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SUPPLEMENTARY INFORMATION

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Targeted neurotechnology restores walking in humans with spinal cord injury

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SUPPLEMENTARY NOTES

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SUPPLEMENTARY METHODS

CLINICAL STUDY AND PARTICIPANTS

Study design and objectives

All experiments were carried out as part of the ongoing clinical feasibility study STIMO ("Stimulation Movement Overground"), which investigates the effects of spatiotemporal EES combined with weight-supported overground locomotor training on the recovery of motor function after SCI. This study was approved by the Swiss ethical authorities (Swissethics protocol number 04/2014 ProjectID: PB_2016-00886, Swissmedic protocol 2016-MD-0002) and was conducted in accordance with the Declaration of Helsinki. All participants signed a written informed consent prior to their participation. More information at clinicaltrials.gov (NCT02936453).

All surgical and experimental procedures were performed at the Lausanne University Hospital (CHUV). The timeline of the study is reported in **Fig. 1b**. Briefly, the study involved assessments before surgery, the surgical implantation of the neurostimulation system, a one-month period during which EES protocols were configured, and a five-month rehabilitation period with physiotherapists taking place four to five times per week for one to three hours (**Extended Data Fig. 10a**), including monthly assessments.

The rehabilitation program was personalized to the participants' improvements. Each session was typically segmented into three parts: (i) conventional physiotherapy (30 min), (ii) locomotor training with gravity-assist and EES (60 to 90 minutes), (iii) Recovery (stretching, massage). The physiotherapy sessions included muscle stretching and/or muscle strengthening, volitional control of movement and standing.

Based on improvements observed in P1 and P2, an optional three-year study extension was submitted and approved by the ethical authorities to allow the use of EES without body weight support outside the laboratory environment. This amendment included 9 sessions of training with gravity-assist, 3 times per week, which was performed by P3 only. There was no serious adverse event during the course of the study.

Study participants

Three individuals who had suffered a traumatic cervical SCI participated in the study. Their neurological status was evaluated according to the International Standards for Neurological Classification of Spinal Cord Injury[47], and is reported in **Extended Data Table 1**. At enrollment, participant P1 was 28 years old and was classified with a C7 lesion that occurred six years earlier during a gymnastics accident. His left leg was completely paralyzed while his right leg retained some residual functions (lower extremity motor scores left: 0/25, right: 14/25). He could ambulate with a walker with no braces and no physical assistance over ten meters (WISCI score: 13). He had followed an extensive rehabilitation program consisting of 262 sessions of Lokomat-assisted locomotor training over a period of three years, which led to very limited improvements of hos overground walking capacities and failed to mediate any neurological improvement of his paralyzed left leg. Participant P2 was 35 years old and was classified with a C4 lesion that occurred six years earlier during a bicycle accident. His impairments were bilateral, with some residual functions in both legs (left: 12/25, right: 13/25). He could ambulate with a walker, braces and physical assistance of one person over ten

meters (WISCI score: 6). A few months prior to his enrollment, he had terminated 50 rehabilitation sessions involving both stepping on a treadmill and overground locomotion. He had plateaued at a very low functional level (WISCI II score of 6). Participant P3 was 47 years old, classified with a C7 lesion that occurred four years earlier during a bicycle accident. He presented bilateral flaccid leg paralysis, with motor scores of 0 on all key leg muscles. He did not have any spasticity in his legs and could neither stand nor ambulate at all (WISCI score: 0), despite extensive participation in physical exercise with adapted devices for home use.

SURGICAL IMPLANTATION

Investigational device

Participants were implanted in the posterior epidural space with a 16-electrode paddle array clinically approved for the treatment of chronic pain (Specify™ 5-6-5 surgical lead for participants P1 and P2, Specify® SureScan® MRI 5-6-5 for participant P3; Medtronic plc, Fridley, MN, USA). This paddle array was connected to an IPG (Medtronic Activa™ RC) standardly used for Deep-Brain Stimulation. These combined elements and associated firmware constitute an investigational device that was tested as part of this clinical study.

Pre-surgical planning

Pre-operative MRI allowed the identification of the conus medullaris. Average spinal cord anatomical dimensions[48,49] were then used to estimate the position of the targeted spinal cord segments (L1-S2) with respect to the vertebrae.

Laminectomy and paddle array insertion

The insertion level was identified under fluoroscopy. An approximately 5 cm midline skin incision was performed, the fascia opened and the muscles retracted bilaterally. Excision of the midline ligamentous structures and L1/L2 flavectomy enabled the insertion of the paddle array that was placed over the midline of the exposed dura and advanced rostrally to the target location.

