Epidural electrical stimulation (EES) of the spinal cord restores locomotion in animal models of spinal cord injury but is less effective in humans. Here we hypothesized that this interspecies discrepancy is due to interference between EES and proprioceptive information in humans. Computational simulations and preclinical and clinical experiments reveal that EES blocks a significant amount of proprioceptive input in humans, but not in rats. This transient deafferentation prevents modulation of reciprocal inhibitory networks involved in locomotion and reduces or abolishes the conscious perception of leg position. Consequently, continuous EES can only facilitate locomotion within a narrow range of stimulation parameters and is unable to provide meaningful locomotor improvements in humans without rehabilitation. Simulations showed that burst stimulation and spatiotemporal stimulation profiles mitigate the cancellation of proprioceptive information, enabling robust control over motor neuron activity. This demonstrates the importance of stimulation protocols that preserve proprioceptive information to facilitate walking with EES.

Spinal cord injury (SCI) has an immediate and devastating impact on movement control. These motor deficits result from the interruption of communication between the brain and spinal cord, depriving the otherwise intact spinal cord executive centers below the injury from essential sources of modulation and excitation to produce movement.

EES applied to the lumbar spinal cord immediately enables the executive centers to coordinate a broad range of motor behaviors including standing, walking in various directions, and even running in rodent, feline, and nonhuman primate models of leg paralysis. When combined with locomotor training, EES promotes an extensive reorganization of residual neural pathways that restored locomotion without the need of stimulation.

EES has also been applied to the human spinal cord for several decades but has been less effective. EES induces rhythmic leg movements in people with complete paralysis, and enables independent stepping when delivered over more than a year of intense rehabilitation. EES also enabled volitional activation of paralyzed muscles to initiate isolated leg movements in individuals with motor-complete paralysis. However, EES has not restored independent, weight-bearing locomotion in humans with severe SCI, as observed in animal models.

The mechanisms underlying species-specific responses to EES remain enigmatic. This understanding is essential for guiding the development of evidence-based approaches that fulfill the potential of EES to improve recovery after SCI. Evidence from computational models and experimental studies conducted in animals and humans suggests that EES recruits afferent fibers conveying proprioceptive information. This recruitment leads to the activation of motor neurons through monosynaptic and polysynaptic proprioceptive circuits, and it increases the overall excitability of the lumbar spinal cord. This modulation enhances the responsiveness of spinal circuits to residual descending signals and sensory feedback. In turn, sensory information modulates the reciprocal inhibitory networks in the spinal cord that gate the excitatory drive produced by EES toward functionally relevant pathways. This mechanism enables the generation of muscle activation underlying standing and walking in animal models of paralysis.

This conceptual framework implies that sensory information plays a central role in motor-pattern formation during EES. However, this viewpoint does not consider that the recruitment of proprioceptive fibers by EES may interfere with the natural flow of information traveling along the same fibers.

Electrical stimulation triggers bidirectional action potentials (APs) along the recruited fiber. EES would thus elicit orthodromic and antidromic APs that travel to the spinal cord and sensory organs. Consequently, we hypothesized that antidromic APs may collide with APs conveying proprioceptive information, preventing its propagation to the brain and spinal cord. The probability of these detrimental interactions is proportional to EES frequency, the firing rate of afferents, and the time required for an AP to travel along the entire length of the fiber. These physiological parameters diverge dramatically between rats and humans. The traveling time of APs along proprioceptive fibers is longer in humans than in rats, and firing rates are lower. The resulting higher probability of
used to enable locomotion in rats (40 Hz).

While delivering EES at frequencies commonly extremely low in rats, regardless of EES frequency and natural firing rate (Fig. 1b). We thus calculated the probability of antidromic collisions along proprioceptive afferent fibers. We modeled realistic interactions between natural and EES-elicited APs and natural APs traveling along the recruited proprioceptive afferent fibers.

We thus developed computational models of proprioceptive afferents. We used these models to study the occurrence probability of antidromic collisions during EES in rats and humans. A schematic illustration of antidromic collisions between EES-induced antidromic APs and natural APs traveling along the recruited proprioceptive afferent fibers is shown in Fig. 1a.

The occurrence probability of antidromic collisions was exceeded 20%.

These probabilities were dramatically different in humans. Even relatively low EES frequencies blocked most of the natural proprioceptive signals from reaching the spinal cord. For distal muscles, the occurrence probability of antidromic collisions reached nearly 100% for EES frequencies of 30 impulses per second at 30-Hz EES frequency (Fig. 1c). The occurrence probability of antidromic collisions was markedly higher along afferents innervating proprioceptors located in distal muscles compared to proximal muscles (Fig. 1c). These results suggest that continuous EES may disrupt proprioceptive information in humans, but not in rats.

**Results**

**Antidromic collisions during EES.** To study the occurrence probability of antidromic collisions along proprioceptive afferents during EES, we developed computational models of proprioceptive afferents that consider the length of axons innervating proximal and distal muscles, as well as the propagation times of APs. We modeled realistic interactions between natural and EES-elicited APs (Fig. 1a). We thus calculated the probability of antidromic collisions in muscle spindle afferents depending on EES frequency and natural firing rate.

The occurrence probability of antidromic collisions was extremely low in rats, regardless of EES frequency and natural firing rate (Fig. 1b). While delivering EES at frequencies commonly used to enable locomotion in rats (40 Hz), this probability never exceeded 20%.

**EES induces antidromic activity along human afferents.** We thus verified whether EES produces antidromic activity along proprioceptive afferents. We recorded the proximal and distal branches of the tibial nerve (mixed nerve), the sural nerve (sensory nerve), and EMG activity from the soleus muscle during continuous EES in two individuals with chronic SCI (Fig. 2a; subjects #2 and #3 in Supplementary Table 1).

We selected an EES configuration that elicited contractions of the soleus and then reduced EES amplitude to elicit a tingling sensation in the corresponding dermatome without visible muscle contraction. In subject #2, each pulse of EES (20 Hz) elicited a weak response in the soleus with a latency of 25 ms, which has been associated with the recruitment of motor neurons via group-Ia afferents. Concurrently, we detected two responses in the proximal branch of the tibial nerve, with latencies of 12.5 and 26.5 ms, and...
Fig. 2 | EES induces antidromic activity along proprioceptive afferents and disrupts proprioception. a, Recordings of antidromic activity from sensory nerves during EES. Needle electrodes were inserted subcutaneously close to peripheral nerves and surface electrodes over the soleus muscle, as depicted in the scheme. Continuous EES (20 Hz, monopolar stimulation, black cathode and red anode) was delivered for approximately 1 min. Averaged evoked potentials (± s.e.m., n = 1,198 and n = 1,180 independent measurements for subjects #2 and #3, respectively). Evoked potentials highlighted in blue, red, and gray were respectively classified as antidromic afferent volleys, efferent orthodromic activity, and far-field potentials (for example, electromyographic potentials). b, Sensory AIS subscores of the lumbosacral (L1–S2) dermatomes for the two subjects that performed the threshold to detection of passive movement (TTDPM) test. AIS, American Spinal Injury Association Impairment Scale. c, Setup of the TTDPM test. Top: randomly selected flexion or extension movements were imposed to the knee joint of subject #1. A movement speed of 0.5° s⁻¹ and a maximum allowed range of motion of 30° was used. EES configurations used to target knee flexor and extensor muscles were applied as indicated. d, Scatter plots reporting the detection angle and plots reporting the error rate (percentage correct trials ± 95% CI, n = 32 and n = 47 independent measurements for subjects #1 and #3, respectively) on the TTDPM test performance without EES and when delivering continuous EES (50 Hz) at 0.8x and 1.5x muscle-response-threshold amplitudes. Grey dots, detection angles for successful trials; pink dots and red crosses, false positives and failure to detect movement within the allowed range of motion, respectively. *P < 0.05, Clopper–Pearson two-sided non-overlapping intervals.

