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Supplementary Materials for

High-frequency epidural electrical stimulation reduces spasticity and facilitates walking recovery in patients with spinal cord injury

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Supplemental Materials and Methods

Neurosurgical procedure

Both participants underwent a surgical procedure of lumbar laminectomy, paddle (CoverEdge X 32 lead, Boston Scientific Spa) lead insertion and internal pulse generator (IPG) implantation under general anesthesia (P1, April 2023; P2, July 2023). After placing the patient in a prone position, an intraoperative x-ray fluoroscopy (Artis Pheno Robotic C-arm Angiography system, Siemens Healthineers GmbH) was performed to map the spine anatomy and to guide the incision. An approximately 6-cm midline skin incision was performed, the muscle fascia was opened, and the paraspinal muscles were retracted bilaterally. Excision of the midline ligamentous structures and laminectomy between L2 and L3 was performed, and the dura mater was exposed to enable the insertion of the paddle in the epidural space. Preoperative MRI of the spine was used to determine the spinal level for laminectomy by identifying the termination of the conus medullaris and to ensure that this area and the beginning of the cauda equina were covered by the lower portion of the paddle. A commercial 67 mm long, 32-contact paddle with contact spacing of 3 mm was used (CoverEdge X 32 lead, Boston Scientific Spa). The paddle was placed over the midline and advanced rostrally to the target location of the intervertebral disc between T11 and T12 with the aim of recruiting the iliopsoas muscles through the more rostral paddle contacts (**Fig. 1**).

EMG of lower limb muscles was conducted intraoperatively using the Neuroexplorer 2019 monitoring (Inomed Medizintechnik GmbH) while EES was applied through the stimulation system (Boston Scientific Spa) connected to the implanted paddle to ensure that all the desired muscles were recruited by EES. Single EES pulses (2 Hz) were delivered at increasing amplitude to elicit muscle responses that were recorded from subdermal or intramuscular needle electrodes. After the neurophysiological evaluations, the paddle was secured using anchors sutured to the ligaments, and a final x-ray fluoroscopy was then acquired to register the final position of the paddle. Thereafter, an IPG (Wavewriter alpha 32, Boston Scientific Spa) was inserted into a subcutaneous pocket in the participant's abdomen. The paddle array cables were then tunneled between both openings and connected to the IPG.

Neuro-rehabilitation protocol

A comprehensive physiotherapy assessment of (i) single joints residual movements, (ii) motor strategies with the ability to isolate individual motor gestures, (iii) muscular recruitment capability, (iv) muscle tone, (v) postural transitions, (vi) postural transfers, (vii) upright stance, (viii) balance and control of trunk, (ix) functional gestures, and (x) walking residual abilities was performed as an initial stage. For patient P1, a reevaluation of the orthotic devices in use and of her compensatory motor strategies was necessary to improve the future rehabilitative procedures. Specifically, to manage the tibiotarsal inversion primarily caused by the hypertonia of the posterior tibialis muscle, the Codeville spring was replaced with the Dyna-Ankle orthosis (Ottobock). In addition, to reduce the elevation of the left heel along with the push on the left forefoot, associated also with contralateral hip vaulting, during the advancement of the right lower limb (during the swing phase of the gait), a full-length 1.5 centimeters height insole was inserted beneath the left shoe. Patient P2 was equipped with orthotic devices to correct improper movements during walking. Two Peromed orthoses were used to manage the inversion of the subtalar joint of both ankles already during treadmill-walking training; two other orthotic devices were added for overground walking to handle dynamic knee valgus and knee hyperextension during the stance phase.

The rehabilitation protocol implemented during the weeks of hospitalization of both participants initially focused on favoring stretching to temporarily inhibit the hypertonia of the ankle plantar flexors and knee extensors, facilitate the training of residual isolated movements, maintaining an upright stance, and walking with body weight support. Then, once identified optimal LF-EES protocols associated with the activation of isolated muscle groups (see section "EES protocols – LF-EES"), single-joint and multi-joint movements were trained in different positions while applying EES. More specifically, we trained hip flexion in both supine and seated positions, knee flexion in supine and seated positions, and ankle dorsiflexion in supine position with the hip and knee supported and flexed at 90 degrees, using the electrode configurations shown in Fig. 2B and Figs. S1 and S2. During training, augmented feedback through Virtual Reality and Inertial Measurement Units (VRRS, Khymeia S.R.L) was also employed to enhance sensorimotor control and guide exercises toward oriented and repetitive tasks (**fig. S8B**). In addition to these exercises, we trained the patients to maintain an upright position on different terrains and control balance in static conditions such as anterior-posterior and medio-lateral displacement of center of pressure, with and without arm support. Gait training was also performed, initially using a self-paced omnidirectional treadmill with instantaneous feedback on performance through Virtual Reality (MoonWalker, Khymeia S.R.L.) (**fig. S8C**). To maximize motor learning of locomotion, a complete 100% body weight support was used in the initial weeks, which was then gradually scaled down to reach 0%. Finally, the two patients proceeded with overground walking, patient P1 with crutches, patient P2 with a two-wheeled walker. Walking in different scenarios was trained using the stimulation protocols combining LF-EES and HF-EES (see section "EES protocols – Final EES protocols"). Functional exercises such as repetitive sit-to-stand, stand-to-sit and climbing stairs (only for patient P1) were also incorporated while the rehabilitation progressed. During the hospitalization period, 15 hours of rehabilitation per week were provided, divided into approximately 3 hours per day. The percentage distribution of the different activities is shown in Fig. S8A. At discharge, the patients were provided with a kit containing a dedicated tablet and wearable sensors (VRRS Home-Kit, Khymeia) to perform exercises based on Virtual Reality and augmented feedback at home (**fig. S8D**). The system automatically reports the exercises and repetitions performed by the patient in a daily diary and compares them to those prescribed by the physiotherapist. Additionally, the physiotherapist contacts the patient every 10 days to monitor the progress and accordingly adjust the type and quantity of the exercises. At home, patient P1 and P2 continued using the same LF-EES and HF-EES protocols for single-joint and multi-joint movements, and the protocol combining LF-EES and HF-EES for walking.