Electrophysiological monitoring

Electrophysiological testing was conducted using the NIM Eclipse® monitoring and stimulation system (Medtronic Xomed Inc, Jacksonville, FL, USA) to optimize the medial and segmental position of the paddle array. EES was delivered at increasing amplitude (0.5 Hz) to elicit muscle responses that were recorded with subdermal (Neuroline Twisted Pair Subdermal, 12 x 0.4 mm, Ambu A/S, Ballerup, Denmark) or intramuscular needle electrodes (Ultra Sharp, 44 mm/27 g, Chalgren Enterprises, Inc. Gilroy, CA, USA), as explained in **Extended Data Fig. 1b**. The final location of the paddle array overlaid lumbar and upper sacral segments in participant P1 and P3. Due to his height (192 cm), the paddle array was too short in P2, which imposed the selection of a position targeting the weak hip flexor muscles rather than ankle extensor muscles.

Pulse generator implantation

The implantable pulse generator (IPG) was inserted into a subcutaneous pocket in the abdomen. An extension cable was then tunneled from one opening to the other and connected to the IPG.

PERSONALIZED COMPUTATIONAL MODEL

Personalization

We personalized computational models to simulate the spread of electric potential and currents generated by single pulses of EES. The model is composed of an anatomically accurate volume conductor model including the human spinal cord that is coupled to a geometrically realistic biophysical model of human primary afferents. First, we elaborated an average human spinal cord model comprising white and grey matter compartments combining information from the Paxinos human atlas and anatomical measurements of segments lengths and widths taken from 15 human cadaveric spinal cords. Second, for each participant we adapted the length of the white and grey matter compartments to patient-specific data extracted at the

L1 vertebral level from pre-operative T2-weighted MRI images. The cerebrospinal fluid (CSF) dimensions were also segmented out of the pre-operative MRI-images.

Anatomically realistic vertebral bones and discs were generated and fitted to post-operative CT-data for each patient and the epidural fat was initialized to fit the inner borders of the bone. Finally, the whole spinal model was placed in a saline conductor to represent the human body[18]. Isotropic dielectric coefficients for each compartment were taken from previous studies[50]. We then estimated the precise position of each electrode by segmentation of post-implantation CT-data and registered it to the vertebral bone.

The model was discretized and Maxwell's equations were solved under quasi-static conditions with the Finite Element Method to determine an accurate representation of electric potentials and currents generated by single pulses of EES in each subject. Monopolar pulses were modelled as Dirichlet-boundary conditions of 1 V at the active electrode site with a duration of 500 µs. Dirichlet-boundary conditions of 0 V were initialized at the outermost boundaries of the model or at contacts selected as anodes in case of multipolar configurations. The models were all implemented in Sim4Life v3.4 (ZMT Zürich MedTech AG).

We used NEURON[51] in Sim4Life to model 50 myelinated Group-I afferent fibers per posterior root as a log-norm function with a mean fiber diameter of 16 µm and a standard deviation of 4 µm. An anatomically accurate trajectory of the afferent fibers was initialized and fitted around their entry and exit point determined by the anatomical parameters extracted from MRI and CT-data. A Sweeney model was used to describe the equation of the active membrane at the nodes of Ranvier and at the passive internodal segments. The electrical compartments were automatically initialized in Sim4Life. The resting potential was set to -80 mV. Recruitment of group-Ia afferents was evaluated by linearly scaling the extracellular voltage applied at each fiber's compartment until an action potential was generated.

Posterior root selectivity index for simulations

For a given electrode configuration and EES amplitude, computational simulations yielded the percentage of fibers activated in each posterior root. We derived a root selectivity index $SI_{computational}(r_i, I)$ for each root r_i and each stimulation amplitude I:

$$SI_{computational}(r_i, I) = \frac{A_{r_i}(I)}{1+\sum_{other\ ipsilateral\ roots} A_{r_j}(I)} \cdot f_+\left(\frac{A_{r_i}(I)-A_{r_{contralateral(i)}}(I)}{A_{r_i}(I)+A_{r_{contralateral(i)}}(I)}\right)$$

where $A_{r_i}(I)$ represents the percentage of fibers activated in root r_i, $r_{contralateral(i)}$ is the root contralateral to root r_i, and $f_+(x) = \begin{cases} x\ if\ x > 0 \\ 0\ if\ x \leq 0 \end{cases}$.