one response (latency: 21 ms) in the distal branch. The responses induced in the proximal (12.5 ms) and distal (21 ms) branches of the tibial nerve (Fig. 2a) likely resulted from the same neural volley propagating toward the periphery. Since the responses recorded in the distal branch occurred before any motor response, they cannot be attributed to orthodromic efferent activity. These responses corresponded to antidromic afferent volleys. The response (22 ms) recorded in the exclusively sensory sural nerve is compatible with this conclusion. The antidromic recruitment of Aβ afferents is the most probable explanation for this response. In subject #3, each EES pulse elicited a distinct response in both proximal (12.5 ms) and distal (22 ms) branches of the tibial nerve, as well as a response in the sural nerve (22.5 ms). No responses were detected in the soleus muscle. These results indicate that EES elicits antidromic activity along proprioceptive afferents, suggesting that EES interferes with natural sensory information in humans.
EES disrupts kinesthesia. Cancellation of proprioceptive information during EES should alter the conscious perception of joint position and movement velocity. To test this hypothesis, three individuals with a chronic SCI (Supplementary Table 1) completed a threshold to detection of passive movement test. Due to impaired sensory function, only subject #1 and subject #3 could complete the task without EES (Fig. 2b).

Participants sat in a robotic system that imposed a passive isokinetic leg movement (Fig. 2c). They were asked to detect the direction of movement as soon as they could perceive it, but before the knee joint angle reached a predefined amplitude. Without EES, subject #1 detected extension and flexion of the knee with 100% success (median detection angle, 7°; 95% confidence interval (CI), 3.9–11.9°). Without stimulation, subject #3 successfully detected movement onset with 100% success (median detection angle, 6.7°; 95% CI, 5.8–8.4°).

We selected electrode configurations that targeted antagonistic muscles of the knee. We first tested amplitudes that elicited a tingling sensation without producing motor responses (0.8x muscle response threshold). At this intensity and over a broad range of frequencies, continuous EES did not alter subject #1’s performance, while detection of movement onset was disrupted in subject #3 (Fig. 2d and Supplementary Fig. 1). At 1.5x muscle response threshold, EES prevented both participants from detecting leg movements. The participants reported a complete loss of awareness of leg position and movement. These psychophysical experiments corroborate our hypothesis that continuous EES disrupts and may even block proprioceptive information in humans. This disruption occurred at EES amplitudes and frequencies commonly used for rehabilitation.

Continuous EES alters afferent modulation of spinal circuits in humans but not in rats. Proprioceptive signals exert a strong influence on the excitability of sensorimotor circuits. The cancellation of proprioceptive information during continuous EES in humans should therefore affect the modulation of reflex responses elicited by EES. To test this hypothesis, we studied the modulation of reflex responses elicited by various EES frequencies (5–60 Hz) during passive oscillations of the ankle or knee joint. The participants were seated in a robotic system that imposed passive rhythmic flexion–extension movements of the ankle or knee at a fixed angular velocity and amplitude (Fig. 3a and Supplementary Fig. 2). Continuous EES was delivered with electrode configurations and intensities that induced reflex responses in flexor and extensor muscles of the targeted joint (Fig. 3a and Supplementary Fig. 2).

The rhythmic flexion–extension movements of the joint induced a significant phase-dependent modulation of reflex responses in the mobilized muscles (normalized modulation depth > 0.25; P < 0.001 for each frequency, bootstrap test). However, the extent of this modulation depth depended on EES frequency. Quantification of angle-dependent reflex responses revealed a pronounced monotonic decrease of the normalized modulation depth with EES frequency increments.

We performed the same experiments in four lightly anesthetized rats with a contusion SCI that had been implanted with an electrode over the lumbar spinal cord (Fig. 3e–h). Robot-controlled oscillations of the ankle induced a robust modulation of reflex responses (normalized modulation depth > 0.15; P < 0.001 for each frequency, bootstrap test). However, we did not detect systematic relationships between EES frequencies and normalized modulation depth (Fig. 3h). Modulation of motor responses was still present at frequencies as high as 100 Hz (Fig. 3g). A linear fit of the median values yielded a slope close to 0 in all rats (median, 0.0003; 95% CI, [−0.0056, 0.0015]; bootstrap test), suggesting a lack of linear dependency between modulation depth and EES frequency. These experiments indicate that continuous EES disrupts the ability of proprioceptive information to modulate the motor output elicited by EES in humans.

Computational models of proprioceptive feedback circuits during locomotion. We next sought to assess the impact of continuous EES on the natural dynamics of proprioceptive feedback circuits during locomotion. Since these interactions cannot be studied in vivo, we synthesized EES properties, proprioceptive feedback circuits, and leg biomechanics into computational models (Fig. 4a). We adapted a previously validated dynamic computational model to the anatomical features of rats and humans. The model includes the minimal proprioceptive neural network responsible for reciprocal activation of antagonist muscles (Fig. 4b). We used species-specific biomechanical and muscle-spindle models to estimate the firing rates of proprioceptive afferents during locomotion. This afferent activity was used to steer the neural networks (Fig. 4c).

We first studied the impact of EES on the activity of proprioceptive afferents. To model increments in EES amplitude and frequency, we scaled up the number of recruited afferent fibers and the rates of both orthodromic- and antidromic-induced activities. In rats, EES did not alter the modulation depth of proprioceptive information (Fig. 4d). In striking contrast, the same parameters of EES dramatically disrupted the modulation of proprioceptive information in humans. At frequencies as low as 40 Hz, antidromic APs abolished the sensory information conveyed by each electrically stimulated fiber. The residual modulation of proprioceptive information resulted solely from the activity of nonrecruited afferent fibers. The percentage of erased proprioceptive information was directly proportional to EES amplitude.

We then evaluated the impact of this cancellation on the ability of EES to steer reciprocal activation of motor neurons innervating antagonist muscles during locomotion. Continuous EES delivered excitation to Ia-inhibitory interneurons and motor neurons. In rats, the modulation of Ia-inhibitory interneurons driven by the natural proprioceptive information led to a reciprocal activation of antagonist motor neurons during the stance and swing phases of gait. Increasing EES frequency or amplitude resulted in higher firing rates of motor neurons, but only during their natural phase of activity.

In the human model, antidromic collisions dramatically disrupted the dynamics of the neural network (Fig. 5b). At low frequency and low amplitude, continuous EES steered the reciprocal activation of antagonist motor neurons, as observed in rats. With higher stimulation parameters, the cancellation of proprioceptive information prevented phase-dependent modulation of Ia-inhibitory interneurons. The resulting imbalance between antagonist pools of Ia-inhibitory interneurons led to a profound asymmetry in the excitatory drive delivered to motor neurons. Extensor motor neuron pools became overactive while flexor motor neuron pools received strong inhibition.

Together, these results suggest that only a narrow range of EES parameters could be exploited to enhance the excitability of the human spinal cord without compromising the critical role of proprioceptive information in the production of locomotion. Therefore, the degree of controllability over human motor neurons may be very limited compared to that achievable in rats.

