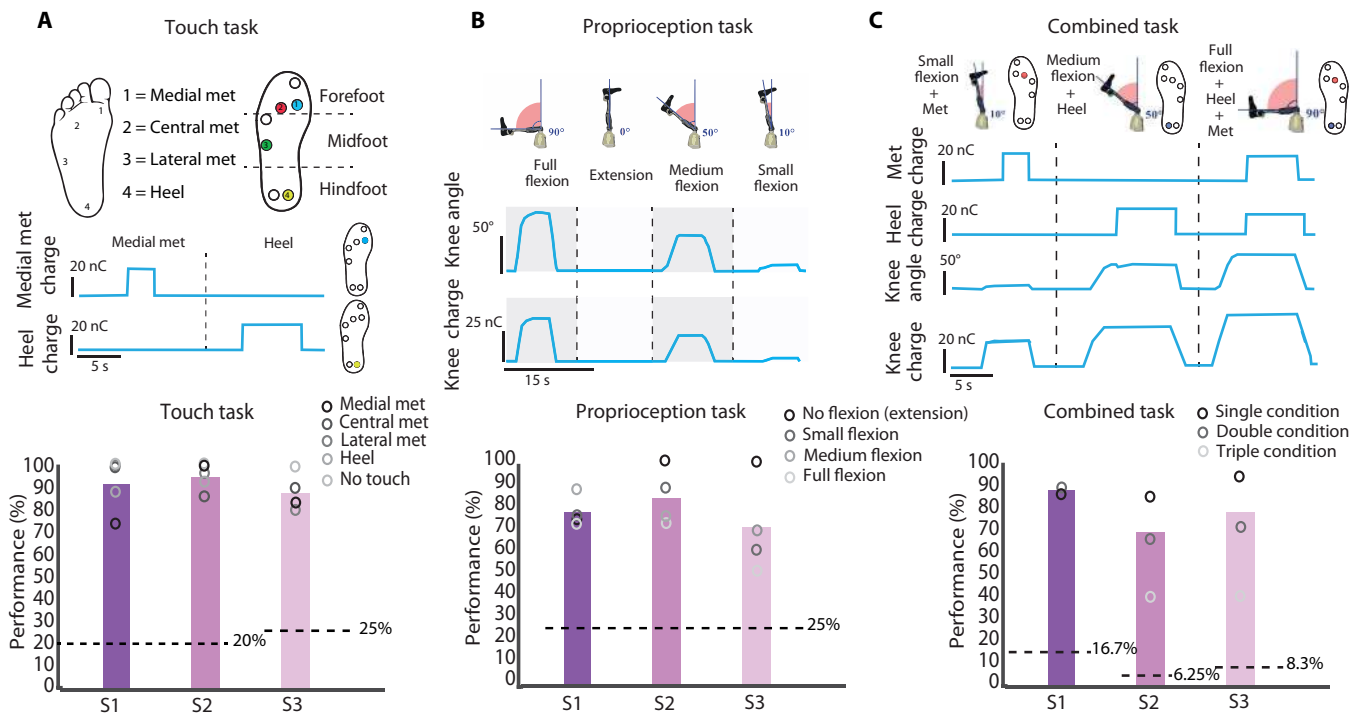During the stairs test, the subjects were asked to walk clockwise on an angular

Fig. 4. Passive recognition tasks. (**A**) The touch task and its performance. Up to four positions on the foot sole were chosen and randomly touched by the experimenter. A nontouch condition was also used. The plots show how the current was injected according to the position touched. For S3, the medial metatarsus position was replaced with the hallux one. $n = 92$ repetitions for S1, $n = 120$ for S2, $n = 140$ for S3. (**B**) The proprioception task and its performance. Four random angles of the knee were transmitted to the subjects. The plots show how the charge was modulated according to the angle. $n = 72$ repetitions for S1, $n = 120$ for S2; $n = 120$ for S3. (**C**) The combined task and its performance. Different combinations of touch and angle were induced to the subjects. The plots show how the current was injected and modulated according to the angle and the position under the sole touched. $n = 72$ repetitions for S1, $n = 308$ for S2, $n = 180$ for S3. In (A) to (C), the circles indicate the partial performance for every condition, whereas the dashed lines show the chance levels.

staircase, climbing up five steps and going down on three steps maximizing the number of laps (intended as going up and down the stairs and reaching the starting position again). Ascending and descending stairs are one of the most challenging situations in an amputee's daily life. The mobility (number of laps) achieved by the three subjects with the PT feedback was higher than the mobility without stimulation (Fig. 5A and movie S2). For S2 and S3, not only PT but also P and T enabled them to climb and descend stairs with a higher velocity than the NF condition (Fig. 5A).

To evaluate the ability of the subjects not to slip and fall, they were instructed to walk on a platform with obstacles at self-selected speed while wearing glasses that impaired the inferior field of vision (Fig. 5B and movie S2). The platform was created ad hoc for fitting between parallel bars, which were used as supports in case of falls of the participants. They were asked to not rely on the bars during walking. Falls were counted when the subjects, while stepping on an obstacle with the prosthesis, lost balance and had to rely on the bars (either by grasping them or leaning the body on them) to prevent a fall.

The falls decreased for all the subjects when touch—alone or in combination with proprioception—was elicited, compared to NF. For all three participants, no difference was found between P and NF conditions. In addition, for S1 and S2, the number of falls was lower with the PT or T conditions than with P (Fig. 5B). During this task, the velocity of execution and the number of obstacles trodden on were similar in all the conditions or higher when stimulation was provided (fig. S5, A and B), which rules out the possibility of a lower number of falls with feedback because of slower walking.

Last, to test the agility gained because of the restored sensations, subjects were asked to walk over a straight line (29, 30), putting one foot after the other without stepping outside the line (Fig. 5C and movie S2). Whenever the subjects failed to precisely walk on the line with the prosthesis, an error was counted. S1, S2, and S3, when using the PT, outperformed the NF condition by 47, 34.5, and 38.2%, respectively. Because the subjects executed the same number of steps per condition on average in all the sessions (fig. S6), we ruled out the possibility that the accuracy in the task was determined by the pace of walking on the line.

