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LETTERS

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Neuromodulation of lumbosacral spinal networks enables independent stepping after complete paraplegia

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Spinal sensorimotor networks that are functionally disconnected from the brain because of spinal cord injury (SCI) can be facilitated via epidural electrical stimulation (EES) to restore robust, coordinated motor activity in humans with paralysis[1–3]. Previously, we reported a clinical case of complete sensorimotor paralysis of the lower extremities in which EES restored the ability to stand and the ability to control step-like activity while side-lying or suspended vertically in a body-weight support system (BWS)[4]. Since then, dynamic task-specific training in the presence of EES, termed multimodal rehabilitation (MMR), was performed for 43 weeks and resulted in bilateral stepping on a treadmill, independent from trainer assistance or BWS. Additionally, MMR enabled independent stepping over ground while using a front-wheeled walker with trainer assistance at the hips to maintain balance. Furthermore, MMR engaged sensorimotor networks to achieve dynamic performance of standing and stepping. To our knowledge, this is the first report of independent stepping enabled by task-specific training in the presence of EES by a human with complete loss of lower extremity sensorimotor function due to SCI.

Scientific evidence has shown that EES of lumbosacral spinal circuitry can generate tonic and rhythmic patterns of motor activity in spinalized animals[5–11] and humans diagnosed with complete SCI[12–15]. More recently, EES in humans with complete loss of motor function after SCI enabled volitional control of flexion and extension leg movements, as well as standing[1,2]. However, EES-enabled stepping after complete loss of lower extremity function due to SCI has not been reported.

Despite a clinical diagnosis of complete loss of sensorimotor function after traumatic SCI, subfunctional neural connections likely remain intact across the injury (that is, discomplete SCI profile)[16–18]. Spared connections across a discomplete SCI are not robust enough to generate clinically observable functions. However, they are capable of modulating excitability of sublesional spinal sensorimotor networks[19–22].

Previously, we reported a case of complete loss of function below the sixth thoracic spinal segment due to SCI that occurred 3 years before study enrollment. During 22 weeks of locomotor training (LT), before implantation of the EES system, evidence of discomplete SCI was observed. The LT approach to functional recovery has been shown to improve walking speed in humans with partially retained motor functions after SCI[23,24], but LT has not enabled recovery of stepping in those with complete loss of motor function in the lower extremity[25,26].

After initial outcomes of standing and intentional control of step-like leg movement using EES were observed, we set out to determine whether the same subject could achieve independent weight-bearing stepping during 43 weeks of MMR. MMR sessions were comprised of subject-driven attempts to perform EES-enabled activities while supine, side-lying, seated, standing, and stepping with trainer assistance and with BWS provided as needed.

Clinical evaluations, performed at several time points throughout the study with EES turned off, indicated no change in function (Supplementary Fig. 1a,b). Transcranial magnetic stimulation (TMS) over the scalp elicited motor evoked potentials (MEPs) of normal latencies in muscles innervated from above the SCI; however, MEPs were not present below the SCI (Supplementary Fig. 1c). Tibial somatosensory evoked potentials (SSEPs) were not present in recordings over the scalp (Supplementary Fig. 1c). Over the course of the study, changes in the Modified Ashworth scale of spasticity were unremarkable (Supplementary Fig. 1d). Magnetic resonance imaging (MRI) of the spine showed intact tissue within the SCI site (Supplementary Fig. 2). Prolonged attempts to maximally contract muscles, with intention focused on increasing activity in either the left or right leg, resulted in bursts of muscle activity across agonist and antagonist leg muscles that increased in amplitude and leg selectivity over time; however, functional movement was not achieved (Supplementary Fig. 3). Together, these results indicate a functionally complete SCI with evidence of a discomplete SCI profile.

The use of a single program of active electrodes and stimulation parameters (voltage, pulse width, and frequency) enabled the

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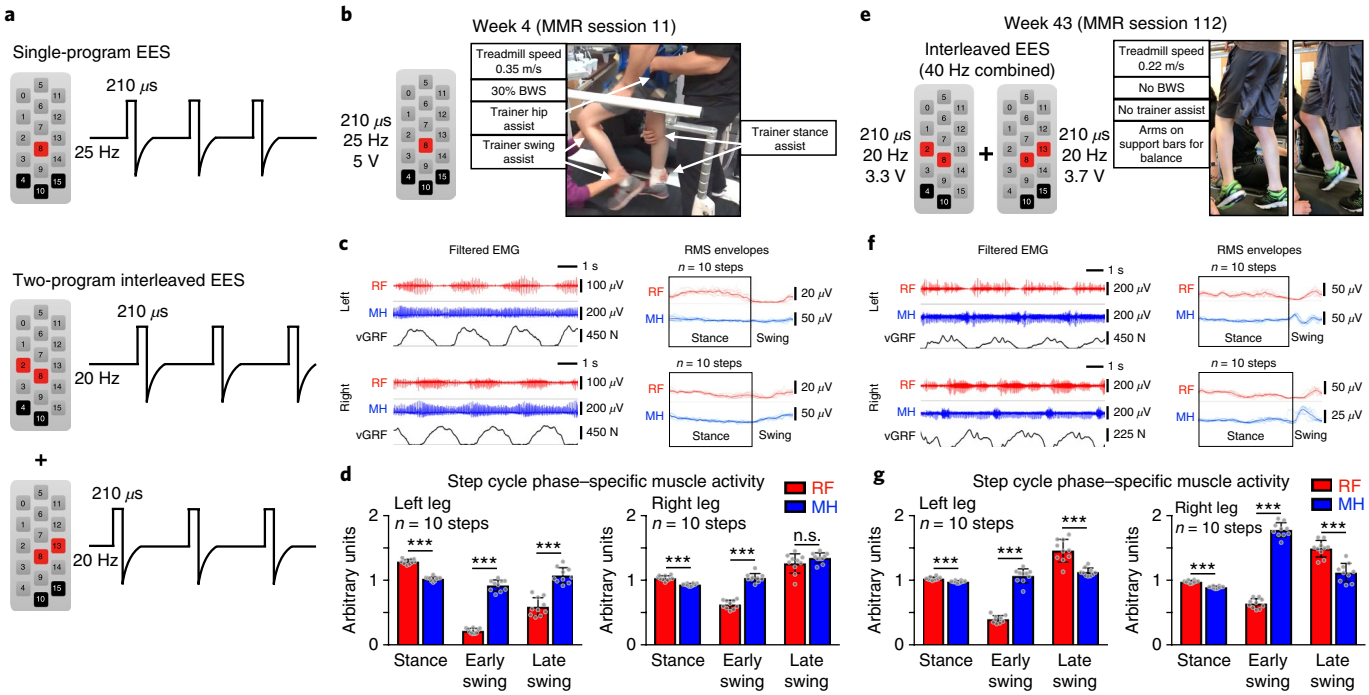