Limited facilitation of locomotion in humans compared to rats. We then evaluated the impact of EES frequencies and amplitudes on leg muscle activity during locomotion in rats and humans. Rats with a clinically relevant contusion SCI and EES electrodes (n = 4 rats) were positioned bipedally in a bodyweight support system over a treadmill (Fig. 6a). Continuous EES (40 Hz) induced robust locomotor movements of the otherwise paralyzed legs (Fig. 6b). As previously reported, increases in EES frequencies (20–80 Hz) led to a linear modulation of leg muscle activity, which gradually adjusted kinematic features such as step height (Fig. 6b,c).
Fig. 3 | Effect of EES on the natural modulation of proprioceptive circuits during passive movements. a, Configuration of the experimental setup for subject #2. The subjects were secured in a robotic system that moved the ankle or knee joint passively within the reported range of motion. EES electrodes were configured to target a muscle that underwent stretching cycles during the selected joint movement (red). Configurations of the experimental setup for subjects #1 and #3 are reported in Supplementary Fig. 2. b, Plots showing EES pulses, EMG activity of the vastus medialis, and changes in knee joint angle during passive oscillations of the knee for two different EES frequencies (20 and 40 Hz) in subject #2; similar results were obtained in subjects #1 and #3. The same plots for 60 Hz are reported in Supplementary Fig. 2. Rectangular windows, muscle responses induced by a single pulse of EES; red and gray arrows, the onset of the stimulation pulse and of the muscle response, respectively. c, The joint oscillation cycle was divided into ten bins of equal durations, during which muscle responses were extracted and regrouped. Superimposed muscle responses are displayed for each bin for two EES frequencies (subject #2). Muscle responses used to compute the normalized modulation depth are depicted in light blue. d, Plots reporting the median and 95% CI of the normalized modulation depth, for each EES condition tested and for each subject. The CI was bootstrapped (10,000 iterations) over frequencies (subject #2). Muscle responses used to compute the normalized modulation depth are depicted in light blue. e, The same plots for 60 Hz are reported in Supplementary Fig. 2. Rectangular windows, muscle responses induced by a single pulse of EES; red and gray arrows, the onset of the stimulation pulse and of the muscle response, respectively. f, The joint oscillation cycle was divided into ten bins of equal durations, during which muscle responses were extracted and regrouped. Superimposed muscle responses are displayed for each bin for two EES frequencies (subject #2). Muscle responses used to compute the normalized modulation depth are depicted in light blue. g, Plots reporting the median and 95% CI of the normalized modulation depth, for each EES condition tested and for each subject. The CI was bootstrapped (10,000 iterations) over frequencies (subject #2). Muscle responses used to compute the normalized modulation depth are depicted in light blue. h, Configuration of the experimental setup for rats with severe contusion SCI (250 kdyn) and results, following the same conventions as in b–d for human subjects. Results in f and g are for rat #1; similar results were obtained for all rats. The CI in h was bootstrapped (10,000 iterations) over n = 1,834, n = 1,982, n = 1,984, and n = 1,983 muscle responses, respectively, for rats #1, #2, #3, and #4.

The three human participants with SCI were supported by a gravity-assist\(^\text{18}\) that provided trunk support to facilitate stepping on a treadmill (Fig. 6d). Using rails located on each side of the treadmill, subject #1 (60% body weight support) and subject #2 (70% body weight support) were able to take some steps on the moving treadmill belt and produce alternating activation of antagonist leg muscles without EES. However, this muscle activity did not translate into functional movements, as both feet dragged along the treadmill belt at the end of stance. The amplitude of leg movements remained limited. Continuous EES (40 Hz, 3–9 mA)
Fig. 4 | Impact of continuous EES on proprioceptive afferent firings during locomotion in rats and humans. a, Layout of the computational models built for rats and humans. Components highlighted in brown are tuned to match the anatomical and physiological features of rats vs. humans. b, Spiking neural network model of muscle spindle feedback circuits for a pair of antagonist muscles. Mn, motor neuron; Ex, excitatory interneurons; Iai, Ia-inhibitory interneurons. Synapses highlighted with an asterisk (*) are tuned to match the known properties of human and rat synapses. c, Estimated stretch profiles and afferent firing rates of ankle flexor and extensor muscles over an entire gait cycle in rats (top) and humans (bottom). Similar results were obtained for $n = 8$ gait cycles in rats and $n = 11$ gait cycles in humans. d, Impact of EES on the predicted natural firing rate profiles of group-Ia afferents innervating a flexor muscle of the ankle during locomotion in rats (left) and humans (right). From left to right: averaged firing rate profiles of the simulated population of afferent fibers over one gait cycle, mean afferent firing rate (± s.e.m., $n = 8$ gait cycles in rats, $n = 11$ gait cycles in humans), modulation depth of afferents firing rate profiles (mean ± s.e.m., $n = 8$ gait cycles in rats, $n = 11$ gait cycles in humans), and total amount of sensory information erased by EES. Results are reported over a range of EES frequencies. Top and bottom: results for EES amplitudes recruiting 40% (top) or 80% (bottom) of the entire population of modeled group-Ia afferents.
facilitated leg muscle activity and kinematic features (Fig. 6e,f and Supplementary Figs. 3 and 4). Contrary to what we observed in rats, however, this facilitation was insufficient to enable coordinated, weight-bearing locomotion. Subject #3 exhibited flaccid paralysis of all leg muscles. Continuous EES increased muscle activity, but failed to produce consistent modulation of this activity to produce stepping (Supplementary Fig. 5). All participants reported a complete loss of limb position awareness during continuous EES, which affected their ability to coordinate the timing of locomotor movements.

Consequently, we sought to augment muscle activity with increases in EES frequency or amplitude. From optimal EES parameters, increases in frequency or amplitude did not improve stepping. The amplitude of EMG activity scaled up in flexor muscles, while EES frequency was set to 60 Hz when the amplitude was increased.

Spatiotemporal EES protocols may remedy the limitations of continuous EES. We next exploited our computational model to identify stimulation strategies that may remedy the identified limitations of continuous EES. We reasoned that, to avoid disrupting the natural network dynamics, the temporal and spatial structure of EES should encode the profile of proprioceptive feedback information. We surmised that the amplitude and frequency of the stimulation targeting a specific muscle should be proportional to the instantaneous firing rate of the proprioceptive afferents originating from the sensory organs located in this muscle. Due to the continuous match between the proprioceptive afferent activity and the stimulation profile, EES would augment the overall excitation delivered to the targeted motor pool without compromising the information conveyed by the proprioceptive afferents. Targeting antagonist motor pools with their specific stimulation profile would contribute to maintaining the modulation of reciprocal inhibitory networks that is necessary to facilitate walking with EES. In turn, we hypothesized that adjusting the amplitude and frequency used to configure the stimulation profiles would enable controlling the activity of motor neurons.

We implemented this stimulation strategy in the computational model. We constructed stimulation profiles that combined the natural modulation of primary and secondary proprioceptive afferents (group Ia, group II, and group Ib; Fig. 7a,b) from the homonymous muscles. We did not explicitly model Golgi tendon organs, although Ib afferents are also recruited with EES and provide strong excitation.
during locomotion\(^\text{9}\). Because of the close correlations between Ib afferent firings and homonymous muscle activity\(^\text{10}\), the EMG envelope was used as a surrogate for the firing profile of Ib afferents.