The first term of this selectivity index represents the rostro-caudal ipsilateral root selectivity, while the second term indicates the medio-lateral selectivity and penalizes configurations that activate the root contralateral to the targeted one. If the contralateral root is more activated than the targeted root, the selectivity index is equal to 0. If only the targeted root is recruited, the selectivity equals 1. This selectivity index was calculated for a range of stimulation amplitudes (number of recruited afferent fibers) and the maximum was taken to characterize the overall selectivity of each electrode configuration.

SINGLE-PULSE EES

Single-pulse EES was used to identify electrode configurations recruiting the targeted posterior roots (L1, L3, L4, S1). This identification was based on the recruitment order of lower-limb muscles with specific segmental innervations in response to single pulses of EES (300 µs pulse width) repeated every 1.5 s with increasing amplitudes.

Participants were lying relaxed in supine position on an examination table. EMG activity was recorded bilaterally from the iliopsoas (Il), rectus femoris (RF), vastus lateralis (VLat), semitendinosus (ST), tibialis anterior (TA), medial gastrocnemius (MG), and soleus (Sol) muscles with wireless bipolar surface electrodes (Myon 320, Myon AG, Schwarzenberg, Switzerland). Each electrode pair was placed centrally over the muscle with a longitudinal alignment and an inter-electrode distance of 3 cm. Abrasive paste (Nuprep,

Weaver and Company, Aurora, CO) was used for skin preparation to reduce electrode-skin resistance and improve EMG signal quality. Stimulation artefacts required to display stimulus-triggered EMG activity were picked up by an additional pair of surface-EMG electrodes placed over the spine at the thoracolumbar junction. EMG signals were amplified with a gain of 500 and band-pass filtered between 20 and 450 Hz. Continuous EMG signals were sampled at 5 kHz and saved to a desktop computer. Stimulus-triggered EMGs were visualized in real-time as superimposed traces for each muscle within a 50-ms time window following each stimulus.

Recordings were performed with graded stimulation amplitudes in order to compute recruitment curves, which indicate the degree of recruitment of each muscle as the stimulation amplitude is increased. Specific electrode sites were tested according to the segmental organization of lower limb muscle innervation[46] and predictions from computer simulations. Each selected electrode was stimulated in monopolar configuration as the cathode, with the case of the implantable pulse generator set as the anode. First, low-amplitude stimulation was applied to identify the lowest response threshold across all recorded muscles. Then, EES amplitude was increased manually to identify the amplitudes at which the responses reached a plateau, limited to levels that did not cause discomfort to the participant. Finally, single-pulse EES at amplitudes ranging from response threshold to saturation was performed automatically, with four repetitions at each EES amplitude, and recorded EMG responses were used to compute recruitment curves and a functional selectivity index (see "Data processing" section).

This procedure was repeated to identify eight sites targeting the left and right L1 (hip flexion), L3 (knee extension), L4 (ankle flexion) and S1 (ankle extension) posterior roots. If the selectivity was not satisfactory, EES was refined with multipolar electrode configurations, using additional anodes and cathodes to steer the electrical field towards the targeted posterior roots. Finally, the optimal electrode configurations for each targeted posterior root were validated during standing with body weight support.

EVALUATION OF SINGLE-JOINT TORQUE PRODUCTION

Assessment of voluntary force production

Maximum torques produced at the knee, ankle and hip in flexion and extension were measured using the Humac Norm Cybex dynamometer system (Computer Sports Medicine Inc., Stoughton, MA). For measuring maximum isometric knee flexion and extension, the participant was seated in upright position, the knee placed in line with the dynamometer axis of rotation, and the lever arm pad of the system secured to the lower leg just above the ankle. Hip, knee, and ankle assumed angles of 90°, respectively. For ankle flexion and extension, the participant was seated reclined, with an angle of 120° between the trunk and the thigh of the leg under examination. The thigh was secured to a stabilization arm provided by the system, and the ankle was strapped to the foot platform attachment, with the joint aligned with the dynamometer axis of rotation. Knee and ankle were at angles of 90°. Hip flexion and extension were assessed in supine position, with the distal thigh strapped to the measuring lever arm, and hip and knee at 90° of flexion. For each single-joint task, the participants were asked to produce a progressive contraction from rest to maximum strength with real-time visual torque biofeedback, with three repetitions and at least a 1-minute resting period between each attempt. If spasticity was induced during the attempted task, the trial was repeated. The torques developed were measured at a sample frequency of 5 kHz, and in parallel, EMG was acquired from the main agonists and antagonists of the respective movement attempt, sampled at 5 kHz. This assessment was carried out at all the main and monthly intermediate time points, with identical settings of the dynamometer system for each participant.