Fig. 1 | Progression of EES-enabled stepping performance on a treadmill. a, Schematic depicting single-program EES used during week 4 of MMR and two-program interleaved EES used during week 43 to enable stepping on a treadmill. Anodes, red squares; cathodes, black squares. **b**,**e**, EES settings and exemplary image from week 4 (**b**) and week 43 (**e**) depicting trainer assistance and BWS needed to achieve stepping. **c**,**f**, Filtered EMG and averaged RMS envelopes from the RF and MH synchronized to vGRF recordings under each foot at week 4 (**c**) and week 43 (**f**). vGRF, vertical ground reaction force. **d**,**g**, Differences in RF and MH activity during stance, early (first 50%) swing phase, and late (last 50%) swing phase are shown as means (±s.d) at week 4 (**d**) and week 43 (**g**). RMS envelopes and means were generated from 10 steps of each leg at week 4 and week 43. n.s., not significant; ***$P<0.001$, two-tailed Mann–Whitney test.

subject to control function in one leg at a voltage intensity that was suboptimal for enabling function in the contralateral leg (Fig. 1a). Over the course of MMR, we discovered that two interleaved EES programs enabled bilateral control of leg functions via independent adjustment of active electrodes and voltage intensities within each program.

During treadmill stepping at week 4 of MMR, single-program EES was applied while the subject attempted to step on a treadmill moving at 0.35 m/s (Fig. 1b). In order to achieve EES-enabled treadmill stepping, 30% BWS and trainer assistance at the hip, knee, and ankle of each leg were required (Fig. 1b). Rectus femoris (RF) and medial gastrocnemius (MG) activity, recorded by skin surface electromyography (EMG), was characterized by bursts of activity during the stance phase with inhibition during the swing phase (Fig. 1c and Supplementary Fig. 4b) of the step cycle. Medial hamstring (MH) activity was characterized by tonic activation throughout the step cycle (Fig. 1c). Root mean square (RMS) envelopes of EMG recordings during 20 consecutive steps showed a lack of coordinated activity across muscles during the swing phase of the step cycle (Fig. 1c and Supplementary Figs. 4 and 5). For both legs, levels of RF and MG activity ($P<0.001$) during the stance phase were significantly higher than that for MH and tibialis anterior (TA), respectively (Fig. 1c, d and Supplementary Fig. 4c). During the early portion of the swing phase, EMG of flexor muscles (MH and TA) showed significantly higher levels of activation than that of antagonist extensor muscles (RF and MG) ($P<0.001$). However, during the later portion of the swing phase (that is, leg extension to transition from swing to initial weight bearing of stance phase), the left MH and TA remained significantly more active than the RF ($P<0.001$) and MG ($P=0.001$), respectively. Similarly, the right TA remained significantly more active than the MG ($P<0.001$); however, right RF and MH activity levels were not significantly different, indicating a state of cocontraction ($P=0.14$) and thereby impairing the ability to independently extend the leg for stance phase load acceptance.

By week 43 of MMR, the use of two interleaved EES programs improved the subject's ability to control each leg in order to achieve independent stepping on the treadmill at a speed of 0.22 m/s without trainer assistance or BWS (Fig. 1e). The subject maintained upper body balance via hand placement on support bars to facilitate anterolateral weight shifting during gait (Fig. 1e and Supplementary Video 1). For both legs, EMG showed bursts of RF activity during the stance phase, with inhibition occurring during the early part of the swing phase (Fig. 1f and Supplementary Fig. 5). MH activity was characterized by tonic activation during stance and bursting activity during swing phase with reciprocal inhibition of RF activity. During early swing phase, MH and TA activity was significantly higher than RF (left $P<0.001$, right $P<0.001$) and MG (left $P<0.001$, right $P=0.002$), respectively, allowing independent leg flexion during swing phase (Fig. 1g, Supplementary Fig. 4f and Supplementary Video 1). Most importantly, activation profiles during late swing demonstrated a significant increase in RF activity compared to MH for both legs ($P<0.001$) (Fig. 1g), allowing independence during load acceptance at the beginning of the stance phase. When EES was turned off, stepping ability was lost.

During step training over ground at week 16 of MMR, single-program EES enabled stepping over ground. In order to step, a front-wheeled walker was required with trainer assistance to facilitate the swing phase of one leg while bracing the contralateral limb to maintain stance (Fig. 2a). Trainer assistance was also required at the hips to facilitate weight shifting and to maintain balance. In both legs, RF activation occurred during the stance phase with inhibition during the swing phase (Fig. 2b). RMS envelopes of EMG recordings from the RF and MH during 20 consecutive steps (10 in each leg) showed a lack of coordinated activity during the swing phase of the left leg,