Longitudinal assessment measures

Medical Research Council (MRC) scale. Evaluates muscle function on six levels:

- 0: no visible contraction;
- 1: flicker or trace of contraction.
- 2: active movement, with gravity eliminated.
- 3: active movement against gravity.
- 4: active movement against gravity and resistance.
- 5: normal power.

When the MRC grade for a muscle was higher than 2, we measured muscle force through a hand-held dynamometer (see "Data acquisition" section for details).

Modified Ashworth scale (MAS). Muscle tone assessment scale used to measure the resistance experienced during passive motion of a joint, on five values:

- 0: no increase in muscle tone.
- 1: slight increase in muscle tone, manifested by a catch and release or by minimal resistance at the end of the range of motion when the affected part is moved in flexion or extension.

- 1+: slight increase in muscle tone, manifested by a catch and release or by minimal resistance throughout the remainder (less than half) of the range of motion.
- 2: marked increase in muscle tone, but easy passive motion.
- 3: considerable increase in muscle tone, passive movement is difficult.
- 4: rigidity in flexion or extension.

Timed up and go test (TUG). Assessment of a person’s mobility. It consists of measuring the time it takes to rise from a chair, walk three meters, turn around 180 degrees, walk back to the chair, and sit down while turning 180 degrees. Patient P1 performed the test assisted by crutches, whereas patients P2 assisted by a walker.

Six-minute walk test (6MWT). Sub-maximal exercise test used to assess aerobic capacity and endurance. The measured outcome is the distance travelled over the six minutes’ time. Patient P1 performed the test assisted by crutches, whereas patients P2 assisted by a walker.

Borg rated perceived exertion (RPE). Measurement of perceived exertion rated by the patient after an activity. In the literature, correlation has been shown between its values and heart rate (estimated heart rate = 10 × Borg RPE). It ranges from 6 to 20, with the following interpretation:

- 6: no exertion at all;
- 7: extremely light;
- 9: very light;
- 11: light;
- 12: moderate;
- 13: somewhat hard;
- 15: hard;
- 17: very hard;
- 19: extremely hard;
- 20: maximal exertion.

Supplementary Figures

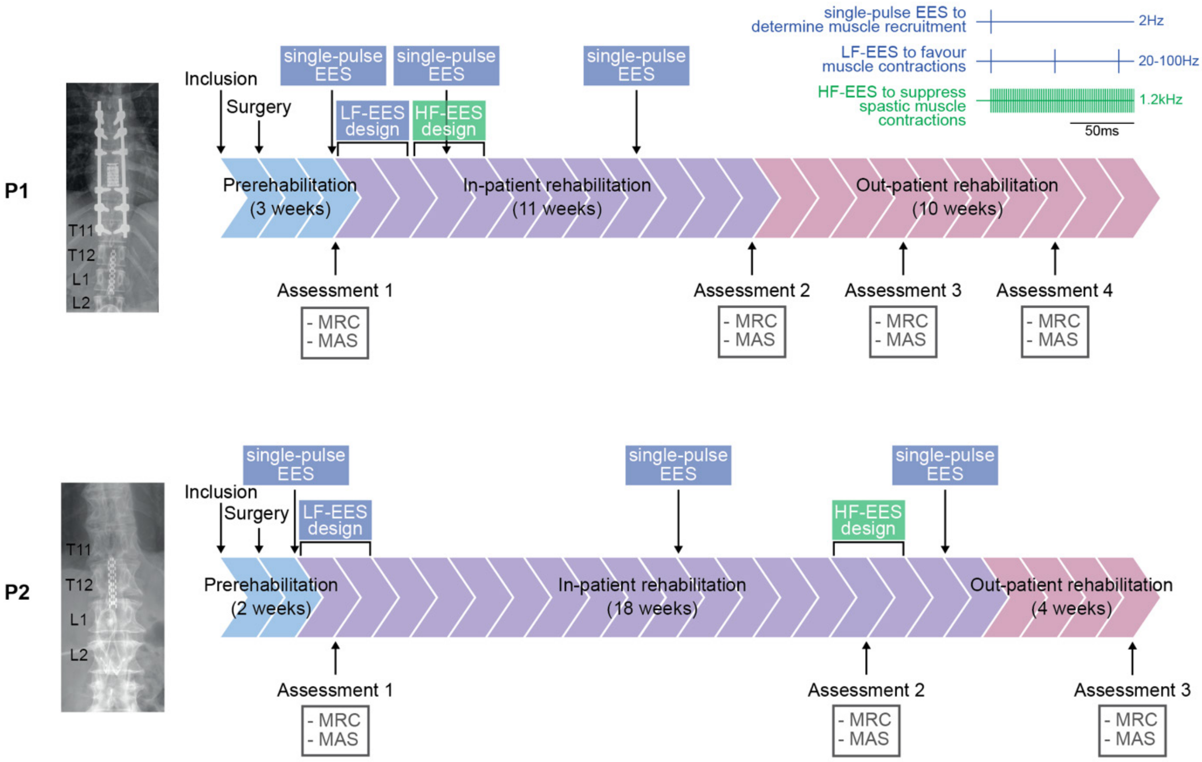


Fig. S1. Experimental protocol. The two patients participated in a 6-month clinical trial with the aim to investigate the effects of epidural electrical stimulation (EES) combined with locomotor training on the recovery of motor function. They were implanted with a commercial epidural paddle, as shown in the X-rays images of the spine. They then underwent a rehabilitation program that included an inpatient and a home therapy phase and was carried out with EES. Single-pulse EES (2 Hz) was performed just after implantation to determine the muscle recruitment for different electrode configurations and identify those that were optimal for specific movements; it was then repeated three times throughout the hospitalization period of the two participants to assess temporal stability. Low-frequency EES (LF-EES) protocols (20-100 Hz) were then designed to enhance voluntary isolated movements and then complex tasks, such as walking. High-frequency EES (HF-EES) protocols (1.2 kHz) were eventually introduced to suppress spastic muscle contractions and thus improve the performance of LF-EES alone. Clinical assessments of muscle function and spasticity were performed throughout the study period. MRC, Medical Resource Council; MAS, modified Ashworth scale.