Impact of EES on voluntary force production

A similar setup was used to study the interactions between targeted EES and voluntary attempts of single-joint movements. EES was applied with parameters optimized to facilitate hip flexion, hip extension, knee extension or ankle extension. Electrode configurations derived from single-pulse experiments were used to target the posterior roots projections to the spinal cord regions containing motoneurons associated with the intended movement. EES amplitude and frequency were adjusted manually. If necessary, EES was refined using multipolar electrode configurations. EES was applied with a delay following the onset of the voluntary

motor task for a duration of a few seconds and was stopped 1-2 seconds after the participant had stopped the voluntary movement. This procedure was repeated three times for each of the studied tasks. The torques produced were assessed by dynamometry in the standardized positions as described above, except for the hip flexion which was assessed in prone position, with the hip at 10° of extension and the knee fully extended.

TECHNOLOGICAL FRAMEWORK

Gait analysis and rehabilitation environment

For assisting treadmill and overground locomotion, we used an overhead support system based on cable robot technology (FLOAT, Lutz Medical Engineering GmbH, Rüdlingen, Switzerland) that allows the application of forces to the trunk through a dedicated harness (Maine Anti-Gravity Systems, Inc. Portland, ME) in each of the Cartesian directions[27]. The robotic interface was integrated within a gait analysis platform, which allowed acquisition of EMG activity, ground reaction forces and whole-body kinematics in real-time. EMG activity during walking was acquired bilaterally at 1 kHz using the 16-channel wireless Myon system, with bipolar surface electrodes placed over the from the iliopsoas (Il), rectus femoris (RF), vastus lateralis (VLat), semitendinosus (ST), biceps femoris (BF), tibialis anterior (TA), medial gastrocnemius (MG), and soleus (Sol) muscles. Kinematic recordings were obtained at a 100-Hz sampling rate using a 3D motion capture system (Vicon Motion Systems, Oxford, UK), consisting of 14 infrared cameras that covered a 12 x 4 x 2.5 m workspace. Head, trunk, and bilateral upper and lower extremity kinematics were captured by these infrared cameras and 34 small infrared-reflective markers (16 mm, Prophysics AG, Schaffhauserstrasse 121, Kloten, Switzerland) positioned over standardized anatomical landmarks. Two force plates (9260AA6, Kistler, Winterthur, Switzerland) were installed in the middle of the workspace to monitor ground reaction forces. We also captured chronophotographic images of participants using a high-definition camera (FUJIFILM X-T2, 5 images/s, ISO 6400, shutter speed 1/250 sec) and overlaid successive images offline for illustrative purposes.

Real-time gait event detection

To perform real-time gait event detection, we placed large infrared-reflective markers (46 mm, Prophysics AG, Schaffhauserstrasse 121, Kloten, Switzerland) on the main joints of the leg (greater trochanter, lateral femoral condyle and lateral malleolus) and an extra marker on the left arm to differentiate body sides. We developed a custom C++ based control software to detect gait events in real time based on kinematic information from the motion capture system and to send EES triggering commands to the neurostimulation system. This control software streamed the real-time 3D-positions of all the large kinematic markers detected. The labeling of these markers was then performed in the control software itself, based on their vertical position and additional geometrical constraints. Then, the control software computed the trajectory of the foot with respect to the hip and the **centroid** of this trajectory over the last few steps. An overlay of past trajectories and the current **centroid** were displayed as feedback for the user. The vector between the current point on the foot trajectory and the **centroid** was used to define an angular variable evolving monotonically throughout the gait cycle. A set of user-defined thresholds were applied on this angular variable to define the occurrence of desired gait events, such as foot off, foot strike, mid-swing or mid-stance. These events were used as triggers to start or stop specific spinal cord stimulation protocols.