Simulations revealed that this strategy erased proprioceptive information to a similar extent as continuous EES (Fig. 7c). Due to the continuous match between the natural proprioception and stimulation profile, however, the proprioceptive signals reaching the spinal cord contained the same amount of information. Naturally generated APs annihilated by antidromic collision were replaced by EES-produced orthodromic APs. While the percentage of erased information increased with EES amplitude (Fig. 7c), the depth of proprioceptive afferent modulation was preserved or even

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**Fig. 6** | Impact of EES frequencies on muscle activity and leg kinematics during locomotion in rats and humans. (a) Experimental setup in rats. Rats with a severe contusion SCI were positioned in a robotic body weight support system located above a treadmill. Continuous EES was applied over L4 and L2 segments through chronically implanted electrodes secured over the midline of the dorsal spinal cord. (b) EMG activity of the tibialis anterior muscle and foot height trajectory over two gait cycles without EES and with EES delivered at 40 Hz, 60 Hz, and 80 Hz in rat #1; similar results were obtained for rats #2, #3, and #4. (c) Scatter plots reporting step heights at different gait cycles for the tested EES frequencies (\(n = 111\), 139, 101, and 123 gait cycles, respectively, for rats #1, #2, #3, and #4). Dashed lines show linear regressions between the EES frequency and the step height. (d) Experimental setup in humans. Subjects were positioned in a gravity-assist system that provided personalized forward and upward forces to the trunk. Subjects were asked to step on the treadmill while holding the handlebars, since they were not able to step independently with the hands free. (e) EMG activity of flexor (semitendinosus and tibialis anterior) and extensor (rectus femoris and soleus) muscles spanning the right knee and ankle joints, together with the changes in the knee ankle angles and foot elevation over 4 gait cycles without EES and with EES delivered at 20 Hz, 40 Hz, and 80 Hz in subject #1; similar results were obtained for 49 gait cycles (analyzed in f). EES amplitude was set to 1.2x the muscle response threshold (thr). Note the opposite modulation of EMG activity in extensor and flexor muscles with increase in frequencies together with co-activation of flexor with extensor muscles. (f) Violin plots reporting root mean square activities of the recorded muscles, ranges of motion of knee and ankle angles, and step heights at different gait cycles for subject #1 (\(n = 77\) gait cycles). Small gray dots, individual data points; large white dots, medians of the different distributions. Boxes and whiskers report interquartile ranges and adjacent values, respectively. *\(P < 0.05\), **\(P < 0.001\), two-sided Wilcoxon rank-sum test with Bonferroni correction for multiple comparisons. The same results are reported for subjects #2 and #3 in Supplementary Figs. 4 and 5.
increased for higher stimulation amplitudes. Consequently, the stimulation artificially drove reciprocal modulation of Ia-inhibitory interneurons, as would natural proprioception during walking (Fig. 7c). Scaling up EES amplitude led to a proportional increase in the firing rates of proprioceptive afferents, which augmented the excitation delivered to motor neurons. Since this excitation was restricted to the active phase of each motor neuron pool, increasing EES parameters enabled a linear modulation of motor neuron firing rates (Fig. 7c). These results suggest that encoding the profile of proprioceptive afferent activity into the spatiotemporal structure of EES protocols may expand and refine control over the amplitude of motor neuron activity, while also reinforcing the modulation of reciprocal inhibitory networks, thereby enhancing the facilitation of walking compared to continuous EES.

High-frequency, low-amplitude EES alleviates the disruptive effects of continuous EES. We finally explored whether alternative strategies based on continuous EES could alleviate the cancellation of proprioception. We sought to design a stimulation strategy that minimizes the amount of erased proprioceptive information.
during continuous EES while providing high postsynaptic excitation to motor neurons. Each Ia afferent synapses onto every motor neuron that innervates the homonymous muscle. Moreover, high-frequency stimulation of nerve afferents leads to a temporal summation of excitatory postsynaptic potentials (EPSPs) delivered to the targeted cell. We concluded that recruiting a limited number of Ia afferents with a stimulation burst of low amplitude but high frequency could theoretically deliver the same excitation to motor neurons as recruiting a large number of Ia afferents with single pulses of high amplitude. We thus hypothesized that each pulse of EES could be replaced by a high-frequency, low-amplitude burst of EES that would provide the same overall excitation to motor neurons while reducing the overall amount of erased proprioceptive information. Indeed, while proprioceptive information traveling along the recruited fibers would still be blocked by the stimulation, the reduced number of electrically recruited afferents would ensure that a large amount of fibers remained able to convey sensory signals to the spinal cord. Finally, the excitation delivered to motor pools could then be controlled by adjusting the interburst interval.

We tested the hypotheses underlying this stimulation strategy using computer simulations with multicompartamental motor neuron models and realistic distribution of Ia afferent synaptic contacts (Fig. 8a). As predicted, the temporal summation of EPSPs elicited by high-frequency, low-amplitude bursts of stimulation enabled recruiting the same number of motor neurons as single pulses of high-amplitude EES (Fig. 8b).

To validate these results experimentally, we conducted electrophysiological experiments in five rats. Figure 8c shows motor responses recorded in the tibialis anterior during single pulses and single bursts of EES (25-ms duration, frequencies: 100–1,000 Hz) at increasing amplitudes. Compared to single pulses, high-frequency burst stimulation decreased the threshold to elicit a motor response by 39.8% (s.e.m.: ±4.4%). The largest reductions were obtained toward 500 Hz (s.e.m.: ±54.8 Hz). Decreases in EES burst amplitude led to increased latencies of motor responses, suggesting that more pulses were necessary to recruit motor neurons through the temporal summation of EPSPs (Fig. 8d).

The pulse generator implanted in the participants could generate waveforms with a maximum frequency of 125 Hz. However, the simultaneous delivery of interleaved waveforms (2-ms hard-coded delay) enabled the configuration of single bursts composed of 4 pulses delivered at 500 Hz. This feature allowed us to evaluate the concept of high-frequency EES in humans. As observed in rats, high-frequency bursts of EES required markedly reduced stimulation amplitudes to elicit a motor response, compared to single pulses (Fig. 8e,f).

We implemented this stimulation strategy in the computational model. We delivered EES bursts, consisting of 5 pulses at 600 Hz, with a stimulation amplitude recruiting 20% of all primary afferents. Compared to continuous EES, this stimulation reduced the amount of erased proprioceptive information (Supplementary Fig. 6). Decreasing the time between each EES burst led to a proportional increase in the excitation delivered to motor neurons. These results suggest that high-frequency, low-amplitude stimulation protocols may alleviate the detrimental impact of continuous EES on the modulation of proprioceptive feedback circuits in humans.

**Discussion**

We have accumulated evidence that the antidromic recruitment of proprioceptive afferents during continuous EES blocks the propagation of naturally generated proprioceptive signals to the brain and spinal cord. Computer simulations suggest that this cancellation of proprioceptive information disrupts the natural modulation of reciprocal inhibitory networks that is essential to produce alternating recruitment of antagonist motor pools during locomotion. Consequently, only a narrow range of EES parameters can facilitate movement in people with SCI, which is insufficient to enable locomotion without extensive rehabilitation. Computer simulations guided the identification of EES protocols that not only preserve proprioceptive information but also enable a robust control over motor neuron activity. Here we discuss the significance of these results, stress the dramatic consequences of the transient proprioceptive deafferentation during EES, and envision the avenues for translating these new stimulation protocols clinically.

**EES erases proprioceptive information in humans, but not in rats.** Evidence indicates that EES primarily recruits large-diameter afferents within the posterior roots. These afferents originate from proprioceptive organs, which sense changes in muscle length and tension, and to a lesser extent, from mechanoreceptors within the skin. EES elicits orthodromic APs along the recruited afferents that mediate the therapeutic effects of the stimulation. However, we show that EES also induces antidromic APs that travel in the opposite direction. Indeed, recordings of peripheral nerve activity identified antidromic volleys propagating toward sensory organs in response to EES in humans. Previous studies documented the presence of antidromic APs traveling along the sensory fibers of the sciatic, peroneal, and sural nerves in rats, dogs, nonhuman primates, and humans in response to EES applied to thoracic segments. Here we establish the high occurrence of antidromic APs when EES targets the lumbar posterior roots.