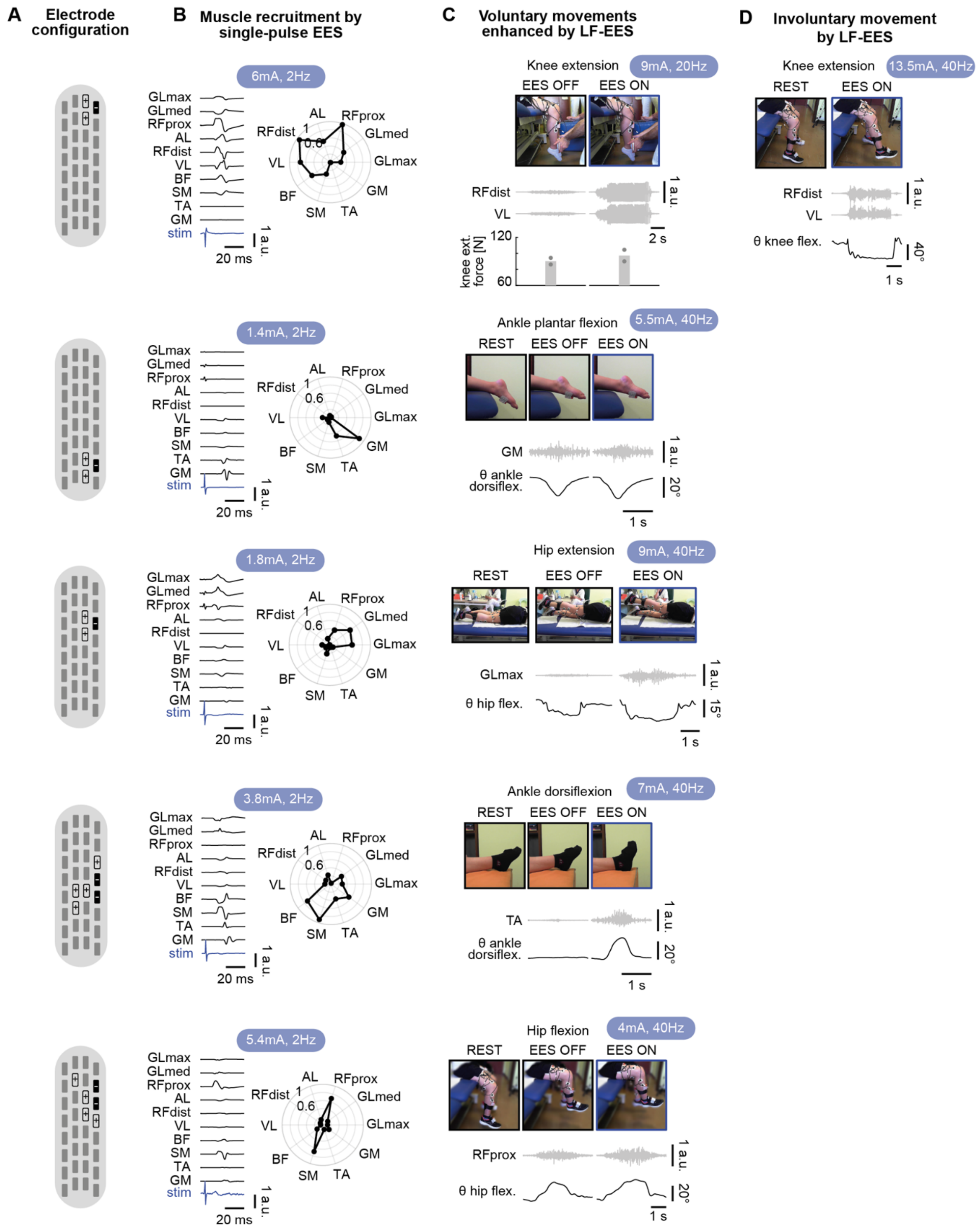
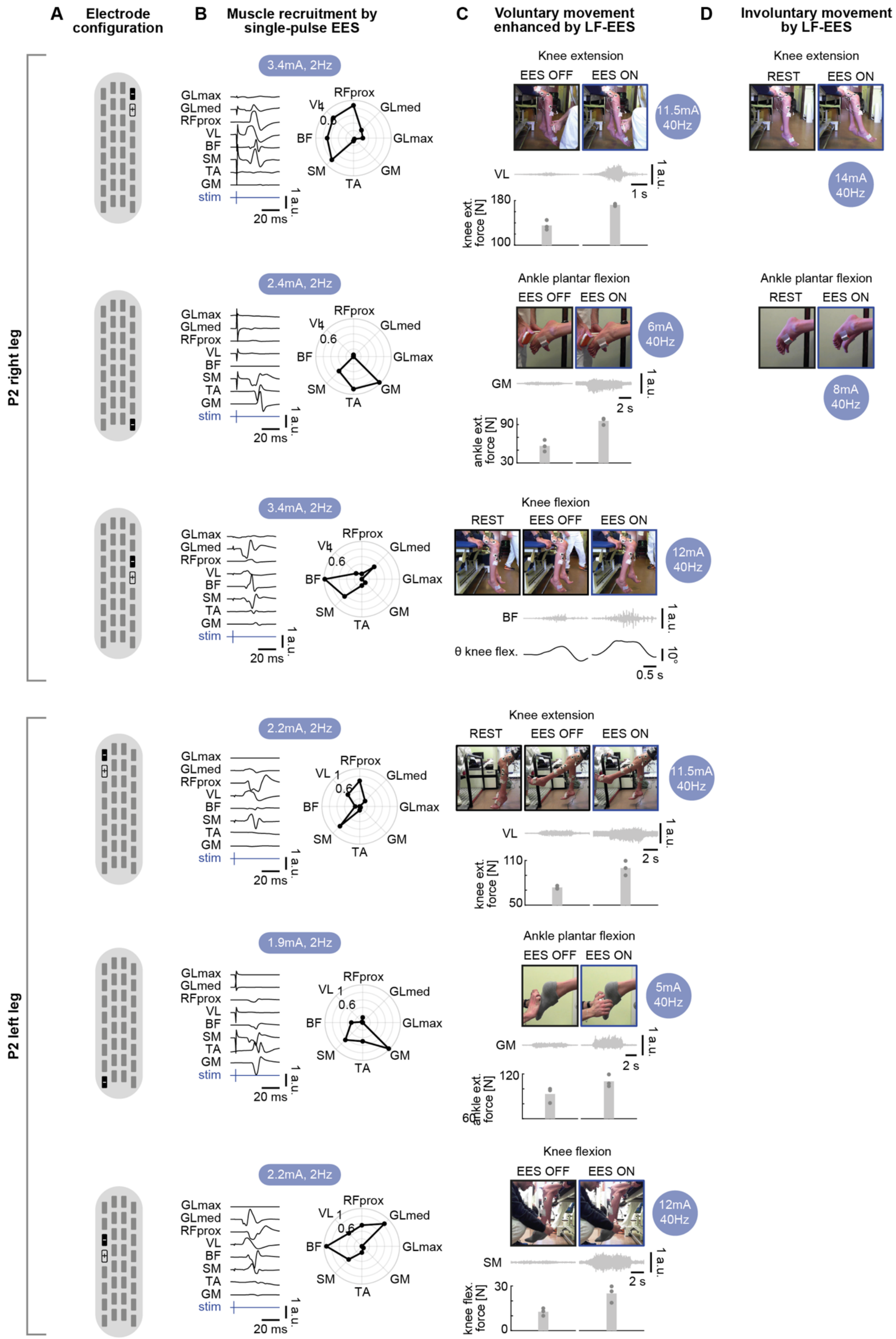