Neurostimulation system

EES was delivered with an IPG (Medtronic Activa™ RC) that enabled monopolar and multipolar stimulation at constant current or constant voltage through one or a subset of the 16 electrodes of the paddle array or the case of the IPG (anode). The IPG was modified from its clinical version with an investigational firmware that enabled real-time communication with a software running on an external computer (NEUWalk Research Programmer Application NRPA, Model 09103, Medtronic). The NRPA acted as a relay between EES triggering commands sent by the control software described in the previous section and the IPG. It communicated wirelessly with the IPG through the following communication chain: the NRPA sent commands via a virtual COM port corresponding to a Bluetooth adapter, a custom wireless bridge consisting of a nano computer (Raspberry Pi) received this command and forwarded it to a virtual COM port

corresponding to a USB adapter, a USB to infrared adapter (ACT-IR224UN-LN115-LE, ACTiSYS Corporation, Fremont, CA, USA) transformed this command into infrared signals that were then read by a modified Medtronic patient's programmer (Sensing Programmer Telemetry Module SPTM, Medtronic), which finally transmitted the command to the patient's IPG by electromagnetic induction through the skin (**Extended Data Fig. 1a**). This constituted our overall investigational system: motion capture system, control software, NRPA relay software, wireless bridge, SPTM and IPG with modified firmware connected to the 16-electrode paddle array. This system allowed real-time triggering of stimulation protocols with a median latency of 110 ms (99^{th} percentile, 135 ms).

CONFIGURATION OF TARGETED EES

After approximately ten days of rest following the surgery, participants started a one-month period during which we configured EES protocols to enable single-joint movements and walking.

Single-joint movements

We delivered EES with electrode configuration targeting the posterior roots projecting to the spinal cord regions containing the motoneuron pools associated with the intended movement. Electrode configurations were selected using the selectivity index calculated from single-pulse EES experiments and optimized with multipolar configurations when necessary (**Extended Data Fig. 2**).

Spatiotemporal EES during walking

These EES protocols were guided by the spatiotemporal maps of motoneuron activation reconstructed from EMG activity of healthy individuals during walking[28,29] (see data processing section). These spatiotemporal maps revealed that walking involved the successive activation of three hotpots restricted to specific spinal cord regions. EES protocols targeting the posterior roots projecting to these hotspots were selected from the selectivity index that was calculated during single-pulse EES experiments. The configuration, amplitude and frequency of EES were optimized during standing and refined during walking. The onset and duration of each EES protocol was optimized using the procedures described in **Extended Data Fig. 6**. EES train durations could be pre-determined or terminated by a gait event (e.g. mid-swing).

Continuous EES during overground walking

Continuous EES was delivered using a variety of locations that covered the broad range of protocols employed in previous studies[11,12,15], both on a treadmill and overground. We report the results from the most efficient protocols for each participant. In general, these protocols involved the widespread activation of the spinal cord through the recruitment of the L1 and S1 roots or L1 and L4 roots on both sides simultaneously, as employed in recent studies[10]. EES pulses were interleaved with a 2 ms interval to avoid superposition of the electric fields generated by each configuration. EES amplitude and frequency were optimized visually.

EEG recordings

Subjects were asked to produce an isometric torque at the right knee without and with targeted EES (**Extended Data Fig. 5**). The subjects seated on the Humac Norm Cybex dynamometer with the knee flexed at 90° and were instructed to follow a sequence displayed on a screen in front of them: movement preparation (auditory cue), GO cue around 2 s after movement preparation, movement execution (about 3 seconds). During trials with EES, 2 s after the initiation of the movement the stimulation was switched on for about 5 s. EEG data were continuously acquired using 64 channels in standard 10–20 configuration (ANT neuro) at a sampling rate of 1024 Hz.

CLINICAL EVALUATIONS

International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI)

Each participant's neurological status was assessed before surgery and after 5 months of rehabilitation based on the ISNCSCI, a comprehensive clinician-administered neurological examination of residual sensory and motor function quantifying SCI severity[47].

WISCI II score
Functional walking ability was assessed by the WISCI II score[52], which evaluates the amount of physical assistance, assistive devices or braces required to walk overground for ten meters.

Muscle mass and quality
Muscle mass and quality were quantified using CT images obtained at abdominal (L_3 vertebra) and mid-thigh (25 cm above femorotibial joint space) levels, assessed before surgery and after rehabilitation. Muscle segmentations were performed semi-automatedly using ImageJ and specific HU thresholds[53] (-29 to 150 HU) (**Extended Data Fig. 11a**). Muscle quality was assessed using CT attenuation numbers, which reflect lipid content in skeleton muscle[54].

10-meter walk test
Walking speed was assessed by a timed 10-meter walk test without body weight support. Participants were instructed to walk with the preferred assistive device as fast as they could. Participants P1 and P2 performed the test with a standard 4-wheel walker. This test was also performed with spatiotemporal EES after rehabilitation and during follow-up after obtaining the authorization to use spatiotemporal EES without body weight support. Participant P_3 performed the test only after rehabilitation using a 4-wheel walker with armrests, spatiotemporal EES, and the presence of the body weight support for safety.