We reasoned that EES-induced antidromic action potentials may collide with APs conveying proprioceptive information. The annihilation of APs following these collisions is due to the refractory period of Ranvier’s nodes. Computer simulations predicted a high occurrence probability of these collisions along the recruited afferents when EES is delivered at frequencies commonly used in human studies to facilitate movements after SCI. Due to the longer length and therefore longer propagation time of APs along human proprioceptive afferents, the incidence of these collisions is considerably higher than in rats. These results suggest that EES may partially cancel proprioceptive information in humans.

To assess this possibility, we conducted experiments that highlighted the consequences of these collisions on the integration of proprioceptive information in the brain and spinal cord of humans. First, we found that the delivery of continuous EES abolishes the conscious perception of leg position and displacement. Second, we showed that proprioceptive information drives the modulation of spinal circuits during movement and that the cancellation of this information during continuous EES disrupts this modulation.

Over the past two decades, EES has been applied to thousands of people to alleviate pain and to improve motor function after SCI. For pain treatments, low-intensity stimulation is applied at the thoracic level. Consequently, there was no obvious loss of sensation in the legs during EES. For SCI, the participants exhibited no or limited sensation in the legs, which may explain why this unexpected cancellation of proprioceptive information remained unnoticed. However, this phenomenon has far-reaching implications for the development of a therapy to restore locomotion with EES. Indeed, this transient proprioceptive deafferentation not only alters the conscious control of movement and the modulation of spinal circuits with EES, but may also compromise the reorganization of residual descending pathways during rehabilitation enabled by EES.

**Proprioceptive information must be preserved to enable locomotion with EES.** Bipedal locomotion requires the integration of information from a multiplicity of sensory modalities, of which proprioception may be the most important. Proprioceptive information gives rise to a conscious perception of limb positions that plays a critical role during walking. For example, the sudden loss of proprioception induces severe gait deficits. Individuals with chronic proprioceptive loss can learn to compensate using...
other sensory modalities, especially vision. While this adaptation enables them to walk, the associated cognitive load obliges them to rely on a wheelchair for daily life. All our participants reported a loss of limb position awareness during EES. Consequently, this disruption of proprioception strongly limits the clinical relevance of continuous EES to support locomotion during daily living activities in people with SCI. In addition to its integration in the brain, the information derived from proprioceptive organs is distributed throughout the spinal cord via a dense network of afferent feedback circuits that directly activate motor neurons and shape motor pattern formation during locomotion. Signals from muscle spindles and Golgi tendon organs determine the timing of phase transitions, substantially contribute to leg motor neuron pool recruitment, and coordinate the adaptions.
of leg movements to unpredictable perturbations and task-specific requirements42–45. Our results suggest that these key mechanisms of motor control are obstructed during continuous EES. Moreover, the interruption of descending pathways reinforces the critical role of these proprioceptive feedback circuits, which become the primary source of control for motor pattern formation46. For example, the integration of proprioceptive information enables the spinal cord to coordinate locomotion across a broad range of speeds, loads, and directions in animal models of complete SCI45. The disruption of proprioceptive information during EES would severely degrade this ability of the spinal cord to coordinate motor pattern formation after SCI.

We previously documented some of the mechanisms through which EES facilitates locomotion in rats. In particular, we showed that the modulation of reciprocal inhibitory circuits via proprioceptive feedback during each phase of gait directs the excitatory drive elicited by EES toward the motor neuron pools that are functionally relevant at that specific time47. This mechanism transforms the unspecific excitatory drive into a spatially and temporally specific pattern of excitation delivered alternatingly to the motor neuron pools whose activation is required in the flexion and extension phases of the step cycle. The spinal cord thus acts as an elegant filter that endows EES with the necessary specificity for therapeutic applications. Due to the cancellation of proprioceptive information in humans, only narrow ranges of EES frequencies and amplitudes can take advantage of this mechanism. Computer simulations indicate that EES disrupts movement-related modulation of reciprocal inhibitory circuits as soon as the stimulation elicits responses in muscles. The resulting destabilization of the network leads to an imbalance in the excitation of antagonist motor pools, favoring one motor pool over the other. Consequently, the modulation of EES parameters fails to enable the graded control over motor neuron activity that is observed in the rodent computational model. Experimental recordings confirmed these results, both in rodents and humans with SCI. We previously showed that this controllability enables targeting lesion-specific gait deficits and mediating task-specific adjustments of leg movements through closed-loop controllers and brain–spine interfaces in rats and nonhuman primates3,5,18. These features may be essential to facilitate the complex postural and propulsive requirements underlying the bipedal gait of humans.

Finally, input from proprioceptive organs plays a determinant role in steering the reorganization of residual descending pathways that helps restore locomotion after SCI. Genetically modified mice lacking functional proprioceptive circuits display defective rearrangements of descending projections after SCI, which abolish the extensive recovery occurring spontaneously in wild-type mice after the same injury48. Similarly, clinical studies reported that the preservation of proprioceptive information is a key predictor of recovery after neurotrauma49, suggesting that this specific sensory channel may also contribute to steering the reorganization of residual neuronal pathways in humans. Therefore, the disruption of natural proprioception may reduce the ability of EES to augment neuromuscular plasticity and recovery when delivered during rehabilitation. The multifaceted roles of proprioceptive information in coordinating locomotor functions and steering functional recovery after SCI emphasize the critical importance of identifying EES protocols that preserve proprioceptive information to fulfill the therapeutic potential of this treatment framework for clinical applications.

EES strategies that replace or preserve proprioceptive information. We exploited this new understanding to design sensory-compliant EES protocols that circumvent the cancellation of natural proprioception during EES. We first conceptualized a strategy that aims to replace the cancelled proprioceptive information with a spatiotemporal stimulation profile that encodes the natural firing rates of proprioceptive afferents from each muscle during locomotion. Computer simulations confirmed that this EES protocol not only preserves proprioceptive information but also augments the control over motor neuron activity, while preserving the alternation between antagonist muscles. Realistically, the afferents originating from a single muscle cannot be targeted specifically with current stimulation technologies. However, these stimulation protocols could be approximated with EES bursts delivered over spatially selective spinal cord regions, using a temporal sequence coinciding with the firing profile of the proprioceptive afferents innervating these specific spinal cord regions. This approach shares similarities with EES protocols that encode the spatiotemporal sequence of motor neuron activation during locomotion47. Compared to continuous EES, this targeted stimulation strategy enables a markedly higher degree of control over motor neuron activity in animal models of SCI47. The alternation of spatially selective bursts also preserves the natural proprioceptive information flowing in the dorsal roots that are not engaged by the stimulation. Our simulations suggest that the delivery of EES bursts should coincide with the profile of proprioceptive afferent firing, which can be partially out of phase with motor neuron activity. However, we believe that this protocol would enhance control over motor neuron activity and maximize the amount of preserved proprioceptive information. Such a stimulation strategy shares striking similarities with biomimetic approaches developed for the delivery of realistic tactile sensations in human amputees49.

We found that the delivery of EES bursts with low amplitude but high frequency may be an alternative or complementary stimulation strategy to minimize the cancellation of proprioceptive information. Due to the low amplitude, the stimulation recruits a limited number of afferents. Each proprioceptive afferent synapses onto all the homonymous motoneurons3,5,18. Consequently, the repeated recruitment of these afferents by EES bursts at high frequency leads to a summation of EPSPs in motor neurons, which receive an overall amount of excitation equivalent to that induced by continuous EES at high amplitude and low frequency. However, all the nonrecruited afferents continue providing essential information about muscle length and tension changes. These results have general implications for EES protocols. First, modulating EES bursts allows them to augment the amount of excitation delivered to motor neurons without the need to increase the stimulation amplitude. Second, the lower amplitude requirements would improve the spatial selectivity of the stimulation, since the volume of the electrical field is proportional to the current amplitude.