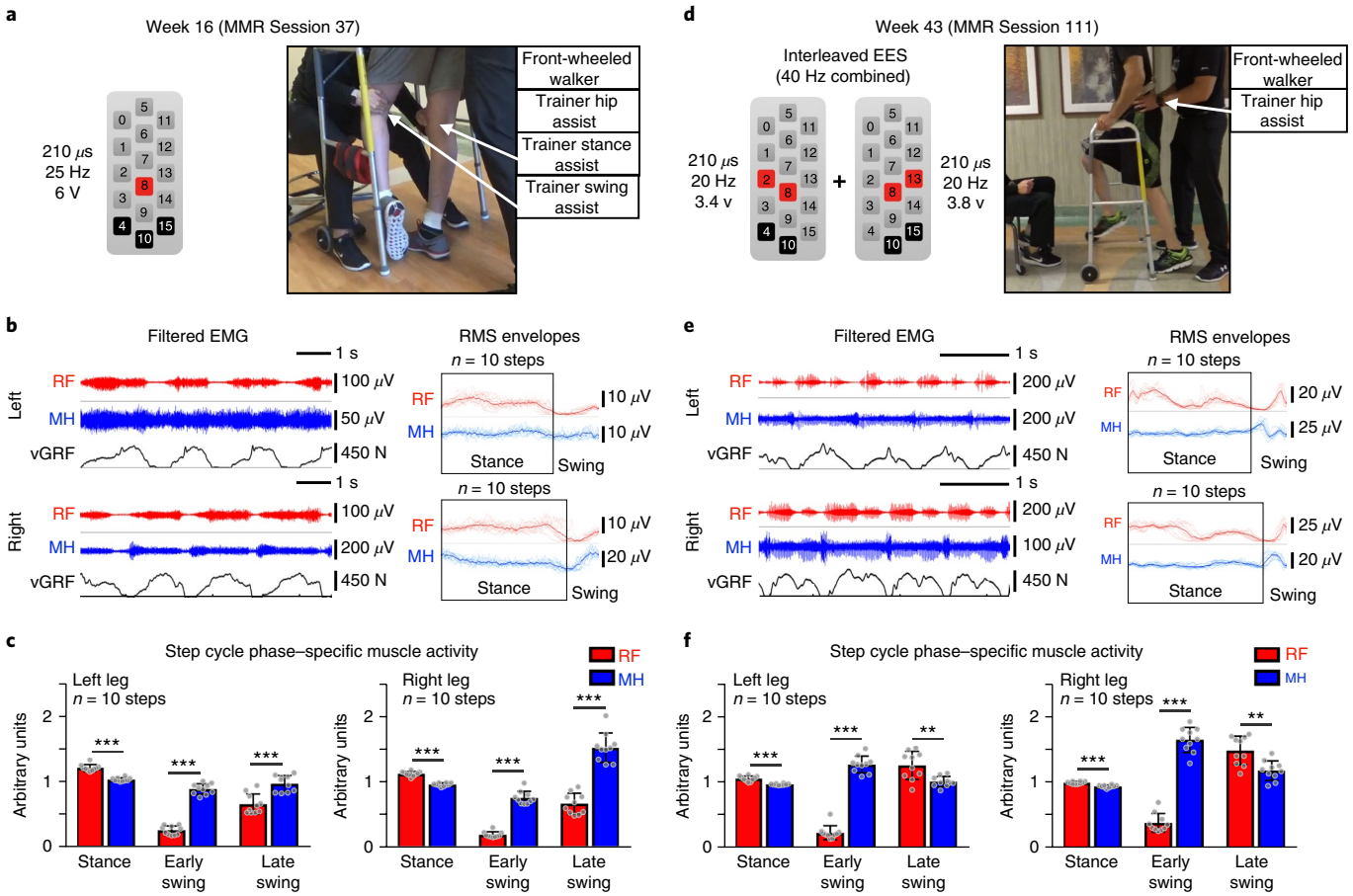

Fig. 2 | Progression of EES-enabled stepping performance over ground. a,**d**, EES settings and exemplary image from week 16 (**a**) and week 43 (**d**) depicting trainer assistance needed to achieve stepping. **b**,**e**, Filtered EMG and averaged RMS envelopes from the RF and MH synchronized to vGRF recordings under each foot at week 16 (**b**) and week 43 (**e**). **c**,**f**, Differences in RF and MH activity during stance, early (first 50%) swing phase, and late (last 50%) swing phase are shown as means (±s.d) at week 16 (**c**) and week 43 (**f**). RMS envelopes and means were generated from 10 steps of each leg at week 16 and week 43. Left leg week 43 $^{**}P = 0.007$, right leg week 43 $^{**}P = 0.005$, $^{***}P < 0.001$, two-tailed Mann–Whitney test.