6-minute walk test
Endurance was assessed by the distance covered overground within 6 minutes with the preferred assistive device. Participants P1 and P2 performed this test in the same conditions as the 10-meter walk test. Participant P_3 was unable to perform this test.

STUDY EXTENSION AND TECHNOLOGIES FOR USE IN THE COMMUNAUTY

Technological framework
The home-based system consists of a tablet (Surface Pro 4, Microsoft) containing personalized EES programs, each associated with a specific training activity. Custom software was developed in C# to allow switching between EES protocols and loading them onto the IPG. A Graphical User Interface (GUI) allows the participant to change the amplitudes of EES protocols, and to start or stop an activity from the tablet or using a commercially available smartwatch (Fossil Q Marshal, Fossil Group, Inc., USA) connected to the tablet via bluetooth. Participants can either tap on the smartwatch screen or use a voice controller implemented using a keyword detection system (Snowboy Hotword Detection, non-commercial license, KITT.AI, Baidu, Inc., China) built on artificial neural networks.

Participants could select activities with EES delivered in open-loop or closed-loop. The controller of closed-loop EES used a pair of inertial measurement units (IMUs, NGIMU, FW v.1.5 HW v1.6, x-io Technologies Limited, UK) connected to the tablet via WiFi, streaming data at 90 Hz.

Closed-loop walking
During closed-loop walking, the pitch angle of IMUs placed on the feet or tibia enabled gait event detection based on a Kalman filter combining the accelerations and angular velocities of IMU signals:

$$\begin{cases} \text{Noisy measurement from accelerometers:} & \theta_{measured} = atan2(A_y, A_x) \\ \text{Kalman observation model:} & \theta_{measured} = H \cdot \begin{pmatrix} \theta_{real} \\ \dot{\theta} \end{pmatrix} + \omega_1 \text{ with } H = (1\ 0) \\ \text{Kalman state-transition model:} & \begin{pmatrix} \theta_{real} \\ \dot{\theta} \end{pmatrix}_{i+1} = A \cdot \begin{pmatrix} \theta_{real} \\ \dot{\theta} \end{pmatrix}_i + \omega_2 \text{ with } A = \begin{pmatrix} 1 & 1/f_s \\ 0 & 1 \end{pmatrix} \end{cases}$$

where A_x and A_y represent the accelerations measured by the accelerometers in the plane of movement, $\dot{\theta}$ represents the angular velocity measured by the gyroscope around the axis of the joint (ankle or knee), $\theta_{measured}$ is an approximation of the pitch angle assuming that the IMU is static and that only gravity contributed to the accelerations, atan2 is the four-quadrant inverse tangent, θ_{real} is the real pitch angle that estimated by the Kalman filter, and ω_1 and ω_2 are Gaussian noises. Gait events are detected when the pitch angle crosses a pre-defined threshold personalized for each participant.

Closed-loop biking

Gait event detection was based on one IMU placed on the crank and another IMU attached on the frame of the trike. These IMUs were used to measure the angle of the crank and the current slope on non-levelled terrains, respectively. Detections were based on the above described Kalman filter that first estimated the measured angle from the accelerometers:

$$\theta_{measured} = atan2(A_y, A_x - r.\dot{\theta}^2)$$

Communication delays with the IPG could result in variability in EES timing as the angular velocity is varied. To avoid this issue, we estimated the angular velocity $\dot{\theta}$ over the last cycle and thresholded the crank angle at $\theta_{threshold} - \dot{\theta}.\tau_{communication}$, where $\theta_{threshold}$ is the desired angular threshold and $\tau_{communication}$ is the communication delay (about 110 ms).

EES protocols promoted knee extension when the crank reached 90° (top) together with hip flexion of the contralateral leg. This system was mounted on a tricycle specially conceived for patients with motor impairments, combining foot and hand pedals and electrically-powered assistance (Trike, GBY SA, Vuisternens-en-Ogoz, Switzerland).