Restored physiologically plausible leg sensations could be connected to an increased sense of ownership (3, 31) and cognitive relaxation (4). To explore this, we thus assessed the level of embodiment of the prosthesis after the execution of functional tasks (Fig. 6A) and the cognitive load due to the use of the prosthesis during functional tasks (Fig. 6B) in two subjects (S2 and S3), with and without intraneural sensory feedback.

The subjects' integration of the prosthesis in the body image was assessed through objective (proprioceptive displacement) and subjective measures (questionnaire) after every condition of execution of the functional tasks (no feedback, tactile, proprioceptive, tactile and proprioceptive feedback during obstacles, stairs, straight line). The proprioceptive displacement was defined as the spatial difference between the position of the phantom hallux after the functional task and the real position of the prosthesis. In other words, the less is the displacement, the more is the embodiment of the phantom lower limb with the prosthesis. For this reason, here, we indicated this measure
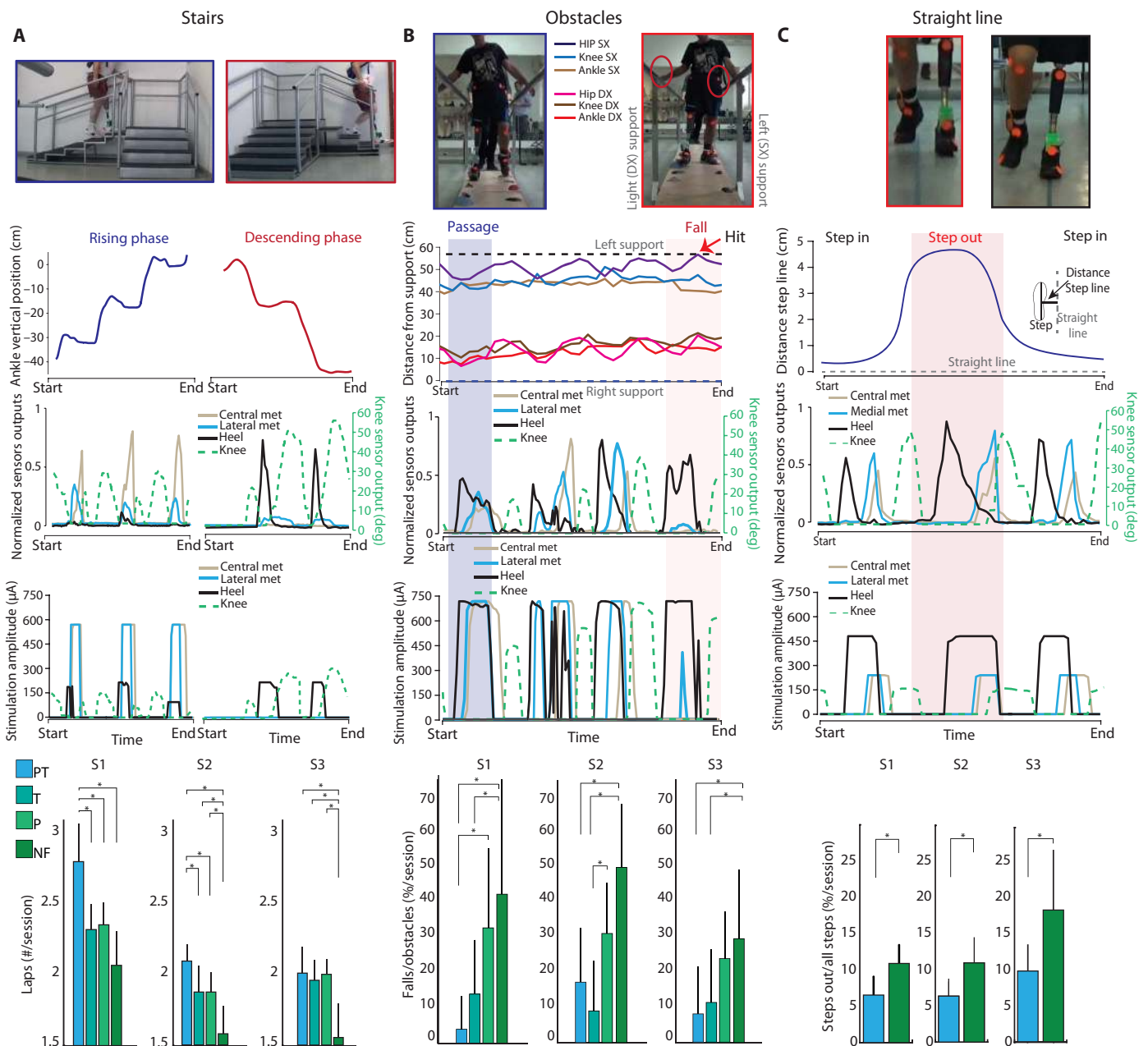
Fig. 5. Sensory feedback–improved walking performance of amputes. (**A**) Top: A subject while climbing (first picture) and descending stairs (last one). Middle (from top to bottom): prosthetic ankle trajectory of a subject while climbing and descending stairs for one lap (extracted from camera recordings in the parallel plane to motion), synchronous sensorized insole and knee encoder readouts, and encoded currents injected into the TIMEs. Bottom: The mean number ± SD of laps/session with proprioception + touch (PT), touch (T), proprioception (P) conditions, and without stimulation (NF) during stairs tests for S1, S2 and S3. $n = 12$ sessions per condition. (**B**) Top: A subject passing over an obstacle without falling (representation on the left) and falling (one on the right). Middle (from top to bottom): trajectories of ankle, knee, and hip of prosthetic and healthy sides of a subject while walking on the obstacle path (extracted from camera recordings in the orthogonal plane to motion). Four steps are shown: passing without falling on an obstacle (also in pictures), two steps with no obstacle, falling due to an obstacle (also in pictures); synchronous sensorized insole and knee encoder readouts, and encoded currents injected into the TIMEs. Bottom: The mean number ± SD of falls versus total trampled obstacles per session with PT, T, P, and NF during obstacles test for S1, S2, and S3. $n = 12$ sessions per condition. (**C**) Top. A subject while executing the straight-line test: a subject steps on the line, then off, and finally on it again. Middle (from top to bottom): distance between the prosthetic foot and the straight line of a subject (extracted from camera recordings as shown in the scheme on the right), during the execution of the three steps, synchronous sensorized insole and knee readouts, and encoded currents injected into the TIMEs. Bottom: The mean number ± SD of steps off the straight line of all the steps performed with PT and NF during straight line tests for S1, S2, and S3. $n = 9$ sessions per condition. *$P < 0.05$. Two-tailed ANOVA test with Tukey-Kramer correction for multiple groups of data was performed.