These stimulation protocols require dedicated implantable pulse generators that allow delivery of EES bursts with high-frequency resolution through independent current sources that are controllable independently in real-time. Various companies are developing next-generation implantable pulse generators that partially meet these requirements. In parallel, we are conducting a clinical study using a commercially available stimulator that we upgraded to enable real-time control of spatially selective EES trains. We found that within 1 week, spatiotemporal stimulation enables independent weight-bearing locomotion in the three participants of the present study49. These combined findings stress the necessity of developing new neurotechnologies that support the implementation of strategies that preserve proprioception to facilitate motor control and steer plasticity with EES in humans.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41593-018-0262-6.

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Author contributions

E.F., M.C., K.M., S.M., and G.C. conceived the study. E.F. and M.C. designed the computational model and E.F. performed the simulations. I.B. performed the surgery in humans. E.F., K.M., F.W., J.B.M., and G.G.I. performed the experiments. A.R. and E.F. built the robotic platform to control rat ankle kinematics. E.F. performed the
data analyses and prepared the figures. G.C. wrote the manuscript with E.F., M.C., and K.M., and all the authors contributed to its editing. G.C., S.M., M.C., and J.B. supervised the work.

**Competing interests**
G.C., J.B., and S.M. are founders and shareholders of GTXmedical SA, a company developing neuroprosthetic systems in direct relationship with the present work. E.F., M.C., G.C., and S.M. hold several patents related to electrical spinal cord stimulation.

**Additional information**
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Methods
Computational simulations. Computer simulations were performed in Python 2.7 using the NEURON \(^1\) simulation environment to run the spiking neural network models and OpenSim \(^2\) for the biomechanical model of rats and humans. Both the NEURON simulation environment and OpenSim are open-source programs.

Model of a proprioceptive afferent fiber recruited by EES. The afferent fiber model was characterized by two parameters: (i) the propagation time required by an AP to travel the whole length of the fiber, and (ii) the firing rate at which APs are generated by the sensory organ. These parameters were adjusted to meet the properties of all the modeled afferent fibers. For each AP, we simulated the propagation from the sensory organ of origin to the spinal cord and the refractory dynamics (mean refractory period ± s.d.: 1.6 ± 0.16 ms) along the fiber. We modeled EES as a periodic event recruiting the most proximal portion of the fiber. The recruitment of a fiber occurred when the fiber was not in its refractory period. When a fiber was electrically activated, an antidromic AP propagated toward the distal end of the fiber. The encounter of this antidromic AP with a sensory AP traveling toward the spinal cord led to an antidromic collision that cancelled both APs.

Estimation of antidromic collisions probability. The developed fiber model was used to assess the probability of antidromic collisions based on EES frequency, the firing rate of the sensory organs, and the propagation time required for an AP to travel along the whole length of the fiber. Propagation times were set to 2 ms in rat afferents. Due to the extended length of sensory fibers in humans, we modeled the human afferents innervating proximal (10 ms) and distal (20 ms) muscles. Antidromic collision probability was defined as the probability of a natural sensory AP to collide with an EES-induced antidromic AP within a single fiber. For each tested model parameter and stimulation frequency, we integrated the dynamic of the fiber over 60 s and evaluated the number of antidromic collisions occurring within this time period. To estimate the antidromic collision probability, we averaged the results of 50 simulations initialized with different EES onset delays varying between 0 and 10 ms.

Rat model of proprioceptive feedback circuits. The rat model of proprioceptive feedback circuits was elaborated from a previously validated model \(^3\), which we modified to integrate a simpler and faster model of the motor neurons with the new model of proprioceptive afferents that considers the occurrence of antidromic collisions.

Briefly, this model is composed of four components: (i) a spiking neural network reproducing the proprioceptive feedback circuits associated with a pair of antagonist muscles, (ii) a muscle spindle model, (iii) a musculoskeletal model of the rat hindlimb, and (iv) a finite-element method model of EES of the rat lumbar spinal cord (Fig. 4a).

The spiking neural network includes populations of group-Ia and group-II afferent fibers, Ia-inhibitory interneurons, group-II excitation interneurons, and pools of alpha motor neurons. The number of cells, the number and the strength of the synapses contacting the different populations of neurons, and the characteristics of the cell models are described in our previous work \(^4\). To speed up the simulation time, we replaced our previous multicompartimental motor neuron model with an integrate-and-fire cell model designed to reproduce realistic membrane response dynamics to excitatory and inhibitory stimuli \(^5\). Specifically, we set the refractory period to 20 ± 1 ms and the membrane time constant τ_membrane to 6 ± 0.3 ms. Excitatory synapses were modeled as instantaneous changes in current, exponentially decaying with time constant τ_synapse to 0.25 ms. Inhibitory synapses were modeled as alpha functions with a rise time constant τ_synapse to 2 ms and a decay time constant τ_synapse to 4.5 ms (Supplementary Fig. 7a). We adjusted the motor neurons synaptic weights to match experimental excitatory and inhibitory postsynaptic potentials (EPSPs/IPSPs). For this, we normalized experimental EPSPs/IPSPs to the minimum depolarization necessary to induce an AP in our multicompartimental model (Supplementary Fig. 7b,c). Afferent fibers were modeled with an AP propagation time of 2 ms. This parameter was estimated to represent rat afferent fibers innervating the antagonist muscles of the ankle.

Musculoskeletal \(^6\) and muscle spindle \(^7\) models were used to calculate the firing rate profiles of group-Ia and group-II afferents innervating the flexor (tibialis anterior) and extensor (gastrocnemius medialis) muscles of the ankle during locomotion. For this purpose, we steered the musculoskeletal model with previously obtained recordings of rat hindlimb kinematics during locomotion to estimate the ankle muscles stretch profiles through inverse kinematics. We then used the muscle spindle model to compute the firing rate profiles. To mimic the alpha–gamma linkage, muscle stretch and stretch velocity were linked to the envelope of EMG activity from the homonymous muscle (equations (1) and (2) \(^\) ).

The estimated antidromic firing rate profiles drove the activity of the modeled proprioceptive afferents. A validated finite element model of EES of the lumbar spinal cord \(^8\) was finally used to estimate the proportion of afferent and efferent fibers recruited at a given stimulation amplitude. Realistic interactions between EES and the natural sensory activity along the modeled afferent fibers were integrated using the developed proprioceptive afferent model.

Ia firing rate = \(50 + 2 \cdot \text{stretch} + 4.3 \cdot \text{sign(stretchVelocity)} \)

II firing rate = \(80 + 13.5 \cdot \text{stretch} + 20 \cdot \text{EMG}_{\text{mean}}\)

\[\text{EMG}_{\text{mean}} = \frac{\text{stretch}}{\text{stretchVelocity}} + 0.6 \cdot \text{EMG}_{\text{mean}}\]

\[\text{percentile}_{90}(\text{MotoneuronsFR}_{\text{rel}}) > 5 \text{ Imp/s}\]
\[\text{percentile}_{90}(\text{MotoneuronsFR}_{\text{rel}}) > 5 \text{ Imp/s}\]
\[1-\text{mean}(\text{MotoneuronsFR}_{\text{rel}} - \text{MotoneuronsFR}_{\text{rel}} > 9)\]
integrates realistic synaptic boutons from group-Ia afferents (Fig. 8a,b). However, simulations on the effect of high-frequency low-amplitude EES on the muscle spindle feedback circuits were performed using the simplified integrate-and-fire motor neuron model (Supplementary Table 1). The multicompartmental model was used in order to obtain a more accurate estimate of motor neurons' soma responses to high-frequency bursts of EES.