DATA PROCESSING

Recruitment curves during single-pulse EES

EMG signals were band-passed filtered between 10 and 450 Hz (4^{th}-order Butterworth filter). EES onset was determined using semi-automatic methods based on stimulation artefact recordings. The evoked responses were extracted and superimposed to isolate the monosynaptic components based on visual inspection by an experienced neurophysiologist. The temporal window of these responses started 10 to 20 ms after EES onset and lasted 40 to 50 ms, depending on the muscle and participant. For each EES amplitude, the responses were quantified as the peak-to-peak amplitude to generate recruitment curves that we displayed in circular plots. Muscles are distributed at different angular positions, while the radial axis corresponds to EES amplitude (**Fig. 2d**, **Extended Data Fig. 2** and **Extended Data Fig. 3**). A greyscale shading reports the normalized EMG activity. The white circle highlights the EES amplitude that corresponds to the highest selectivity index. The polygon describes the muscle selectivity at the optimal EES amplitude: the edges of the polygon represent the normalized EMG activity on the radial axis for a particular muscle, scaled so that the polygon fills the circle.

Selectivity index

We targeted the posterior roots projecting to L1, L3, L4 and S1 segments to modulate motoneurons associated with hip flexion, knee extension, ankle flexion and ankle extension. To identify the relevant electrodes, we computed a selectivity index $SI_{experimental}(g_i\ I)$ for each targeted muscle group g_i and EES amplitude I. This selectivity index contains two terms, one indicating the selectivity on the targeted side, and one indicating the mediolateral selectivity for the targeted side s_i:

$$SI_{experimental}(g_i, I) = SI_{targeted\ musles}(g_i, I)\ .\ SI_{left\ vs\ right}(s_i, I)$$

$$\begin{cases} SI_{targeted\ muscles}(g_i, I) = \frac{\sum_{muscles\ in\ g_i} \omega_{i,j} . \log\left(1+A_j(I)\right)}{\log 2} \\ SI_{left\ vs\ right}(s_i, I) = f_+\left(\frac{max_{ipsilateral(s_i)}\left(A_j(I)\right) - max_{contralateral(s_i)}\left(A_j(I)\right)}{max_{ipsilateral(s_i)}\left(A_j(I)\right) + max_{contralateral(s_i)}\left(A_j(I)\right)}\right) \\ f_+(x) = \begin{cases} x\ if\ x > 0 \\ 0\ if\ x \leq 0 \end{cases} \end{cases}$$

where $A_j(I)$ represents the normalized EMG activity of muscle *j* in response to EES amplitude *I*. $\omega_{i,j}$ is a weight associated with muscle *j* for the targeted muscle group g_i. For each targeted muscle group, weights of agonists (respectively antagonists) are positive (respectively negative), and the sum of weights over agonists (respectively antagonists) is equal to 1 (respectively -1). These weights were chosen empirically to capture the respective contributions of each muscle in the targeted muscle group:

	Ilio-psoas (Il)	Rectus femoris (RF)	Vastus lateralis (VLat)	Semi-tendinosus (ST)	Tibialis anterior (TA)	Gastrocnemius medialis (MG)	Soleus (Sol)
Hip flexion (L1)	0.8	0.2	-	-1	-	-	-
Knee extension (L3)	-0.5	0.2	0.8	-0.5	-	-	-
Ankle flexion (L4)	-	-	-	-	1	-0.2	-0.8
Ankle extension (S1)	-	-	-	-	-1	0.2	0.8

Torque and EMG activity during voluntary contractions

The recorded torque was low-pass filtered below 5 Hz and EMG activities were band-pass filtered between 10 and 450 Hz (4^{th}-order Butterworth filter). The root mean square (RMS) of the EMG activity was calculated with a 500-ms centered running window. During maximum voluntary contractions without EES, the torque produced was quantified as the average within a 500-ms time window centered at the peak torque. EMG activities were quantified as their RMS value at the peak torque. During maximum voluntary contractions with EES, the task was divided into four time periods: onset of voluntary contribution, EES onset, end of voluntary contribution, EES end. Because the sudden onset of EES created a transient peak in the torque and EMG activities, we further split the analysis between the transient (EES onset to 1.1 s) and sustained effects after EES onset (**Extended Data Fig. 4**).

EMG activity during walking

EMG activities were processed according to the SENIAM (Surface Electromyography for the Non-Invasive Assessment of Muscles) standards for EMG recordings. All displayed EMG activities during walking were band-pass filtered between 10 and 450 Hz (4^{th}-order Butterworth filter). A moving average of the rectified EMG signal within a centered 250-ms time window was used to generate normalized EMG envelopes for quantification. With EES, the sudden EES onset created transient peaks of EMG activity that did not translate into sustained muscle contractions but nevertheless contributed to the computation of EMG envelopes. To avoid this issue, we systematically clipped EMG responses within a time window of 50 ms around the onset of EES trains.