Fig. S2. LF-EES protocols to enhance single-joint movements of the right leg of participant P1. (A) Electrode configurations used to deliver LF-EES to enhance the different movements. **(B)** Muscle recruitment by single-pulse EES (2 Hz) delivered with the electrode configurations shown in (A). Normalized EMG recordings of the indicated muscles during a single stimulation pulse (left). Scale, y-axis, 1 a.u.; x-axis, 20 ms. Radar plots of the normalized muscle recruitment by the same stimulation pulse (right). Scale, 1. **(C)** Volitional motor response with and without enhancement with LF-EES delivered with the electrode configurations shown in (A). For each movement, representative photographs showing the targeted joint during rest, during attempt at voluntary movement with LF-EES off, or with LF-EES on. Normalized EMG activity of the agonist muscle with LF-EES off or on. Scale, y-axis, 1 a.u. Kinematic measurement of joint angle (when the patient could not perform a full-range movement without EES) or force measurement (when the patient could perform a full-range movement without EES). **(D)** Involuntary knee extension movement evoked by LF-EES. Representative photographs showing the targeted joint during rest and with LF-EES on. Normalized EMG activity of the agonist muscle with LF-EES on. Scale, y-axis, 1 a.u. Kinematic measurement of knee flexion angle. In all panels and for each target movement, stimulation amplitude and frequency are indicated in blue. GLmax, gluteus maximus; GLmed, gluteus medius; RFprox, proximal rectus femoris; AL, adductor longus; RFdist, distal rectus femoris; VL, vastus lateralis; BF, biceps femoris; SM, semimembranosus; TA, tibialis anterior; GM, gastrocnemius; stim, stimulation. Illustrations created with Adobe Illustrator.



A Electrode configuration
B Muscle recruitment by single-pulse EES
C Voluntary movement enhanced by LF-EES
D Involuntary movement by LF-EES
P2 right leg
P2 left leg
3.4mA, 2Hz
2.4mA, 2Hz
2.2mA, 2Hz
1.9mA, 2Hz
GLmax
GLmed
RFprox
VL
BF
SM
TA
GM
stim
20 ms
1 a.u.
0.6
Knee extension
Ankle plantar flexion
Knee flexion
REST
EES OFF
EES ON
11.5mA 40Hz
14mA 40Hz
6mA 40Hz
8mA 40Hz
12mA 40Hz
5mA 40Hz
1 s
2 s
0.5 s
knee ext. force [N]
ankle ext. force [N]
knee flex. force [N]
θ knee flex.
10°

Fig. S3. LF-EES protocols to enhance single-joint movements of both legs of participant P2. (A) Electrode configurations used to deliver LF-EES to enhance the different movements. **(B)** Muscle recruitment by single-pulse EES (2 Hz) delivered with the electrode configurations shown in (A). Normalized EMG recordings of the indicated muscles during a single stimulation pulse (left). Scale, y-axis, 1 a.u.; x-axis, 20 ms. Radar plots of the normalized muscle recruitment by the same stimulation pulse (right). Scale, 1. **(C)** Volitional motor response with and without enhancement with LF-EES delivered with the electrode configurations shown in (A). For each movement, representative photographs showing the targeted joint during rest, during attempt at voluntary movement with LF-EES off, or with LF-EES on. Normalized EMG activity of the agonist muscle with LF-EES off or on. Scale, y-axis, 1 a.u. Kinematic measurement of joint angle (when the patient could not perform a full-range movement without EES) or force measurement (when the patient could perform a full-range movement without EES). **(D)** Involuntary movements evoked by LF-EES. Representative photographs showing the targeted joint during rest and with LF-EES on. In all panels and for each target movement, stimulation amplitude and frequency are indicated in blue. GLmax, gluteus maximus; GLmed, gluteus medius; RFprox, proximal rectus femoris; VL, vastus lateralis; BF, biceps femoris; SM, semimembranosus; TA, tibialis anterior; GM, gastrocnemius; stim, stimulation. Illustrations created with Adobe Illustrator.