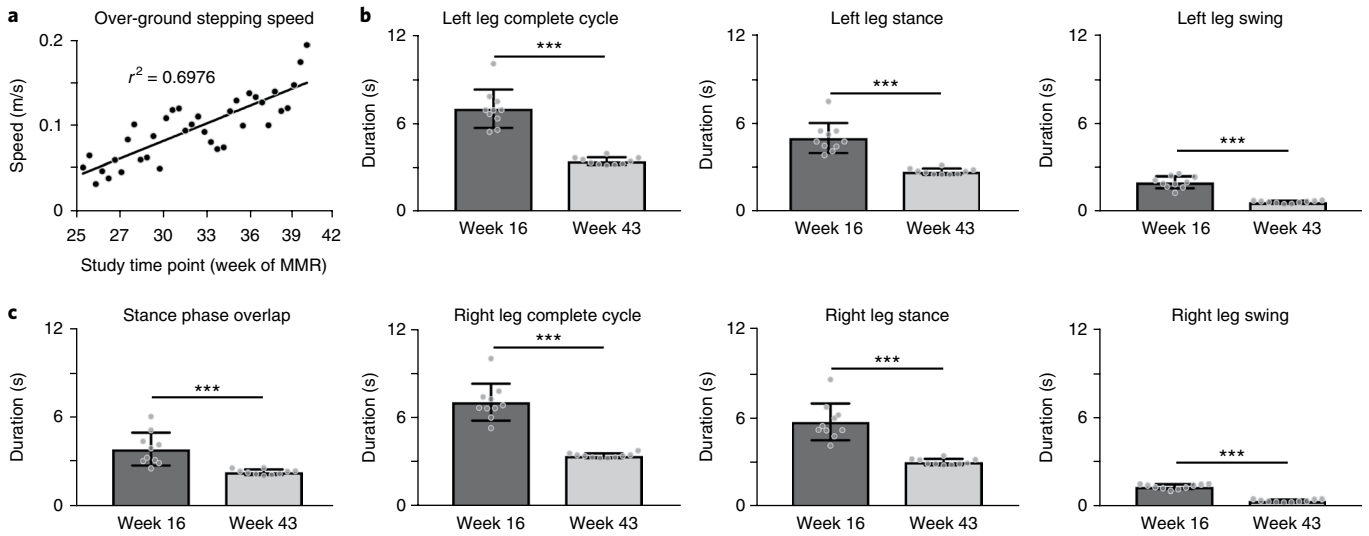

Fig. 3 | Step cycle characteristics during EES-enabled stepping over ground. a, Stepping speed improved from week 25 to 42. r^2 goodness of fit linear regression analysis. **b**, Step cycle, stance phase, and swing phase durations decreased significantly between weeks 16 and 43 of over-ground stepping ($n = 10$ steps of each leg). $^{***}P < 0.001$, two-tailed Mann–Whitney test. **c**, Duration of overlapping stance phase decreased significantly between weeks 16 and 43 of over-ground stepping ($n = 10$ steps of each leg). $^{***}P < 0.001$, two-tailed Mann–Whitney test.

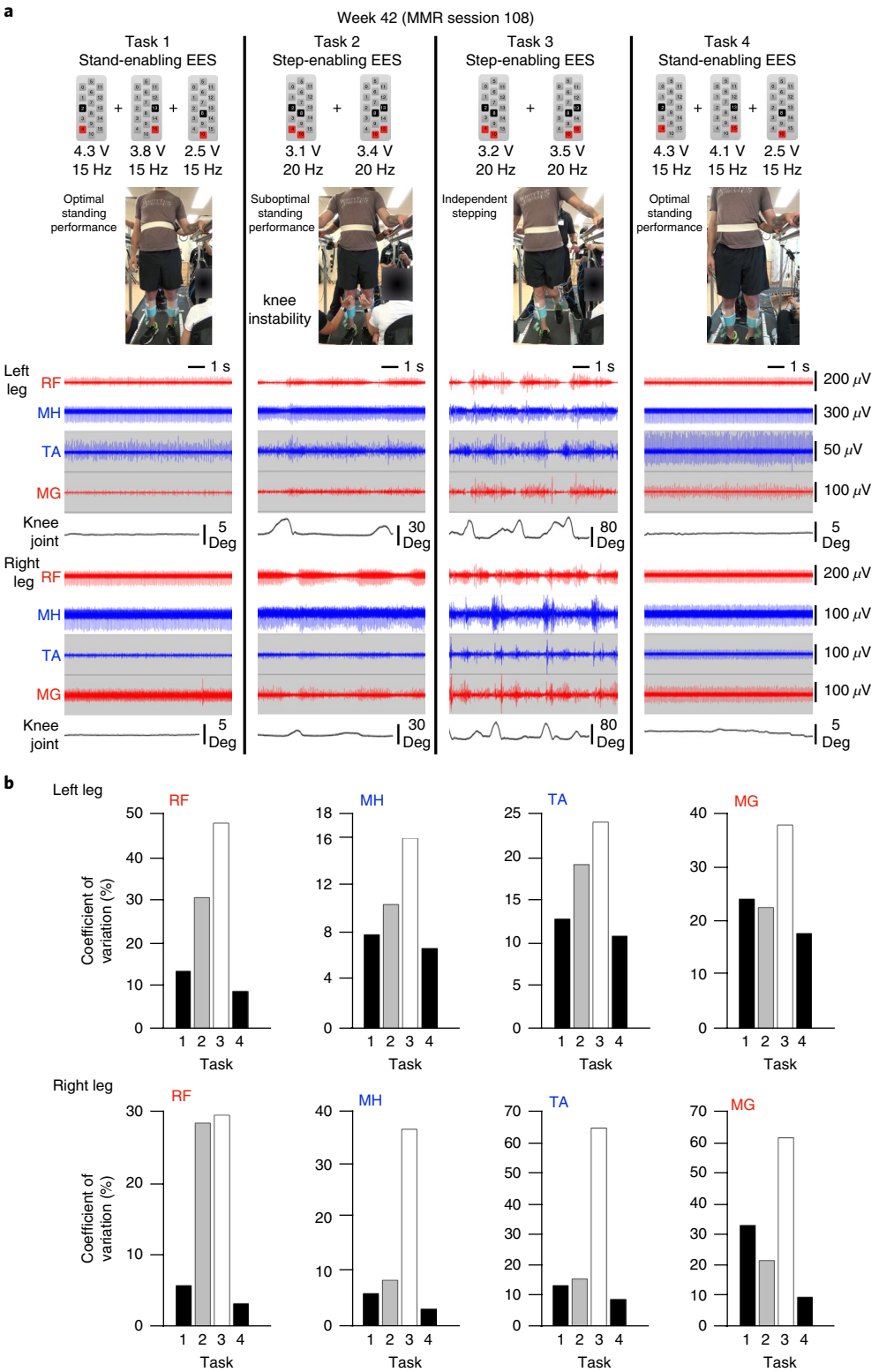

Fig. 4 | Independent standing and stepping during a single MMR session. a, Within a single MMR session, four task-specific activities were performed using EES parameters that enabled independent standing or stepping tasks. Task 1, standing was performed without BWS or trainer assistance using optimal EES settings to enable standing. Task 2, standing was performed using step-enabling EES parameters. Task 3, step-enabling EES parameters combined with the subject's intention to step allowed independent stepping. After stepping, independent standing (task 4) was achieved using optimal stand-enabling EES parameters. Deg, degrees. **b**, Coefficient of variation (%) for each muscle (RF, MH, TA, MG) during each task (1–4), indicating rhythmic and tonic patterns of muscle activity. Coefficient of variation was calculated as the ratio of the s.d. to the mean expressed as a percentage[12].

while the right leg muscles showed modest levels of coordination (Fig. 2b and Supplementary Fig. 7). Similar to week 4 of treadmill stepping, statistical analyses indicated significantly higher levels of RF activity than MH activity occurred during the stance phase of each leg ($P<0.001$), followed by significantly higher levels of MH activity than RF activity during the early swing phase ($P<0.001$).