Limitations of the human computational model. Microneurographic recordings of group-Ia and group-II afferents during slow movements reported that firing rates rarely exceed 30 Imp/s in humans (Swinderby and Brown, 1975). In the human computational model, we thus limited muscle spindle firing to 50 Imp/s during gait, which is markedly lower than peak firings of up to 200 Imp/s reported during locomotion in quadrupedal mammals. Nevertheless, we cannot exclude the possibility that human muscle spindle afferents fire at higher rates during gait. Indeed, locomotion involves higher gait velocities than those customarily used during microneurographic recordings in humans. Consequently, the actual range of firing rates underlying the activity of group-Ia fibers during human gait remains unknown. While higher firing rates might affect the predictions of our model, the overall conclusions would remain unchanged, since EES would still block a substantial amount of proprioceptive information for high frequencies. Therefore, the degree of disruption may scale with the actual range of afferent firings, but the conclusion derived from this model would still hold.

Experimental procedures in humans. Spinal cord stimulation system implanted in human subjects. Experiments evaluating the activity of the knowledge and human spinal model in SCI were carried out within the framework of an ongoing clinical study (ClinicalTrials.gov identifier: NCT02396453) which has been approved by Swiss authorities (Swissethics protocol number 04/2014 project ID: PB_2016-00886, Swissmedic protocol 2016-MD-0002), and were in compliance with all relevant clinical regulations. The study is conducted at the Lausanne University Hospital (CHUV). All subjects were informed about the procedures and their potential risks and benefits in the presence of their legal representative, and provided written informed consent. The Ethics Committee of the University of Lausanne approved the study (Swissethics protocol number 04/2014 project ID: PB_2016-00886, Swissmedic protocol 2016-MD-0002). At the time of the experiment, participants remained relaxed in a supine position. EES was delivered to produce motor responses in the muscles spanning this joint. The Humac Norm Cybex was used to impose passive joint movements with a maximum displacement of 15° (Fig. 2b). A trial was considered successful if the movement was detected within the allowed range of movement. Subject #2 was not able to perceive the imposed movements and was thus excluded from this experiment.

To quantify the modulation of muscle responses during the passive movements, we extracted the timing of each EES pulse with the recorded stimulation artifacts. We then extracted the muscle responses and grouped them according to the phase of the cyclic movement (n = 10 bins; Fig. 3b). When more than one EES pulse occurred within a given bin, only the response with highest amplitude was selected. We bootstrapped the normalized modulation depth median and 95% confidence interval (equation (4)) by computing the median peak-to-peak amplitudes (mp2P) of the responses in the different bins. Normalization was performed to account for frequency-dependent depression of EES-induced muscle responses (Swinderby and Brown, 1975).

\[ \text{Normalized Modulation Depth} = \frac{[\text{max}(mp2P) - \text{min}(mp2P)]}{\text{min}(mp2P)} \]
Continuous EES during locomotion on a treadmill. The FLOAT robotic suspension system (Lutz Medical Engineering AG, Rudlingen, Switzerland) was used to provide the participants with personalized upward and forward forces to the trunk during locomotion on a treadmill13–15. EES was delivered through four independent configurations of electrodes. Each configuration involved one or multiple anodes and cathodes. We configured these electrode combinations to target the left and right posterior roots projecting to the L1 and L4 segments. For this purpose, we searched the electrode configurations that activated preferentially the iliopsoas and the tibialis anterior. These motor pools spanned the L1/L2 segments and L4/L5 segments, respectively. The amplitudes and frequencies of these four electrode configurations were optimized by visual inspection of the induced EMG activity and facilitation of kinematics when subjects were asked to step on the treadmill. Different EES frequencies and amplitudes were tested to characterize the ability of EES to modulate the motor output. The order of the tested parameters was randomized. EMG recordings were performed with wireless surface electrodes (Myon 320, Myon AG, Schwarzenberg, Switzerland) and recorded at 1,000 Hz. Leg kinematics was recorded using the Vicon motion capture system (Vicon Motion Systems, Oxford, UK) at 100 Hz. Subjects were allowed to use the handrails of the treadmill to facilitate their leg movements. Analysis of EMG activity and kinematics were conducted using methods reported in detail previously16.

Electrophysiologic recordings of high-frequency, low-amplitude EES. EES was delivered through electrode configurations used to facilitate locomotion. Motor responses to both single pulses and bursts of 4 pulses at 500 Hz were recorded from different lower limb muscles with wireless surface electrodes at a sampling rate of 5,000 Hz (Myon 320, Myon AG, Schwarzenberg, Switzerland). The responses of the most-recruited muscle were used for analyses. During the experiment, the participants were in the supine position.

Experimental procedures in rats. Animal husbandry. All procedures and surgeries were approved by the Veterinary Office of the canton of Geneva, Switzerland. All animals were in compliance with all relevant ethical regulations. All interventions were performed in aseptic conditions and under general anesthesia. Briefly, rats received a severe thoracic (T8) contusion SCI (250 kdyn) on a light/dark cycle of 12 h.

Surgical procedures. Surgical procedures have been described in detail previously4,15. All interventions were performed in aseptic conditions and under general anesthesia. Briefly, rats received a severe thoracic (T8) contusion SCI (250 kdyn) by force-controlled spinal cord impactor (IH-9400 Impactor, Precision Systems and Instrumentation LLC, USA). During the same surgery, EES-electrodes were sutured to the dura overlying the midline of S1 and L2 spinal segments in Lewis rats and of L4 and L2 spinal segments in Long-Evans rats. Electrodes were created by removing a short length of insulation (~400 µm) from Teflon-coated stainless-steel wires (AS632, Cooner Wire, USA). A common ground wire electrode (~1 cm of active site) was placed subcutaneously over the right shoulder. Finally, bipolar electrodes (same type as used for EES) were implanted unilaterally in the left and right tibialis anterior muscles to record the EMG activity.

Assessment of EES-induced responses modulated during passive joint movements. Lewis rats (n = 4) were lightly anesthetized (ketamine: 75 mg/kg and xylazine 5 mg/kg, i.p.) and positioned in a prone position within a support system that let the hindlimbs hang freely. The right paw was secured within a 3D-printed pedal connected to a stepper motor (Q5H4241-510-1049, Trimanic Motion Control GmbH, Waterloohain, Germany). We used this robotic platform to impose cyclic movements of the ankle with a fixed amplitude (70°) and frequency (0.54 Hz), while continuous EES was delivered to evoke responses in the tibialis anterior muscle (Fig. 3c). EES was delivered using an I2ZH Stimulator controlled by a RZ2 BioAmp Processor (Tucker-Davis Technologies, Alachua, US). EES amplitude was set to approximately 1.2x the muscle response threshold. We tested EES frequencies ranging from 5 to 100 Hz, delivered in a random order. EMG activity of the tibialis anterior was amplified and filtered online (10–10,000-Hz bandpass filter) by a Differential AC Amplifier (A-M System, Sequim, US) and recorded at 2,000 kHz with the Vicon system.