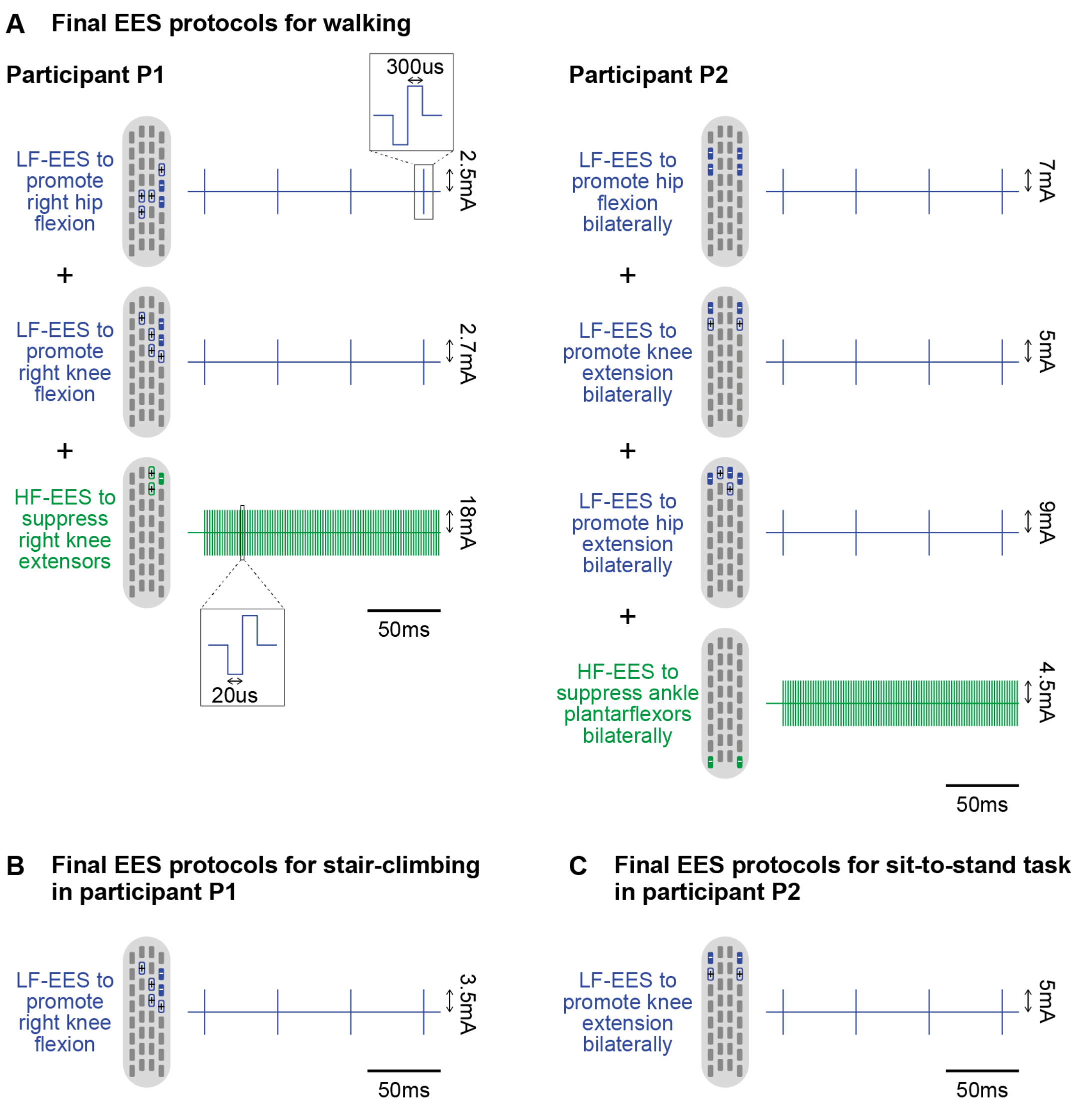


Fig. S4. Final EES protocols in the two patients. **(A)** Final protocols for walking in the two participants, which combined LF-EES to promote specific single-joint movements and HF-EES to suppress undesired muscle co-contractions. (**B**) Final protocol for stair-climbing in participant P1, which was based on LF-EES applied with the electrode configuration that promoted right knee flexion. (**C**) Final protocol for sit-to-stand task in participant P2, which was based on LF-EES applied with the electrode configuration that promoted bilateral knee extension. In all panels, for each subprotocol, the electrode configuration and the applied stimulation wave are shown. Scale, x-axis, 50 ms. Illustrations created with Adobe Illustrator.