However, during the late swing phase, MH activity remained significantly higher than RF ($P<0.001$), preventing independent leg extension during swing-to-stance transitions.

At week 43 of MMR, interleaved EES programming, used to enable independent stepping on the treadmill, also enabled stepping over ground while using a front-wheeled walker and only intermittent trainer assistance to facilitate weight shifting and to maintain balance (Fig. 2d and Supplementary Video 2). For both legs, RF activity was characterized by EMG bursts during the stance phase followed by inhibition during the early swing phase (Fig. 2e). Normalized muscle activity of the left and right leg showed significantly higher levels of RF activity than MH activity during the stance phases ($P<0.001$) followed by significantly higher activation of the MH while the RF was inhibited ($P<0.001$) during the early swing phase (Fig. 2f). During the late swing phase, activation profiles demonstrated a significant increase in RF activity compared to MH (left $P=0.007$, right $P=0.005$) (Fig. 2f), which allowed independent swing-to-stance load acceptance.

From week 25 to 42 of MMR, interleaved EES programming and a front-wheeled walker were used for over-ground stepping activities. During this time, EES-enabled step speed improved from 0.05 m/s at week 25 to 0.20 m/s at week 42 (Fig. 3a). The maximum number of steps taken during a single MMR session was 331, and the maximum distance traveled was 102 m (Supplementary Fig. 8). For both legs, total step cycle, stance, and swing phase durations all decreased significantly ($P<0.001$) from week 16 to week 43 (Fig. 3b). Additionally, the duration of overlapping stance phase across legs decreased significantly over time (Fig. 3c, $P<0.001$).

Contrary to findings reported by Rejc et al.[28], which suggest that EES-enabled task-specific activities have a detrimental downstream effect on subsequent EES-enabled activities (that is, EES-enabled standing training negatively impacts stepping performance), the dynamic approach of stand and step training during each MMR session enabled independent standing and stepping (Fig. 4 and Supplementary Video 3). We observed that task-specific EES parameters enabled independent standing and, when parameters were adjusted to enable stepping, standing was still possible; however, stability decreased and rhythmic patterns were observed in EMG recordings (Fig. 4a, b). Nevertheless, standing was achieved with little to no trainer assistance when the subject used step-enabling EES with intent to stand. When the subject changed his intention from standing to stepping, he was able to independently step with both legs on a treadmill. Subsequent to stepping, EES parameters were adjusted to enable optimal standing performance, and as a result of task-specific EES with dynamic training of spinal sensorimotor networks during MMR, independent standing was achieved.

In summary, during 43 weeks of MMR, task-specific EES training enabled independent stepping on a treadmill, stepping over ground while using a front-wheeled walker and intermittent trainer assistance, and independent standing. For both treadmill and over-ground stepping, the use of two interleaved EES programs enabled a significant change in muscle activity profiles during the swing phase of the step cycle. This shift in coordination coincided with an ability to step without trainer assistance or BWS on the treadmill and with minimal trainer assistance and no BWS over ground. These results, combined with prior evidence of enabling motor control via EES following SCI in humans[1,2,28–33], emphasize the need to reassess our current understanding of the biological underpinnings of complete SCI and how spinal neuromodulation in the presence of task-specific training progressively enables functions that were once thought to be permanently lost following SCI[19,21,34–36].

The interleaved EES paradigm identified in this study likely played a key role in enabling stepping abilities. Additionally, the dynamic MMR paradigm may have re-educated spinal neural networks associated with locomotor activities[37]. During MMR, the subject was encouraged to use his arms on support bars to manipulate his body during EES-enabled stepping. In addition, trainer assistance and BWS were adjusted as performance improved in order to maximize independence. To our knowledge, the use of EES during task-specific training, including both standing and stepping activities, is novel and therefore warrants further investigation in a larger sample size of subjects to determine its validity and efficacy.

We have demonstrated that human spinal networks can be transformed years after SCI to reach physiologic states that generate coordinated and robust spinal motor outputs to generate independent stepping and standing. The outcomes we have reported suggest that the MMR paradigm, which was focused on dynamically training multiple motor tasks in the presence of EES-facilitated spinal network activity, enhanced a synergistic functional reorganization of supraspinal–spinal connectivity. The new outcomes we have reported support the concept that spinal neuromodulation with sensorimotor rehabilitation facilitate functional reorganization of the supraspinal–spinal connectome to recover functions lost due to SCI.

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/s41591-018-0175-7.