Calculation of motoneuron activation maps

Motoneuron pools innervating hip flexors (Il, RF), knee extensors (VLat, RF), ankle flexors (TA) and ankle extensors (MG, Sol) are spatially distributed along the rostro-caudal axis of the lumbosacral spinal cord[42]. We modelled the activation of motoneuron pools in each spinal segment S_i as a linear combination of the normalized EMG activities of leg muscles M_j:

$$S_i = \frac{\sum_{muscles} W_{i,j} . M_j}{\sum_{muscles} W_{i,j}}$$

If all muscles reach their maximum activity, the activation of each spinal segment is equal to 1. The coefficients $W_{i,j}$ represent how much each muscle reflects the underlying activity of a spinal segment[28]. As an approximation, we took for $W_{i,j}$ the ratio of motoneurons innervating the muscle *j* and present in the spinal segment *i*, with respect to all motoneurons innervating the muscle *j*.

These coefficients were derived from anatomical studies[46]:

	Iliopsoas (Il)	Rectus femoris (RF)	Vastus lateralis (VLat)	Semi-tendinosus (ST)	Tibialis Anterior (TA)	Gastrocnemius Medialis (MG)	Soleus (Sol)
L1	0.4	0	0	0	0	0	0
L2	0.4	0.12	0.12	0	0	0	0
L3	0.2	0.19	0.19	0	0	0	0
L4	0	0.19	0.19	0.12	0	0.77	0
L5	0	0	0	0.4	0.23	0.23	0.09
S1	0	0	0	0.4	0.77	0	0.45
S2	0	0	0	0.08	0	0	0.45

Motoneuron activation maps derived from single-pulse EES experiments were calculated from the peak-to-peak responses, while motoneuron activation maps during functional tasks were computed with the instantaneous or average value of EMG envelopes. Motoneuron activation maps were interpolated and superimposed onto a 3D illustration of the human spinal cord.

Extraction of hotspots underlying walking

Three hotspots were extracted from the spatiotemporal maps of motoneuron activation underlying walking of healthy individuals. Hotspots were extracted from the isopotential lines at 45, 55, 65 and 75 % of the maximum activation in the maps[21]. The correlations between each targeted hotspot and EES-induced EMG activity were calculated based on the average motoneuron activation maps of each hotspot ($S_{i,hotspot}$) and those obtained from the EMG activity induced by a 500 ms train of EES during standing ($S_{i,stim}$). The Pearson correlation coefficient was calculated as:

$$\rho_{stim,\ hotspot} = \frac{\sum_{segments}\left(S_{i,stim} - \overline{S_{stim}}\right).\left(S_{i,hotspot} - \overline{S_{hotspot}}\right)}{\sqrt{\sum_{segments}\left(S_{i,stim} - \overline{S_{stim}}\right)^2 . \sum_{segments}\left(S_{j,stim} - \overline{S_{stim}}\right)^2}}$$

where $\bar{S} = \frac{1}{N_{segments}} . \sum_{segments} S_i$ and $N_{segments}$ is the number of segments.

Processing of EEG signals

Raw EEG signals were filtered (1-40 Hz, zero-phase IIR filters), down-sampled at 128 Hz, and re-referenced to a common average. 'Eye blink' and 'eye movements' artifacts were removed by Independent Component Analysis. EES evoked activity was computed by averaging EEG signals from -0.6 to 1 s after stimulation artifact. During volitional movements, epochs were extracted from -1.5 to 1 s after GO cue and from -500 to 500 ms after movement termination, defined as the torque reaching 2 % of the local maximum value. Time frequency spectra were calculated using sliding Hamming windows of 200 ms with time steps of 32 ms. Power spectra were normalized to the average values of the corresponding frequency bin. Beta (17-30Hz) power spectra over leg sensorimotor area (Cz) was calculated during event related resynchronization (ERS), defined 0 to 500 ms after movement.

Statistics

All quantifications show the mean and standard error of the mean of the represented variables, in addition to all individual data. Normality of the data was confirmed using the Kolmogorov-Smirnov test. Comparisons between two conditions were performed using a two-tailed Student's t-test. Comparisons involving more than two categories were performed using a 1-way ANOVA, followed post hoc by Tukey's Honest Significance Difference tests. *, **, *** indicate a p-value smaller than 0.05, 0.01, 0.001 respectively. Significant differences for EEG data were obtained by randomly permuting single trial beta ERS values of the two conditions. The number of permutations was determined to reach $\alpha = 0.001$.

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