as proprioceptive displacement and not drift as it is done in the literature (31, 32). The questionnaire was composed of eight questions (31, 32). Three of them were a direct measure of the embodiment, three were control statements for assessing suggestibility, and two were a measure of vividness, how life-like and realistic the illusion was, and prevalence, the percentage of time that the illusion was experienced.

The subjects reported that their phantom leg was more displaced toward the LLP [proprioceptive drift (3, 31)] after functional tasks
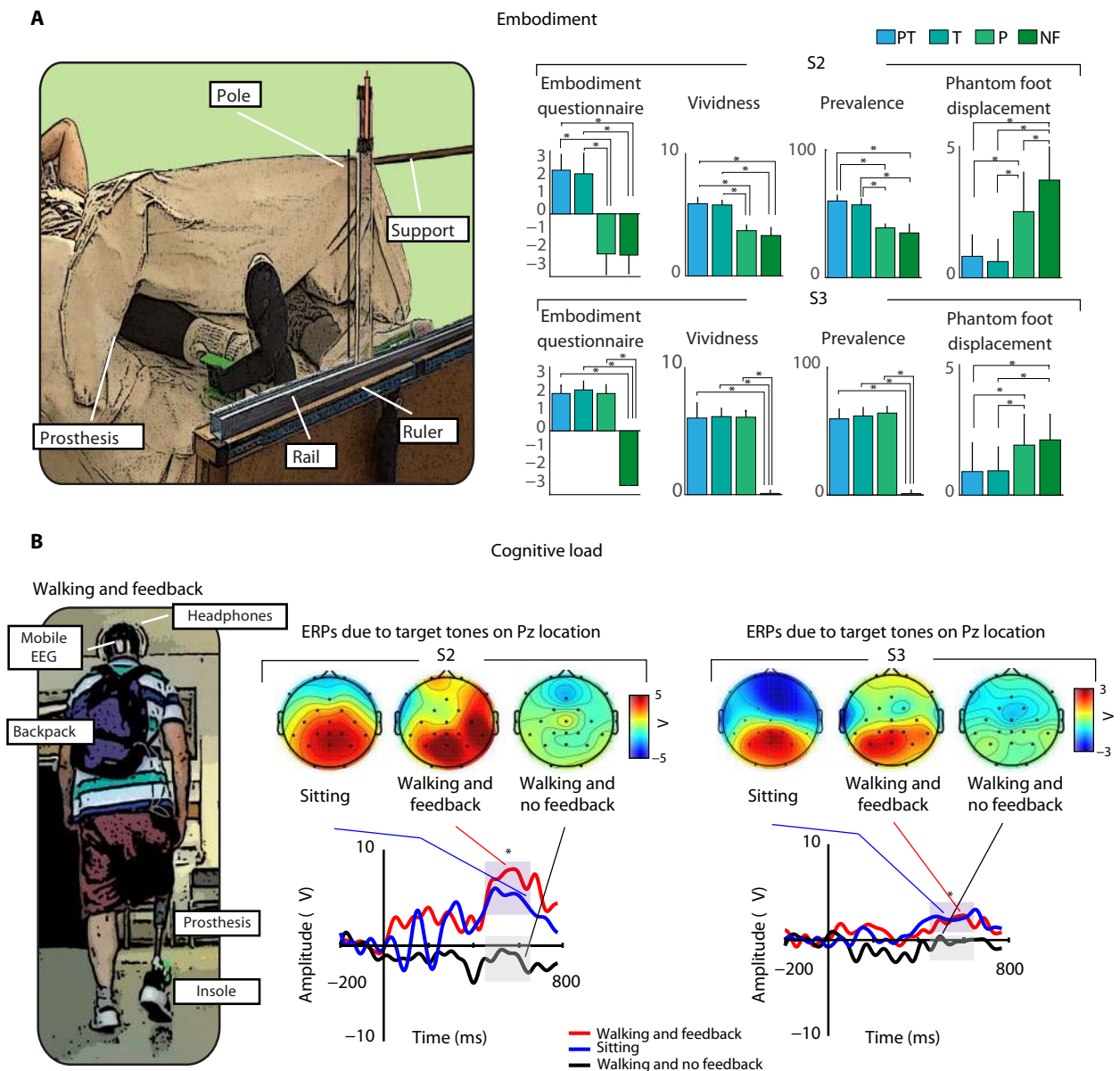