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References

1. Harkema, S. et al. Effect of epidural stimulation of the lumbosacral spinal cord on voluntary movement, standing, and assisted stepping after motor complete paraplegia: a case study. *Lancet* **377**, 1938–1947 (2011).
2. Angeli, C. A., Edgerton, V. R., Gerasimenko, Y. P. & Harkema, S. J. Altering spinal cord excitability enables voluntary movements after chronic complete paralysis in humans. *Brain* **137**, 1394–1409 (2014).
3. van den Brand, R. et al. Restoring voluntary control of locomotion after paralyzing spinal cord injury. *Science* **336**, 1182–1185 (2012).
4. Grahn, P. J. et al. Enabling task-specific volitional motor functions via spinal cord neuromodulation in a human with paraplegia. *Mayo Clin. Proc.* **92**, 544–554 (2017).
5. Gerasimenko, Y. P. et al. Epidural spinal cord stimulation plus quipazine administration enable stepping in complete spinal adult rats. *J. Neurophysiol.* **98**, 2525–2536 (2007).
6. Ichiyama, R. M., Gerasimenko, Y. P., Zhong, H., Roy, R. R. & Edgerton, V. R. Hindlimb stepping movements in complete spinal rats induced by epidural spinal cord stimulation. *Neurosci. Lett.* **383**, 339–344 (2005).
7. Lavrov, I. et al. Epidural stimulation induced modulation of spinal locomotor networks in adult spinal rats. *J. Neurosci.* **28**, 6022–6029 (2008).
8. Capogrosso, M. et al. A brain–spine interface alleviating gait deficits after spinal cord injury in primates. *Nature* **539**, 284–288 (2016).
9. Wenger, N. et al. Spatiotemporal neuromodulation therapies engaging muscle synergies improve motor control after spinal cord injury. *Nat. Med.* **22**, 138–145 (2016).
10. Wenger, N. et al. Closed-loop neuromodulation of spinal sensorimotor circuits controls refined locomotion after complete spinal cord injury. *Sci. Transl. Med.* **6**, 255ra133 (2014).
11. Lavrov, I. et al. Facilitation of stepping with epidural stimulation in spinal rats: role of sensory input. *J. Neurosci.* **28**, 7774–7780 (2008).
12. Dimitrijevic, M. R., Gerasimenko, Y. & Pinter, M. M. Evidence for a spinal central pattern generator in humans. *Ann. NY Acad. Sci.* **860**, 360–376 (1998).
13. Minassian, K. et al. Stepping-like movements in humans with complete spinal cord injury induced by epidural stimulation of the lumbar cord: electromyographic study of compound muscle action potentials. *Spinal Cord* **42**, 401–416 (2004).
14. Danner, S. M. et al. Human spinal locomotor control is based on flexibly organized burst generators. *Brain* **138**, 577–588 (2015).
15. Rattay, F., Minassian, K. & Dimitrijevic, M. R. Epidural electrical stimulation of posterior structures of the human lumbosacral cord: 2. quantitative analysis by computer modeling. *Spinal Cord* **38**, 473–489 (2000).
16. Kakulas, B. A. Pathology of spinal injuries. *Cent. Nerv. Syst. Trauma* **1**, 117–129 (1984).
17. Dimitrijevic, M. R., Dimitrijevic, M. M., McKay, W. B. & Sherwood, A. M. EMG evidence of supraspinal influence on motor unit activity in paralyzed muscles. *Clin. Neurophysiol.* **56**, S68 (1983).

18. Dimitrijević, M. R. Residual motor functions in spinal cord injury. *Adv. Neurol.* **47**, 138–155 (1988).
19. Taccola, G., Sayenko, D., Gad, P., Gerasimenko, Y. & Edgerton, V. R. And yet it moves: recovery of volitional control after spinal cord injury. *Prog. Neurobiol.* **160**, 64–81 (2018).
20. Dimitrijevic, M. R. et al. Human spinal cord motor control that is partially or completely disconnected from the brain. *Am. J. Neuroprot. Neuroregen.* **8**, 12–26 (2016).
21. Minassian, K. & Hofstoetter, U. S. Spinal cord stimulation and augmentative control strategies for legmovement after spinal paralysis in humans. *CNS Neurosci. Ther.* **22**, 262–270 (2016).
22. Sherwood, A. M., Dimitrijevic, M. R. & McKay, W. B. Evidence of subclinical brain influence in clinically complete spinal cord injury: discomplete SCI. *J. Neurol. Sci.* **110**, 90–98 (1992).
23. Field-Fote, E. C. & Roach, K. E. Influence of a locomotor training approach on walking speed and distance in people with chronic spinal cord injury: a randomized clinical trial. *Phys. Ther.* **91**, 48–60 (2011).
24. Harkema, S. J., Schmidt-Read, M., Lorenz, D. J., Edgerton, V. R. & Behrman, A. L. Balance and ambulation improvements in individuals with chronic incomplete spinal cord injury using locomotor training-based rehabilitation. *Arch. Phys. Med. Rehabil.* **93**, 1508–1517 (2012).
25. Forrest, G. F. et al. Neuromotor and musculoskeletal responses to locomotor training for an individual with chronic motor complete AIS-B spinal cord injury. *J. Spinal Cord Med.* **31**, 509–521 (2008).
26. Dobkin, B. et al. Weight-supported treadmill vs over-ground training for walking after acute incomplete SCI. *Neurology* **66**, 484–493 (2006).
28. Carhart, M. R., He, J., Herman, R., D'Luzansky, S. & Willis, W. T. Epidural spinal-cord stimulation facilitates recovery of functional walking following incomplete spinal-cord injury. *IEEE Trans. Neural. Syst. Rehabil. Eng.* **12**, 32–42 (2004).
29. Rejc, E., Angeli, C. & Harkema, S. Effects of lumbosacral spinal cord epidural stimulation for standing after chronic complete paralysis in humans. *PLoS One* **10**, e0133998 (2015).
30. Gerasimenko, Y. P. et al. Noninvasive reactivation of motor descending control after paralysis. *J. Neurotrauma* **32**, 1968–1980 (2015).
31. Lu, D. C. et al. Engaging cervical spinal cord networks to reenable volitional control of hand function in tetraplegic patients. *Neurorehabil. Neural Repair.* **30**, 951–962 (2016).
32. Gad, P. et al. Weight bearing over-ground stepping in an exoskeleton with non-invasive spinal cord neuromodulation after motor complete paraplegia. *Front. Neurosci.* **11**, 333 (2017).
33. Huang, H., He, J., Herman, R. & Carhart, M. R. Modulation effects of epidural spinal cord stimulation on muscle activities during walking. *IEEE Trans. Neural. Syst. Rehabil. Eng.* **14**, 14–23 (2006).
34. Shah, P. K. & Lavrov, I. Spinal epidural stimulation strategies: clinical implications of locomotor studies in spinal rats. *Neuroscientist.* **23**, 664–680 (2017).
35. Courtine, G. & Bloch, J. Defining ecological strategies in neuroprosthetics. *Neuron* **86**, 29–33 (2015).
36. Moritz, C. T. Now is the critical time for engineered neuroplasticity. *Neurotherapeutics* **15**, 628–634 (2018).
37. Shah, P. K. et al. Variability in step training enhances locomotor recovery after a spinal cord injury. *Eur. J. Neurosci.* **36**, 2054–2062 (2012).