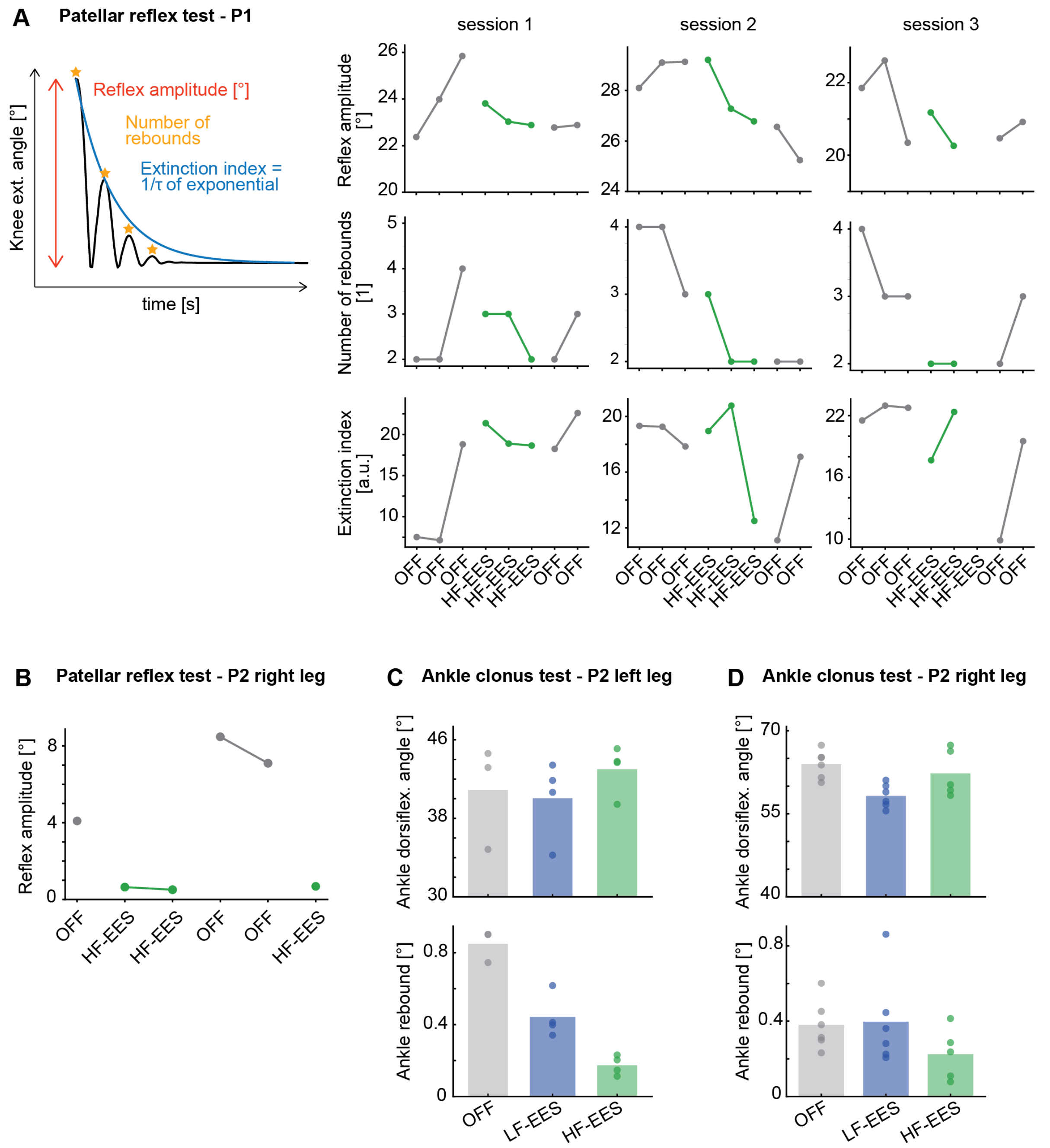


Fig. S5. Effect of HF-EES on stretch reflexes. (A) Left: schematic representation of the three kinematic features extracted on the temporal trace of the knee extension angle, that is, reflex amplitude, number of rebounds, and extinction index, during the patellar reflex test in patient P1. Right: values of reflex amplitude, number of rebounds, and extinction index for three different days (sessions) of patellar reflex test in participant P1, showed for subsequent trials under stimulation off or HF-EES condition. The kinematic signals recorded for the third repetition under HF-EES in session 3 were corrupted, thus they were excluded. **(B)** Reflex amplitude during subsequent trials (under stimulation off or HF-EES condition) of patellar reflex test in patient P2, performed on a single day. **(C)** Ankle dorsiflexion angle (range of motion) and ankle rebound during the clonus test, a test of rapid dorsiflexion stretch, of the left ankle in patient P2 with no stimulation, LF-EES, or HF-EES. Each point represents a repetition of the test (n = 3 repetitions for the no stimulation condition; n = 4 repetitions for the LF-EES and HF-EES conditions; repetitions performed on two different days), bars indicate the average across repetitions. **(D)** Ankle dorsiflexion angle (range of motion) and ankle rebound during the clonus test of the right ankle in patient P2 with no stimulation, LF-EES, or HF-EES. Each point represents a repetition of the test (n = 6 repetitions for the no stimulation and LF-EES conditions; n = 5 repetitions for the HF-EES condition; repetitions performed on two different days), bars indicate the average across repetitions.

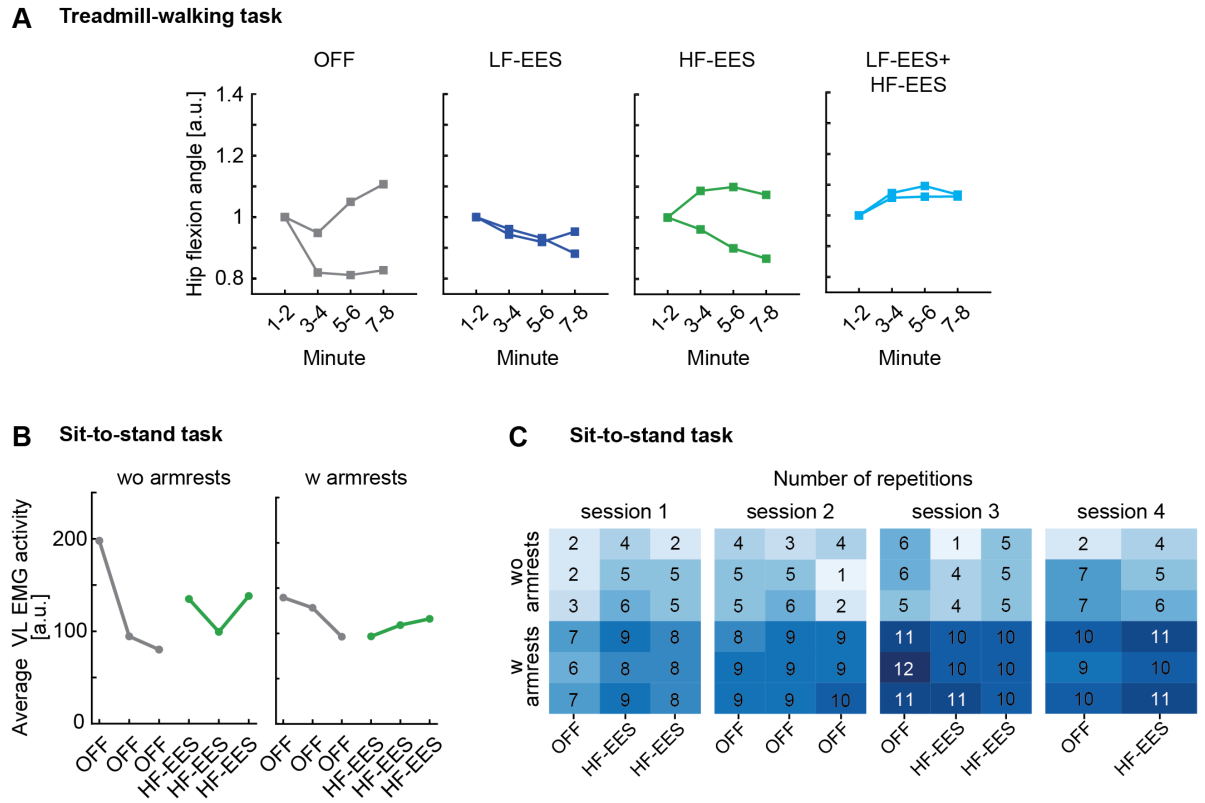


Fig. S6. Effect on HF-EES in functional tasks in patient P1. (A) Hip flexion angle for the treadmill-walking task under four stimulation conditions (LF-EES, HF-EES, and LF-EES plus HF-EES). Each point represents the average of the hip flexion angle every 2 minutes of the task, normalized with respect to the first point; connected points represent one repetition of the task (*n* = 2 repetitions per stimulation condition, each repetition performed on a different day). **(B)** Average EMG activity of the vastus lateralis (VL) during the sit-to-stand task in an exemplary session; each point represents the average EMG activity across one repetition of the test. **(C)** Number of sit-to-stand raises (successes) performed in the sit-to-stand task under different conditions, that is, with or without armrests, and under stimulation off or HF-EES conditions, and in different sessions.