Fig. 6. Sensory feedback-improved embodiment and cognitive burden. The embodiment was assessed through proprioceptive displacement and questionnaires after the execution of the functional tasks. Four conditions were evaluated: NF, P or T, and PT. (**A**) On the left, a subject lies down on a bed during the measurement of the proprioceptive displacement executed with the aid of a shaft moving into a rail. The subject cannot see his feet. From the left to the right, after the picture, there are the: answers to embodiment questions 1 to 3 (control questions were always replied with −3), vividness and prevalence rating, and the proprioceptive displacement, respectively. S2 and S3 are in order on the top and on the bottom. For embodiment questionnaire $n = 30$, for vividness and prevalence $n = 10$, for phantom foot displacement $n = 30$. Two-tailed ANOVA test with Tukey-Kramer correction for multiple groups of data was performed. *$P < 0.05$. The cognitive effort made by the subjects during the walking activity was evaluated through acoustic event-related potentials (ERPs). (**B**) The subjects were asked to repeatedly count sounds that were delivered during three conditions: sitting, walking with (PT) and without neural stimulation. Because the same participant was repeating several sessions of the experiment, we varied the occurrence timings of all tones and the number of target and deviant tones: The number of standard tones was always the same (342), whereas the number of target and deviant tones varied between 41 and 46. The topographical representations of the voltage distribution in the P300 time window are depicted, confirming that the P300 component was present in the target trials because it is the most prominent over the parietocentral scalp locations. The ERPs are plotted in Pz location. Red and black represent walking with and without feedback, whereas blue is sitting. 3 × 2 ANOVA (control/walking with stimulation/walking without stimulation × target/non-target) was executed among averages computed in the P300 time window [450, 650] ms. The two-tailed Greenhouse-Geisser correction for multiple groups of data was applied. Averages are on $n = 38$ trials for both the target and deviant tones, which were not disregarded after data processing. *$P < 0.01$.

with PT and T sensory feedback, while this was not reported by both S2 and S3 with NF or P (Fig. 6A). In addition, embodiment questionnaires confirmed that both the subjects perceived the prosthesis more as being part of the body with PT and T (Fig. 6A).

To measure the cognitive burden, we ran a three-tone auditory oddball task [in which the subjects had to silently count the target (oddball) sounds only, similarly to the setup proposed in (33, 34)] while they were sitting (control condition) or walking with PT or NF [dual task (35)]. We expected that if the task was well executed, then the attention of the subjects to the target tones would have been higher than the attention to the deviant and standard tones. This would have generated a P300 event-related potential (ERP) component due to target tones higher than the P300 ERP component due to deviant and standard tones (33, 34). Electroencephalographic (EEG) recordings showed that only in the control conditions and when walking with PT were the subjects able to allocate sufficient cognitive resources to differentiate between the target and nontarget tones (higher P300 in the first case than in the second one; fig. S7, A and B). Such resources were higher compared to the NF condition (Fig. 6B). These findings demonstrate that subjects can allocate their attentional resources more efficiently (meaning that subjects have cognitive ease) to a task secondary to walking, when the intraneural sensations from the LLP are provided.

DISCUSSION

The lack of sensory feedback does not enable the correct sensorimotor integration (36) between the user's central nervous system and the artificial limbs. Cutaneous feedback is involved in the correct positioning of the foot during normal locomotion or after perturbations induced by mechanical obstruction (37). Potentially, the central nervous system can integrate and benefit from even limited restored sensory inputs (37), and our results support this.

Specifically, during the obstacle task where we only accounted for slip falls (and not trips because the prosthesis does not provide an active control of the knee), the use of emulated proprioception alone had a limited effect, as expected because the information involved during this task is connected mainly to the foot sole. On the other hand, the results achieved with the sensory modalities with touch from the foot sole show the improvements with respect to the nonfeedback condition in all the three subjects. We hypothesize that the awareness triggered by the different feedback evoked when stepping on an obstacle with respect to steps without obstacles prompts the user to stabilize the walking (for example, by abruptly transferring the weight with consequent stop of the prosthetic knee or making an extra stabilizing step). In other words, the subjects manage to use this information to drastically reduce the falls.

Similarly, during the stairs experiment, knowing both the amount of flexion of the knee and the contact of the foot with the stair step is important and beneficial for the subjects. The knee flexion informs about whether the following contact between the foot and the step will be reliable (if the knee is fully extended, then the foot will approach the step diagonally, and the contact with it will be unstable). Equally, it is important to be aware of how the foot is touching the step to be sure to have enough support for starting the next step. The users hesitate to quickly move the other leg while not being sure whether the prosthetic foot is correctly placed over the stair. For these reasons, in this experiment, P and T conditions led to a better (for S2 and S3 but not for S1) performance than NF. The PT condition, which is the closest to the natural situation, restored the best functional abilities (compared to P, T, NF) and promoted cognitive integration.

The additional hypothesis that we make is that not only can the subjects exploit the restored tactile and emulated proprioceptive feedback but they can also better use the other feedback sources (visual-vestibular cues) because their cognitive load on the prosthesis control is reduced. It follows that for all the tasks the subjects can make better movement planning for walking with both the healthy and LLP. Yet, the main goal of this paper was to perform a proof of concept of real-time intraneural sensory restoration to transfemoral prosthetic users and to assess its benefits regarding mobility, agility, embodiment, and cognitive load, aiming to the future translational medical applications. Mechanisms of fine sensorimotor integration are extremely complex and difficult to assess with certainty, especially in amputees. However, we give an interesting insight that could trigger some future works to be more oriented to the mechanistic-related topics.

The subjects reported a multitude of sensation types, many of which can be perceived in real life with the healthy extremity (including touch, pressure, or vibration, which hence we consider natural or similar to the ones perceived with the healthy leg). Similar results were found in a long-term study with hand amputees implanted with transversal intrafascicular multichannel electrodes (TIMEs) (26). Some of the sensations reported by the three subjects were unnatural (for example electricity). We believe that the quality of the restored sensations has a role in acceptability of the device and thus that new encoding strategies must be identified in the future to increase the number of usable active sites eliciting more natural sensory feedback (27, 38–40).