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Author contributions

V.R.E., P.J.G., M.L.G., K.H.L., and K.D.Z. initiated the project. V.R.E., D.I.D., Y.P.G., M.L.G., P.J.G., I.A.L., K.H.L., A.R.T., D.G.S., J.A.S., M.G.v.S., and K.D.Z. designed the experiments with contributions from all authors. L.A.B., J.S.C., M.L.G., I.A.L., M.B.L., J.A.S., and K.H.L. performed clinical assessments. L.A.B., M.L.G., M.B.L., J.A.S., D.D.V., and M.G.V.S. designed and performed rehabilitation. V.R.E., Y.P.G., P.J.G., I.A.L., J.A.S., L.A.B., K.H.L., and D.G.S. performed intraoperative assessments, and K.H.L. performed surgical implantation of the device. L.A.B., J.S.C., M.L.G., P.J.G., M.B.L., I.A.L., A.R.T., M.G.V.S., D.D.V., D.G.S., Y.P.G., and V.R.E. contributed to stimulation setting refinement. L.A.B., J.S.C., P.J.G., A.R.T., C.L., M.G.V.S., D.D.V., D.G.S., Y.P.G., V.R.E., M.L.G., I.A.L., K.H.L., K.D.Z., and J.A.S. contributed to data collection, analysis, and interpretation. J.S.C., M.L.G., P.J.G., M.B.L., and I.A.L. drafted the manuscript with subsequent contribution from all authors. K.H.L. and K.D.Z. supervised all aspects of the work.

Competing interests

V.R.E. and Y.G. are shareholders in NeuroRecovery Technologies and hold inventorship rights on intellectual property licensed by the regents of the University of California to NeuroRecovery Technologies and its subsidiaries. K.H.L. previously served as a consultant to Medtronic's Department of Technology Development focused on deep brain stimulation.

Additional information

Supplementary information is available for this paper at https://doi.org/10.1038/s41591-018-0175-7.

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Methods

Ethical approval. This study was conducted in one subject who provided informed consent to all procedures which were performed under the approval of the Mayo Clinic Institutional Review Board with a US Food and Drug Administration Investigational Device Exemption (IDE G150167, ClinicalTrials.gov identifier: NCT02592668).

Description of study participant. A 26-year-old male sustained a traumatic fracture and dislocation of the eighth thoracic vertebra resulting in a SCI and immediate loss of function below the sixth thoracic spinal segment three years before study enrollment. Following injury onset, he received emergency medical care, and his spine was surgically fused from the 5th to 11th thoracic vertebrae. Next, 8 weeks of inpatient rehabilitation and 5 weeks of outpatient compensatory rehabilitation were completed, all of which focused on gaining independence for activities of daily living from a wheelchair. For the next 132 weeks, the subject did not perform rehabilitation (Supplementary Fig. 1a).

Clinical evaluation of spinal cord injury. Clinical and neurophysiologic evaluations were performed at the start of the study, after 22 weeks of LT, after 3 weeks of surgical recovery, at week 25 of MMR, and at the end of the study (Supplementary Fig. 1b). At these time points, motor and sensory functions were assessed using the American Spinal Injury Association (ASIA) Impairment Scale (AIS). The Modified Ashworth scale was used at several time points to evaluate spasticity below the level of injury. T2-weighted nondiagnostic MRI and computed tomography (CT) were used to visualize the spine, spinal cord, injury site, and spine fusion hardware.

Neurophysiologic evaluation of spinal cord injury. To evaluate the connectivity of descending motor signals passing through the injury, TMS MEPs were recorded bilaterally over the RF, vastus lateralis (VL), MH, TA, MG, and soleus (SOL) muscles using skin surface EMG at a sampling rate of 4 kHz (PowerLab, ADInstruments, Colorado Springs, CO). SSEPs were recorded over the scalp and lumbar spine region to detect ascending sensory signals across the injury. To test for the presence of a discomplete SCI profile, the subject was positioned supine, and while epidural electrical stimulation (EES) was off, he attempted to maximally contract muscles both above and below the injury with intention focused on increasing motor activity in either the left or right leg.

Locomotor training. After study enrollment, 61 sessions of LT were performed over 22 weeks before EES system implantation. As previously described, the main goal of LT focused on recovering normal, preinjury movement through trainer-driven facilitation of sensorimotor activity below the level of injury[38]. LT emphasizes four main principles: (i) maximizing lower extremity weight bearing, (ii) optimizing sensory cues while (iii) optimizing kinematics during each motor task, and (iv) maximizing recovery while also minimizing compensation strategies[39]. During LT sessions, the subject's legs were loaded while maintaining appropriate posture by using a BWS system while trainers manipulated each leg to achieve stand or step-like kinematics. The subject was repeatedly encouraged to attempt to perform task-specific activities, such as maintaining seated trunk balance, standing, and generating any portion of the step cycle, during trainer-assisted BWS treadmill training. During each LT session, task-specific activities were performed in two environments: (i) on the treadmill, and (ii) over ground, which typically lasted 45 min and 30 min, respectively.