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Continuous EES during locomotion on a treadmill. Behavioral experiments during locomotion were performed in n = 4 Long-Evans rats. Following 1–2 weeks of rehabilitation using previously described procedures23, we evaluated the impact of different EES frequencies on the modulation of muscle activity and hindlimb kinematics during bipedal locomotion on a treadmill. Locomotion was recorded without EES and with EES at frequencies ranging from 20 to 80 Hz, delivered in a random order. EES amplitude was kept fixed at the optimal value found during training. For each experimental condition, approximately 10 gait cycles or 20 s of minimal leg movements were recorded.

Hindlimb kinematics was recorded with the Vicon motion capture system (Vicon Motion Systems, Oxford, UK) at a sampling frequency of 200 Hz. EMG recordings were obtained using bipolar Ag/AgCl electrodes (1–2 mm in diameter) connected to a differential AC amplifier (A-M System, Sequim, US) at 2,000 kHz with the Vicon system.

Statistics. No statistical methods were used to predetermine sample sizes, but our sample sizes are similar to those reported in previous publications using similar experimental procedures16,18,23. Data collection and analysis were not performed blind to the conditions of the experiments. No data were excluded from the analyses. Different EES conditions were tested on the same animals or participants, and thus no control groups were used. In each experiment, the order of the tested EES conditions was randomized as described in the relevant Methods sections and in the Nature Research Reporting Summary. Data are reported as mean ± s.e.m. or median values ± 95% confidence interval (CI). Confidence intervals and significance were analyzed using nonparametric two-sided Wilcoxon rank-sum tests with Bonferroni correction for multiple comparisons, two-tailed Wald tests, the Clopper–Pearson interval based on a beta distribution, or a bootstrapping procedure based on the Monte Carlo resampling scheme (n = 10,000 iterations). Linear regression between step height and EES frequency (Fig. 6c) was performed assuming a normal distribution of the residuals around zero, but normality was not formally tested for. We made no other assumptions.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

Acquired data are available from the corresponding author upon reasonable request.

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
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- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
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- Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated
- Clearly defined error bars
  State explicitly what error bars represent (e.g. SD, SE, CI)

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection

- NIM Eclipse system software (Medtronic plc, Fridley, Minnesota, USA), to record the neural activity induced by epidural electrical stimulation (EES) in humans.
- Custom code developed using the TwinCat software system (Beckhoff Automation GmbH & Co. KG, Verl, Germany), to record joint kinematic and surface EMG signals during the assessment of proprioceptive functions, and during the assessment of EES-induced responses during passive joint movements performed in humans.
- The Vicon motion capture system software (Vicon Motion Systems, Oxford, UK), to record kinematic and EMG signals during treadmill locomotion in rats and humans, and to record the ankle kinematic during the assessment of EES-induced responses during passive joint movements performed in rats.
- Custom code developed in RPvsEx to control an RZ2 BioAmp Processor (Tucker-Davis Technologies, Alachua, US), to record EMG signals in rats.

Data analysis

Computer simulations were performed in python 2.7 using the NEURON simulation environment to run the spiking neural network models and OpenSim for the biomechanical model of rats and humans. The code to perform and analyze the neural simulations is available as supplementary information and at https://github.com/FormentoEmanuele/MuscleSpindleCircuitsModel.git. Custom python 2.7 code was developed for data analyses, using the SciPy and StatsModels modules for statistical tests.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.
Data

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All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | No statistical methods were used to pre-determine sample sizes but our sample sizes are similar to those reported in previous publications using similar experimental procedures. |
| Data exclusions | No data were excluded from the analyses. |
| Replication | All experimental findings were replicated different times. For example, experiments in humans and rats during walking were collected multiple times on different days with the same outcomes |
| Randomization | - The assessment of proprioceptive functions during epidural electrical stimulation, performed in humans, was performed randomizing both the sequence of the tested stimulation parameters and the sequence of imposed passive movements.  
- The assessments of EES-induced responses during passive joint movements, performed in humans and rats, were performed by randomizing the sequence of the tested stimulation parameters.  
- The experiments on continuous EES during treadmill locomotion, performed in humans and rats, were performed by randomizing the sequence of the tested stimulation parameters. |
| Blinding | The investigators were not blinded to tested conditions. |

Reporting for specific materials, systems and methods

Materials & experimental systems

| n/a | Involved in the study |
| n/a | Unique biological materials |
| n/a | Antibodies |
| n/a | Eukaryotic cell lines |
| n/a | Palaeontology |
| | Animals and other organisms |
| | Human research participants |

Methods

| n/a | Involved in the study |
| n/a | ChIP-seq |
| n/a | Flow cytometry |
| n/a | MRI-based neuroimaging |

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

| Laboratory animals | Experiments in animals were conducted in:  
- Female Lewis rats, 11 weeks old.  
- Female Long-Evans rats, 11 weeks old. |
| Wild animals | The study did not involve wild animals. |
Human research participants

Policy information about studies involving human research participants

Population characteristics

Three male individuals, aged 28-47 y, all with a traumatic cervical spinal cord injury participated in the study. All participants had completed standard of care rehabilitation following their injury and were in a chronic state, 4-6 y post-injury. All displayed low motor scores in the lower limbs or complete motor paralysis, which bound them to a wheelchair.

Recruitment

Participant recruitment was done via the clinicaltrial.gov website where the principal investigators' contact details were disclosed (NCT02936453). Patients and physicians contacted them directly to communicate their interest to participate or to refer a patient to the STIMO study. The clinical study nurse communicated with the patients or the referring physician and reviewed the clinical status of the patient for compliance with the inclusion and exclusion criteria listed below. Participants meeting the inclusion criteria were given the study's flyer and the informed consent form to understand further their implications and involvement within this clinical study. The participants' selection was also based on their ability to live independently and their autonomy in their daily living activities.

Inclusion Criteria:

- Age 18-65 (women or men)
- Incomplete SCI graded as AIS C & D
- Level of lesion: T10 and above, based on AIS level determination by the PI, with preservation of conus function
- The intact distance between the cone and the lesion must be at least 60mm
- Focal spinal cord disorder caused by either trauma or epidural, subdural or intramedullary bleeding
- Minimum 12 months post-injury
- Completed in-patient rehabilitation program
- Able to stand with walker or 2 crutches
- Stable medical and physical condition as considered by Investigators
- Adequate care-giver support and access to appropriate medical care in patient’s home community
- Agree to comply in good faith with all conditions of the study and to attend all required study training and visits
- Must provide and sign Informed Consent prior to any study related procedures

Exclusion Criteria:

- Limitation of walking function based on accompanying (CNS) disorders (systemic malignant disorders, cardiovascular disorders restricting physical training, peripheral nerve disorders)
- History of significant autonomic dysreflexia
- Cognitive/brain damage
- Epilepsy
- Patient who uses an intrathecal Baclofen pump.
- Patient who has any active implanted cardiac device such as pacemaker or defibrillator.
- Patient who has any indication that would require diathermy.
- Patient who has any indication that would require MRI.
- Patient that have an increased risk for defibrillation
- Severe joint contractures disabling or restricting lower limb movements.
- Haematological disorders with increased risk for surgical interventions (increased risk of haemorrhagic events).
- Participation in another locomotor training study.
- Congenital or acquired lower limb abnormalities (affection of joints and bone).
- Women who are pregnant (pregnancy test obligatory for woman of childbearing potential) or breast feeding or not willing to take contraception.
- Known or suspected non-compliance, drug or alcohol abuse.
- Spinal cord lesion due to either a neurodegenerative disease or a tumour.
- Patient has other anatomic or co-morbid conditions that, in the investigator’s opinion, could limit the patient’s ability to participate in the study or to comply with follow-up requirements, or impact the scientific soundness of the study results.
- Patient is unlikely to survive the protocol follow-up period of 12 months.