In the three participants, a subgroup of the elicited percepts were described as muscle contraction (S1 and S2) or pressure over muscle (S3) and then connected to the prosthetic knee angle position and its variation (knee flexion/extension). Muscle contraction is a sensation, which is conveyed by Golgi tendon organs (41). Hence, muscle contraction can be considered as a close modality to the proprioceptive sensation because it is connected to a proprioceptive receptor. To restore feedback about the knee flexion/extension, we used muscle contraction from the gastrocnemius, which is responsible for the movement of the ankle but is also activated during the movement of the knee. During physiological walking, the activation of the calf muscles is proportionally related to knee flexion (42). The combination of muscle contraction and prosthesis motion was perceived by the subjects as knee angle position variation. In other words, a specific value of injected charge produced a specific muscle contraction that was interpreted by the subjects as knee-specific position, whereas a change of the charge injected produced a change of the muscle contraction, thus a change of specific knee position, which was interpreted as motion of the knee. The subjects did not need any training to associate the muscle contraction with the flexion/extension of the knee. We just explained the neuroprosthesis functioning to the subjects the first time they used it. In conclusion, the restored sensation for the knee extension/flexion resulted to be both quasihomologous and quasisomatotopic (sensations were elicited from one of all the lower limb muscles involved in the proprioception of the knee), differently to approaches such as noninvasive vibration (15) that is nonhomologous and nonsomatotopic. However, our approach is different than the one recently published (43) that used the illusion of phantom motion evoked by vibration applied on the residual muscles of upper limb amputees to restore the information of motion of the prosthesis. The main difference is in the fact that, when vibration is applied on the muscle, with or without prosthesis, the subjects perceive the motion of the phantom limb. Their

approach is in fact both homologous and somatotopic but is achieved in patients who have undergone targeted muscle reinnervation in the upper limb, without a portable setup.

The use of one of the most advanced microprocessor-controlled knees placed the users in the best conditions with regard to device actuation (44), enabling us to estimate the pure influence of restored sensory feedback on functionality. In future experiments, fully controllable devices should be merged with this neuroprosthesis to achieve close-to-natural bionic replacement of the lost lower limb.

The work has limitations. The study was conducted on only three subjects. Evidence from a larger population, including both transfemoral and transtibial amputees, should be gathered to demonstrate the benefits of sensory feedback restoration through direct nerve stimulation. The trial was conducted for only 3 months per each volunteer because the device had a transcutaneous passage of the electrode cable. A fully implantable solution should be adopted in the future to discover the benefits of this technology for home uses and for longer periods of time. Another set of tests should be conducted for in-home use to show the benefits of the technology in a different setup than the controlled condition one of the laboratory.

MATERIALS AND METHODS
Study design
Objectives of the study were to: (i) prove the feasibility of the restoration of sensations from the missing leg of above-knee (transfemoral) amputees and its real-time connection to the LLP; (ii) test the functional effects of the sensory restoration within the walking experiments over obstacles, stairs and straight line; (iii) indirectly measure cognitive load of walking through a dual-task paradigm; (iv) test the level of the embodiment of prosthetic device induced by the neuroprosthetic intervention. The subjects received the same intervention (artificial sensory feedback elicited through direct nerve stimulation), which was compared to the case in which the same subjects were not provided with sensory feedback through nerve stimulation (within-subject comparison or control condition). These conditions were randomly presented to the subjects. The sample size was not predetermined through statistical methods. The experiments were randomized. The investigators were not blinded to allocation during experiments and outcome assessment. Three subjects were involved in the study. Sensation characterization was distributed in the first month, whereas passive (not involving walking with the prosthesis) and active tasks (walking with the prosthesis) were in the second and third months. Tasks were randomized.

Subjects' recruitment
The subjects were recruited among a population of K4 (the most active users of prostheses) transfemoral amputees. S1 lost the left leg 7 years before the trial, and S2 and S3 lost the right leg 3 and 12 years before, respectively, all as a consequence of trauma. They were all active users of passive prosthetic devices (Ottobock 3R80). Because the amount of time necessary for the experiments did not comply with his job, one subject preferred to not participate in cognitive and embodiment experiments. The study was approved by the ethical commission of the Clinical Center of Serbia and the national competent authorities. All the subjects read and signed the informed consent.

Surgical procedures
The tibial branch of the sciatic nerve of each subject was implanted with four TIMEs (25). The tibial nerve conveys most of the somato-sensory innervation of the foot and ankle along with sensory-motor innervation of the leg. TIMEs were chosen because they have been proven to restore selective and natural sensations in upper limb amputees, whose intensity is proportional with the charge or frequency of the stimulation injected through them, and because of their stability over non-acute periods of time (21, 26, 27, 38). An additional electrode was implanted in the peroneal branch to avoid the (low likelihood) possibility of an anatomical variant in which the peroneal nerve was switched with the tibial nerve. The implant of the intraneural electrodes was performed in an operating room under general anesthesia. The thigh was incised over the sulcus between the biceps femoris and semitendinosus muscles, starting 4 to 5 cm from the amputation stump end. The nerve was isolated by moving the semitendinosus medially and the biceps femoris laterally. The electrode cables were tunneled through the lateral thigh and routed outside the body, to enable connection with the neurostimulator, through five small skin incisions 3 to 5 cm higher than the pelvis ilium.

Sensation characterization
After the implantation, each channel of all the electrodes was connected to a stimulator purposely developed to drive the stimulation of TIMEs (STIMEP, Axonic, and University of Montpellier) (45). The first month after each implant was used to characterize the sensations induced by the stimulation of the residual nerves in detail. For this scope, the sensation characterization (or mapping) procedure was performed, which allowed us to explore the subjects' sensation related to the stimulation from different electrodes and active sites.

Short trains of current pulses with variable intensity, pulsewidth, and frequency were delivered through each active site. Charge-balanced, biphasic, rectangular stimulation pulses were applied versus the TIME ground electrode in a monopolar configuration (46). The single-pulse intensity varied between 10 and 980 µA (steps of 10 µA), whereas duration was set between 10 and 120 µs (according to the active site), and pulse frequency was set to 50 Hz. Each specific set of stimulation parameters was delivered at least three times. Pulse trains with duration of 1 s were delivered with a pause of 2 s.