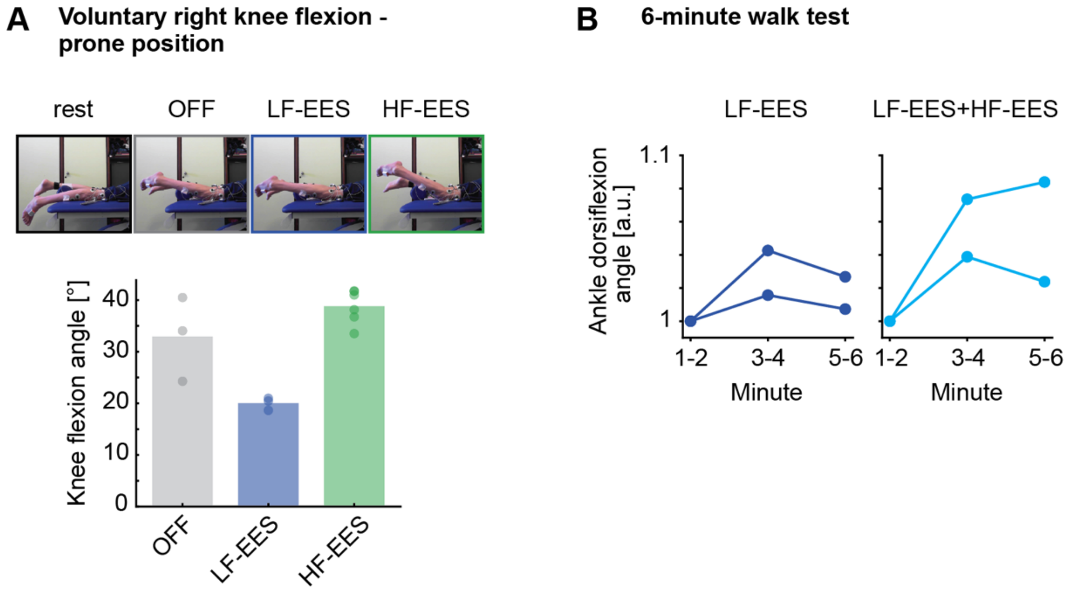


Fig. S7. Effect of HF-EES in functional tasks in patient P2. (A) Voluntary knee flexion in prone position with stimulation off, LF-EES targeting the knee flexors, and HF-EES targeting the knee extensors. Snapshots showing the maximum deviation of knee flexion for example repetitions of the test (top). Range of motion of the knee flexion angle (bottom); each point represents a repetition of the test (n = 3 for the stimulation off and LF-EES conditions; n = 6 for the HF-EES conditions; repetitions performed on a single day), bars indicate the average across repetitions. **(B)** Ankle dorsiflexion angle during the six-minute walk test with the LF-EES protocol for walking, and with the protocol combining LF-EES and HF-EES. Each point represents the average of the ankle dorsiflexion angle every 2 minutes of the task, normalized with respect to the first point; connected points represent one repetition of the task (n = 2 repetitions per stimulation condition, each repetition performed on a different day).