A custom-made graphical user interface specifically designed for the study was used to control and record the stimulation parameters and to record the subjects' reports on the sensations evoked by the intraneural stimulation. The subjects had to describe the evoked sensations in terms of type, location, extent, and intensity. Regarding the sensation type, they could not only select from a list of words [inspired by the one prepared by Kim and colleagues (47)] but also describe and add a new word, if needed, to avoid a bias (as forcing the participant to associate a sensation with the requirements of the test). Location and extent could be described, indicating one or more surfaces on a virtual foot sole and/or leg. The size of this area was a measure of the extent of the sensation. A number between 0 and 10 could be inserted for the intensity of the sensation [as in (26)]. For kinesthetic percepts (motion of a phantom joint to a specific angle), ankle and knee motions could be assigned to one angle range. If needed, subjects could also freely describe the evoked sensation, correcting any of the predetermined parameters.

The minimum threshold to sensation and the saturation values of the electrical charge were defined. The former parameter was considered as the lowest stimulus charge at which the subject reliably feels a sensation and the latter one as the stimulus charge at which the sensation becomes close to uncomfortable or painful. A map of the sensations reported that referred to the correspondent active sites was

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obtained and used for the calibration of the sensory feedback restoration system.

Prosthesis restoring sensory feedback from the phantom leg

The custom-made LLP was provided and fitted by employees of Össur R&D and included the following commercially available components: RHEO KNEE XC, Pro-Flex XC foot and transfemoral flexible brim socket fitted to an Iceross Seal-In X5 TF silicon liner. Within 1 day, adequate accommodation time and instructions were given (48). The microprocessor-controlled knee has an integrated 14-bit knee encoder, and the knee angle can be communicated with resolution of 1° via Bluetooth low energy at 50 Hz. A sensorized insole, purposely developed for this neuroprosthesis (SensArs Neuroprosthetics), was placed under the prosthetic foot. The insole constituted of a substrate of fabric, on which seven pressure sensors were positioned. The sensors had a resolution of 0.05 kg and a maximum measurable weight of 100 kg. The acquisition and amplification system of the sensorized insole had a sampling frequency of 75 Hz and a Bluetooth module. An external controller (implemented on Raspberry Pi 3, Raspberry Pi Foundation) was wired, through real-time SPI communications, to the external stimulator and communicated via Bluetooth with both the sensorized insole and the RHEO KNEE XC. This portable processor managed the acquisition and recording of sensor readouts and the encoding algorithm, transducing it into stimulation parameters needed for driving the stimulator. The instance with acquisition, recording, and encoding lasted less than 50 ms.

Sensors and active sites were coupled following the results of the sensation characterization procedure (Fig. 3). The readouts of three insole sensors and the knee encoder were used to drive the intraneural stimulation of four active sites. The electrode active sites selected for the neuroprosthetic insole elicited a sensation of touch, pressure, or vibration in a position of the phantom foot that minimized the distance with the position where the insole sensors were placed (fig. S2): One sensor placed in the forefoot area related to a sensation in the forefoot, one to the midfoot, and one to the hindfoot. The knee encoder controlled an active site eliciting a muscle contraction (for S1 and S2 and pressure on the muscle for S3) of the gastrocnemius. We indicate the first three as tactile feedback and the last one as emulated proprioceptive feedback in the manuscript. Active sites with lower operating charge (which is the values of charges between the one eliciting the minimum perceived sensation and the one eliciting the maximum sensation intensity before pain) were preferred. Figure S1 shows the operating charges used for the neuroprosthesis.

The amplitude of the train of pulses was modulated, following the linear relationship described below

$$c = (c_{max} - c_{min}) * (s - s_0)/(s_{max} - s_0) + c_{min}, \quad \text{when } s_0 \leq s \leq s_{max} \tag{1}$$

$$c = 0, \quad \text{when } s < s_0 \tag{2}$$

$$c = c_{max}, \quad \text{when } s > s_{max} \tag{3}$$

where: the refreshment frequency is 20 Hz; c is the amplitude of the pulses of current, s is the readout of sensors; s_0 and s_{max} represent for the insole sensors, the minimum and maximum load recorded

when the subject was walking on a flat rigid surface; s_0 and s_{max} represent 10° and 55° for the knee encoder during functional tasks (see after for passive tasks instead) (31). c_{min} and c_{max} are the current pulse amplitudes that evoked the minimum and below pain threshold sensations, respectively, that were determined during the sensation characterization procedure. The frequency of the stimulation was 50 Hz as in previous literature (21, 26, 49). The same setup was used for each of the subjects. Only the insole size was adapted to best fit with the dimension of the prosthetic foot.

Passive recognition tasks

To demonstrate that the participant could effectively and reliably associate in real-time the sensory feedback to the touch under the insole, a prosthesis movement, or both, three experiments were designed and carried out: touch recognition task ($n = 92$ repetitions for S1; $n = 120$ for S2; $n = 140$ for S3), proprioception recognition task ($n = 72$ repetitions for S1; $n = 120$ for S2; $n = 120$ for S3), and combined task ($n = 72$ repetitions for S1; $n = 308$ for S2; $n = 180$ for S3).

The subjects, lying comfortably on a bed, performed these trials blindfolded and acoustically isolated. They did not receive any systematic and prolonged training. The experiments for each subject were conducted during different days.

During the touch recognition task, the subjects were instructed to recognize four (for S1 and S2) or three (for S3) different positions under the insole randomly touched by the experimenter in addition to the no-touch condition. The subjects had to indicate the perceived location of solicitation on a rubber foot they were holding and to declare by voice. The positions on the insole were medial, central and lateral metatarsus, and heel for S1 and S2 (Fig. 4A), whereas the positions on the insole were hallux, central metatarsus, and heel for S3.

For the proprioception recognition task, the subjects were instructed to discern four different prosthetic knee flexion/extension angles randomly applied by the experimenter. In Eqs. 1 to 3, s_0 and s_{max} were set to 0° and 95°, respectively, only for the purpose of this exercise. The subjects had to report the perceived flexion/extension. The knee angles were: extension (0°), small flexion (5° to 15°), medium flexion (45° to 55°), and full flexion (85° to 95°) (Fig. 4B). During the combined task, the subjects were instructed to recognize three (extension, small, and full flexion for S1 and extension, medium, and full flexion for S3) or four (extension, small, medium, and full flexion for S2) knee angles combined with two (heel on and heel off for S1) or four (central metatarsus off and heel off, central metatarsus on and heel off, central metatarsus off and heel on, and central metatarsus on and heel on for subjects 2 and 3) touch conditions randomly reproduced by the experimenter. The volunteers had to report perceived combination.

The subjects were asked to execute tasks of similar difficulty. However, sometimes, a level of difficulty made it hard to accomplish for some subjects; therefore, we simplified the task: For example, S3 was confused by the recognition of four positions under the foot sole (with respect to S1 and S2), so he performed the task with three positions.

During the control condition experiments, we compared the performance in the tasks when intraneural stimulation was not provided and the prosthesis was donned. All the experiments conditions were randomized.

Functional tasks

Stairs and obstacle tests were run in four different conditions: NF, T, P, and PT. Straight-line tests were run in two conditions: NF and PT for all subjects. This experiment was repeated only with these two

conditions because the subjects found it very challenging and did not feel comfortable with performing it many times. PT was selected among the stimulation conditions because it is the one restoring the most informative sensory feedback, being the combination of P and T. All the stimulation conditions were randomly presented to the volunteers.

During the stairs test, the subjects were asked to go through a course of stairs in sessions of 30 s per 12 times per condition. The setup was configured as an angular staircase endowed with six steps with height of 10 cm and depth of 28 cm on one side, and four steps with height of 15 cm and depth of 27.5 cm on the other. Subjects had to walk clockwise climbing up the six steps and going down on the four steps. A lap is intended as going up and down the stairs and reaching the starting position again. Velocity was the selected feature to asses mobility, being one of the most relevant and straightforward indicators in clinical practice. Medicare uses it to assess the ambulation capability of LLA (50).

During the obstacle test, the subjects were asked to walk at their self-determined maximum comfortable speed, through a platform endowed with randomly disposed climbing grips of different sizes (from $3 \times 3 \times 1$ cm to $7 \times 7 \times 7$ cm). The platform was created ad hoc for fitting between parallel bars (5.50×0.56 m), which were used as supports in case of falls of the participants. They were asked to not rely on the bars during walking. They wore modified glasses that prevented them from watching their steps and therefore from knowing where the obstacles were placed. Errors (indicated as falls in Fig. 5) were counted when the subjects, stepping on an obstacle with the prosthesis, lost balance and had to rely on the bars (either by grasping them or leaning the body on them) to prevent a fall. The number of falls was counted by two operators visioning slow-motion videos of the sessions. Experiments were configured in 12 sessions of 30 s per feedback condition. The error count was computed as the ratio between falls and number of total trampled obstacles (with the prosthesis) in the session. Only slip falls (traction/misbalance between feet and obstacle surface, when stepping on it, resulting in loss of balance) were counted, whereas the trip-related falls (foot hits an obstacle and upper body continues moving, resulting in loss of balance) were excluded because the users were not able to counteract them, being unable to actively control the knee movement. However, the number of trip-related falls was computed and resulted to be balanced among feedback conditions.

In the straight-line test [adapted from (29, 30)], subjects were asked to walk on a straight line (5 m long) putting their feet one after the other. Whenever the subjects failed to precisely walk on the line, an error was counted (indicated as steps out/all steps in Fig. 5). Only the steps executed with the prosthesis were taken into account. The experiment was repeated nine times per feedback condition. One repetition constituted of travelling the line twice (in two different directions). The order of the conditions was randomized.

Embodiment evaluation
After every condition of execution of the functional tasks (no feedback, tactile, proprioceptive, tactile, and proprioceptive feedback during obstacles, stairs, straight line), the subjects' integration of the prosthesis in the body image was assessed, through objective (proprioceptive displacement) and subjective measures (questionnaire). The proprioceptive displacement was defined as the spatial difference between the position of the phantom hallux after the functional task and the real position of the prosthesis. In other words, the less is the displacement, the more is the embodiment of the phantom lower limb

with the prosthesis. For this reason, in the manuscript, we indicated this measure as proprioceptive displacement and not drift as it is done in the literature (31, 32, 51). To determine this value, the subject, after the functional task, laid down on a bed. At its bottom, the experimenter moved a little pole along a rail in the frontal plane (Fig. 6). A ruler was disposed in parallel to it. The starting point for the shaft was changed randomly to avoid any learning process and hence bias for the subjects. A support for the bed sheets was disposed to avoid the subject to look at the feet.

The questionnaire was composed of eight questions (31, 32). Three of them were a direct measure of the embodiment, referring to the extent of sensory transfer into the prosthetic foot and self-attribution of it during the trial; three were control statements for assessing suggestibility; and two were about vividness, how life-like and realistic the illusion was, and prevalence, assessing the percentage of time that the illusion was experienced (equivalent to the continuance of the illusion), during the active tasks. The first six were rated with a score between −3 and 3, the vividness from 0 to 10, and the prevalence from 0 to 100%. Questions were presented in random order.

Both the questionnaire and the proprioceptive displacement measurements were repeated 10 times per stimulation condition. Each measure of the proprioceptive displacement was determined as the average of three recordings. The full questionnaire is in table S2.

Cognitive load assessment
To evaluate the effect of sensory feedback on the cognitive load due to use of the prosthesis during walking, a three-class oddball auditory task was executed. The protocol proposed by Zink et al. (33) and Polich et al. (34) was reproduced. A standard sound (900 Hz) and two deviant tones (attended or target and non-attended or deviant tones at 600 and 1200 Hz), lasting 80 ms, were delivered to the participants in a random order through commercial headphones. The participants were told that the target tone was either 600 or 1200 Hz.