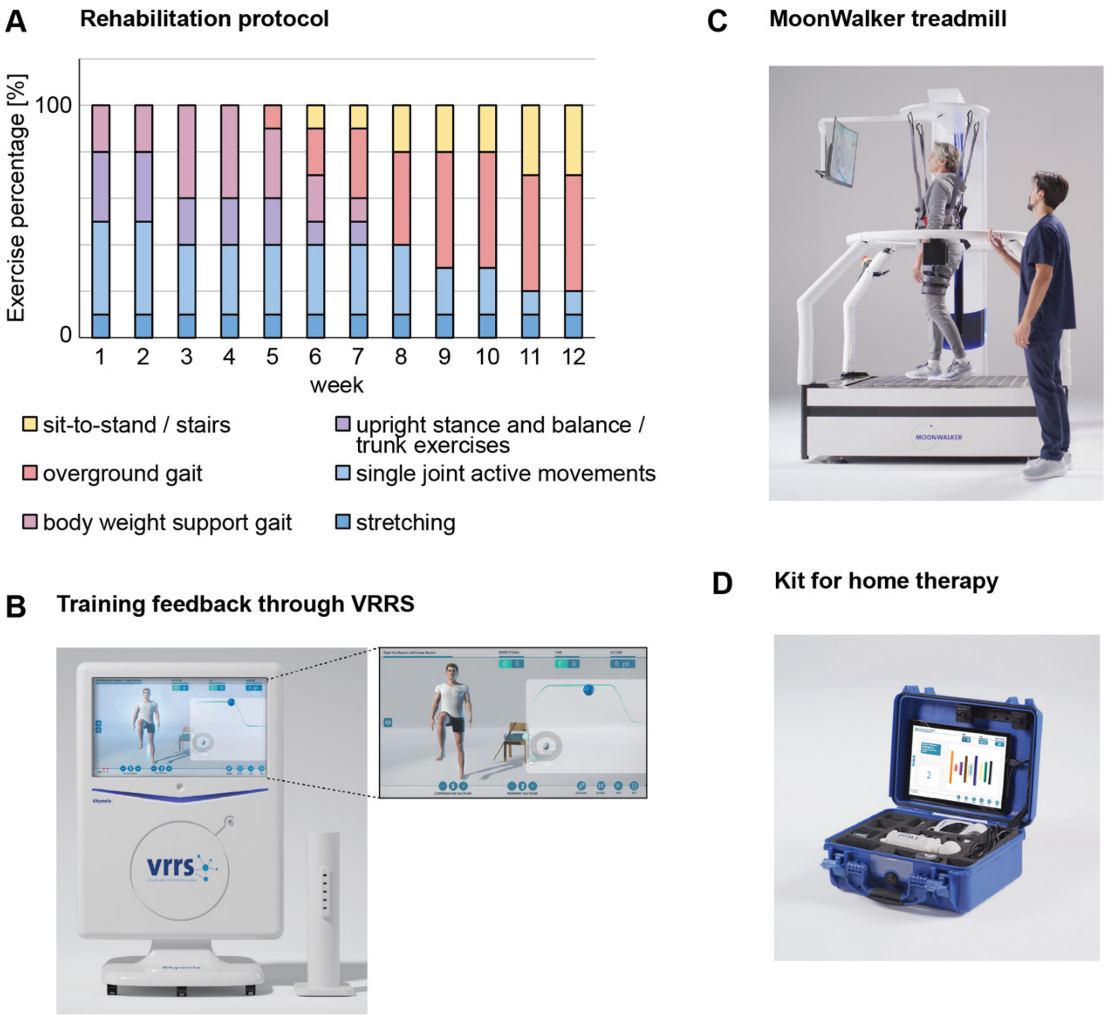


Fig. S8. Tools and methods for neurorehabilitation. (A) Percentage distribution of the different rehabilitation activities over weeks. **(B)** Picture of the Virtual Reality Rehabilitation System (VRRS, Khymeia S.R.L.), a device for augmented feedback based on Virtual Reality and Inertial Measurement Units used to enhance sensorimotor control and guide exercises toward oriented and repetitive rehabilitation tasks. **(C)** Picture of the MoonWalker (Khymeia S.R.L.), a self-paced omnidirectional treadmill with instantaneous feedback on performance through Virtual Reality. **(D)** Picture of the kit for home therapy (VRRS Home-Kit, Khymeia) containing a dedicated tablet and wearable sensors to perform exercises based on Virtual Reality and augmented feedback. (B), (C), (D) are a courtesy of Khymeia S.R.L.

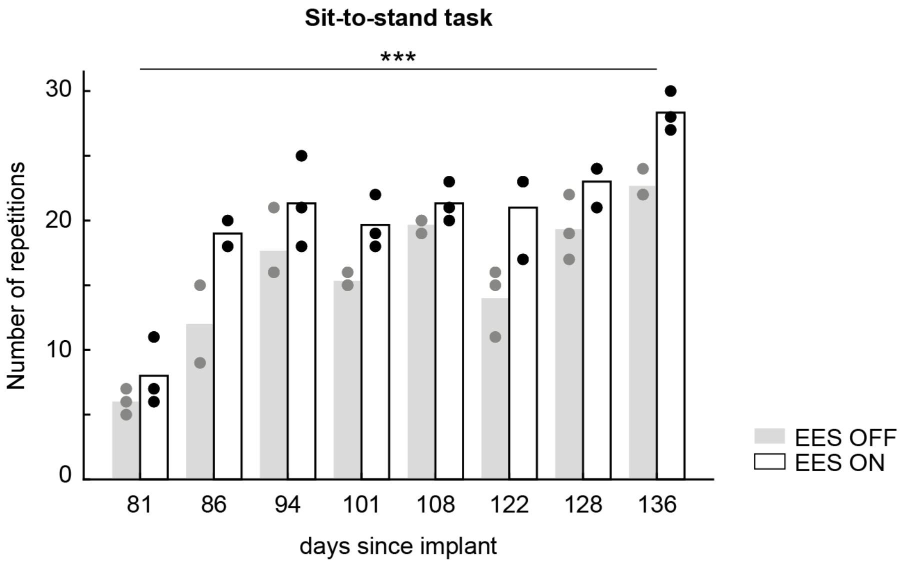


Fig. S9. Longitudinal performance of participant P2 in the sit-to-stand task. Number of sit-to-stand-to-sit complete movements in 60 seconds. Each point represents a repetition of the task (*n* = 3 repetitions per stimulation condition and day), bars represent the average across repetitions. Statistical analysis by z-score test of linear regression model. *** $P < 0.001$.

Supplementary Tables

Table S1. Presence of increments in muscle strength with stimulation off of leg muscles in patients P1 and P2 compared to the two patients of study *[13]* (see radar plots in the left part of Figure 5D).

	P1	**P2**	**First patient**	**Second patient**
Ankle flex R	0	0	0	0
Knee flex R	0	0	+1	+1
Hip flex R	+1	+1	+1	+1
Ankle flex L	+1	0	0	0
Knee flex L	0	+1	+1	+1
Hip flex L	0	+1	0	+1
Ankle ext R	+1	+1	0	0
Knee ext R	+1	0	+1	0
Hip ext R	0	0	+1	+1
Ankle ext L	+1	+1	0	0
Knee ext L	0	0	0	0
Hip ext L	+1	0	0	+1
Adduction R	+1	+1	NA	NA
Adduction L	0	+1	NA	NA
Total	**+7(+6)**	**+7(+5)**	**+5**	**+6**

Data file S1. All individual-level data in tabular format.

Movie S1. Voluntary isolated movements enhanced by LF-EES. Attempted voluntary movements of single or multiple joints with LF-EES off and with LF-EES on in the two participants. LF-EES enhances voluntary movements.

Movie S2. Effect of HF-EES in participant P2. Inhibition of patellar reflex and ankle clonus by HF-EES in participant P2. HF-EES causes an immediate and notable reduction of the amplitude of the patellar reflex; HF-EES suppresses ankle clonus without reducing the range of motion like LF-EES. Facilitation of voluntary isolated movements, specifically ankle dorsiflexion and knee flexion, by HF-EES applied to antagonist muscles in participant P2. HF-EES suppresses the co-contraction of antagonist muscles and increases the ability of the patient to perform the two movements.

Movie S3. Longitudinal effect of the neurorehabilitation program in participant P1. The rehabilitation program led to remarkable longitudinal improvements in walking in participant P1. The patient eventually regained the ability to walk outdoor.

Movie S4. Longitudinal effect of the neurorehabilitation program in participant P2. The rehabilitation program led to remarkable longitudinal improvements in walking in participant P2.