We expected that if the task was well executed, then the attention of the subjects to the target tones would have been higher than the attention to the deviant and standard tones. This would have generated a P300 ERP component due to target tones higher than the P300 ERP component due to deviant and standard tones (34). The hypothesis (33) was that if more cognitive power was consumed to pay attention to walking, then less attention would have been used for counting the tones with consequently low P300 amplitude recorded.

An android operating system mobile phone ran the Neurobs Presentation for mobile software (to deliver stimuli and synchronous trigger) and was connected to the wireless SMARTING mobile EEG amplifier (mBrainTrain) via Bluetooth to guarantee synchronous stimuli and EEG data recording. A mean interstimulus interval of 1000 ms (with jitter timing of 200 ms) was used.

Because the same participant was repeating several sessions of the experiment, we varied the occurrence timings of all tones and the number of target and deviant tones: The number of standard tones was always the same (342), whereas the number of target and deviant tones varied between 41 and 46. The participants had to count the target tones in silence and ignore the other tones while sitting (control condition) or walking on a hard flat surface with and without the nerve stimulation (PT). The walking route was predefined and practiced before the start of the experiment. The order of the conditions was balanced and randomized between subjects. Every condition was repeated twice. The distance that was covered during walking by each subject in both stimulation conditions was similar.

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EEG data acquisition was performed with a sampling frequency of 500 Hz and 24-bit data resolution. The EEG cap was a 24-channel sintered Ag/AgCl cap (Easycap). Electrodes were placed according to the international 10-20 system. The electrodes were referenced to FCz and the ground electrode was AFz.

EEGLAB (34) and MATLAB 2016b (MathWorks Inc., Natick, MA, USA) were used to process EEG signals offline. EEG data were bandpass filtered in the 1- to 30-Hz range, and then the signals were referenced to the average of EEG from the mastoid channels (Tp9 and Tp10). To remove artifacts, an independent component analysis was executed [as explained in (52, 53)]. After data preprocessing, we considered the ERP epochs in the interval [−200, 800] ms with respect to the auditory stimulus timestamp. Then, we excluded the trials, where the ERP amplitude exceeded the artifact threshold of ±100 µV. After this, we were left with 38 trials for plotting and statistical analysis. Last, the P300 amplitude was computed as the average of the data from Pz electrode in the interval [450, 650] ms after the stimulus presentation. The P300 component is mostly represented over the parietocentral scalp location for our setup (54). We computed the grand average ERPs for all the conditions.

Data collection

During the experiments with the prosthesis, the following information was recorded: seven sensor readouts of the sensorized insole under the prosthetic and healthy foot, RHEO KNEE XC encoder, reported sensations, stimulation current amplitude, frequency, and pulse width for each channel used to encode sensations during the task.

Two cameras were used for recording videos from two planes of motion in all the tasks. Lateral hip, lateral ankle, and knee position data from both legs were offline extracted for the obstacle task, LLP lateral ankle data were extracted for the stairs task, and LLP foot tip and dorsal side of the ankle data were extracted for the straight-line task. Data extraction was done in Kinovea (freeware). The main goal of the motion capture was to track macroscopic kinematic data as velocity, stepping in/out, and falling/not falling over the bars, rather than detailed kinematic analysis.

The markers were tracked with respect to a fixed point in the frames of the video recordings (for example, the stairs corner touching the floor). The calibration was executed, by using the known dimension of the setup involved in the tests (namely, height of the stairs or obstacles support and width of the straight line). Embodiment questionnaires were recorded for each subject. EEG data were recorded as explained above. Technical data concerning the use of STIMEP were logged to monitor stimulator behavior and electrode states through contact check. These data confirmed the high reliability of the system as no failure, and no embedded software crashes were detected. Moreover, embedded safety procedures ensured charge injection limits independently from the high-level software. Last, contact check procedures were executed to follow-up functionality of the used contacts.

Statistical analysis

The acquired data were exported and processed offline in MATLAB R2016b (MathWorks). All data were reported as mean values ± SD (unless elsewise indicated). The normality of data distributions was verified. In case of Gaussian distribution, two-tailed analysis of variance (ANOVA) test was applied. Elsewise, we performed two-tailed Kruskal-Wallis test. Post hoc correction was executed in case of multiple groups of data. Significance levels were 0.05 unless differently reported in the figures' captions. In the captions of the figures, we reported the used statistical tests for each analysis and its result, along with the numerosity of the distributions. In the Supplementary Materials, the raw data for distribution with less than 20 samples are reported.

SUPPLEMENTARY MATERIALS

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Fig. S1. Perceived magnitude scale of the evoked sensations.
Fig. S2. Stimulation parameters in the neuroprosthesis.
Fig. S3. Passive task individual confusion matrices.
Fig. S4. Control condition passive tasks.
Fig. S5. Validation measures during obstacles.
Fig. S6. Validation measures during straight line.
Fig. S7. Validation measures of cognitive load assessing.
Table S1. Active sites-sensations correspondence.
Table S2. Embodiment questionnaire.
Movie S1. Neuroprosthesis restores missing leg sensations.
Movie S2. Neuroprosthesis working principle and active tasks.
Data file S1. Raw data for distributions from all the figures in the main text and supplementary materials.

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Enhancing functional abilities and cognitive integration of the lower limb prosthesis

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Good vibrations
The lack of sensory feedback from the leg prosthesis in lower limb amputees is associated with risk of falls, low mobility, and perception of the prosthesis as external object. Here, Petrini *et al.* tested a leg neuroprosthesis, which provided real-time on-demand tactile sensory feedback through nerve stimulation in three transfemoral amputees. The stimulation improved mobility, decreased falling episodes, and increased the perception of the prosthesis as part of the body. Active complex tasks were accomplished with reduced effort when the nerve stimulation was turned on. The results suggest that real-time nerve stimulation could help restore natural sensation in lower leg amputees.

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