EES system implantation. A 16-contact electrode array (Specify 5-6-5, Medtronic, Fridley, MN) was implanted onto the dorsal epidural surface of the spinal cord at the lumbosacral region. Array positioning within the T11-L1 vertebral region was guided via intraoperative X-ray fluoroscopy. Once positioned, various active electrode configurations were applied on the array to evoke motor potentials. Epidurally-evoked motor responses were recorded via intramuscular EMG of the RF, VL, MH, TA, MG, and SOL of each leg to localize the array over the lumbosacral spinal cord region that innervates leg muscles (L2-S1 spinal segments)[4,40–42]. The array was connected to an implanted pulse generator (RestoreUltra SureScan MRI Neurostimulator, Model 97712, Medtronic, Fridley, MN) placed subcutaneously in the right upper quadrant of the abdomen. The subject was instructed to rest at home for 3 weeks before returning to the laboratory for initial EES testing.

Refinement of electrical epidural stimulation settings to enable motor functions. The EES waveform was comprised of biphasic, charge-balanced pulses with a positive pulse width of 210 µs. Initial active electrode configurations were chosen after review of intraoperative epidurally evoked motor responses recorded from leg muscles[4] with reference to previously established topographical maps of electrically evoked motor responses[40–42]. A frequency range of 15–40 Hz was used on the basis of prior literature reporting EES facilitation of tonic and rhythmic motor activity generated by the human spinal cord[1,2,12–14,28]. During 14 sessions over 4 weeks, EES electrode configurations, stimulation frequencies, and voltage intensity ranges were identified that enabled voluntary control of leg flexion and extension, standing, and step-like leg movements while suspended. Outcomes from refinement sessions were previously reported in detail[2].

Multimodal rehabilitation: dynamic training of EES-enabled functions. Over the next 43 weeks, 113 sessions of task-specific training in multiple environments was completed in the presence of EES, termed MMR. The focus of each MMR activity was to provide extrinsic facilitation of the sensorimotor networks, with the goal of combining compensatory, adaptive, and restorative rehabilitation techniques to maximize performance of EES-enabled functions and motor activity patterns at subject-selected performance levels (for example, amount of BWS or trainer assistance provided, or speed of the treadmill). Compensatory strategies focused on encouraging the subject to use intact motor functions above the level of injury to assist in manipulating motor output enabled by EES (for example, shifting his upper body by using his arms to facilitate appropriate posture). Adaptive strategies focused on using assistive devices to improve movement patterns and ensure safety, while also allowing engagement of sensorimotor networks which aligns with restorative approaches to rehabilitation. MMR activities included seated trunk balance, standing balance and stepping, as well as lower extremity active range of motion while positioned supine, side-lying, seated, and standing with varied amounts of BWS and trainer-assistance provided.

EES parameter adjustment during MMR. Observed changes in functional performance during MMR were addressed by adjusting EES parameters, task-specific activities, and rehabilitation approaches. For example, if step training performance improved or declined either within a single session or over several sessions, either on the treadmill or over ground, adjustments in walking speed, trainer assistance, BWS, and EES voltage intensities were made with a goal of maximizing performance and independence. Periodically, EES-enabled leg muscle activity was recorded and displayed in real-time during MMR while adjusting active electrode configurations, stimulation frequencies, and voltage amplitudes to refine parameters with a goal of improving motor outcomes.

For each MMR session, EES settings were typically optimized by incrementally adjusting the voltage within a narrow range for each EES program. This was performed with the goal of enabling stepping ability of each leg while the subject was standing. If EES-enabled task performance improved to require less than 10% BWS, the support harness was removed and the subject was allowed to use his upper extremities for assist on support bars to achieve and maintain appropriate posture and balance during attempts to stand or step.

Home-based EES exercise sessions. In addition to 113 sessions of MMR during 43 weeks, the subject independently completed 72 home-based exercise sessions with EES. Home-based EES sessions were performed on days when an MMR session in the laboratory was not performed. Home-based EES-enabled tasks included attempts to control active range of motion of the legs while supine or sitting, control of seated balance and trunk posture, as well as standing with an assistive device. For repetitious activities, the subject was asked to complete 2 sets of 10 repetitions. He was also instructed to limit EES-enabled exercise to a maximum of 3 h per session. Performance during home-based exercises was self-reported in a subjective fashion upon returning to the laboratory to perform an MMR session.

Data recordings during MMR sessions. To analyze muscle activity profiles during EES-enabled activities, skin surface EMG was recorded bilaterally over the RF, VL, MH, TA, MG, and SOL muscles at a sampling rate of 4 kHz (PowerLab, ADInstruments, Colorado Springs, CO). The vertical component of ground reaction force (vGRF) was recorded under each foot at a sampling rate of 50 Hz (Tekscan, South Boston, MA). Knee joint angles were recorded using 2-D electronic goniometers (Noraxon USA Inc., Scottsdale, AZ). Stance and swing phase transitions were determined by calculating a transition threshold that was defined as 10% of the maximum vGRF value of each step. All data modalities were synchronized to video recordings (Labchart, ADInstruments, Colorado Springs, CO).

Data analyses. Bandpass (Butterworth, 20–1,000 Hz) and notch (infinite impulse response, 60 Hz) filters were applied to EMG data to reduce environmental noise using a custom written MATLAB script (MATLAB 2015a, The MathWorks Inc., Natick, MA). RMS envelopes were calculated from full-wave-rectified EMG using a moving window of 600 samples and an overlap of 200 samples (MATLAB 2015a, The MathWorks Inc., Natick, MA). The mean EMG RMS value of each muscle was calculated for each step cycle. In order to compare EES-enabled muscle activities, mean EMG RMS values from 10 steps were averaged and normalized across muscles by defining each muscle's mean RMS from 10 steps as 1 arbitrary unit. Normalized RMS values above 1 were interpreted as EES-enabled activation, and values below 1 were interpreted as inhibition of muscle activity. Normalized values were calculated for the stance, early swing, and late swing phases of the step cycle. The first 50% of the swing phase was defined as early swing and the latter 50% was defined as late swing. The duration of overlapping stance phases was calculated as

the amount of time that both feet were in stance phase during each step cycle for 10 steps of each leg. To evaluate rhythmicity of leg muscle activity across EES-enabled tasks, and EES parameters, the coefficient of variation (COV) was calculated as the ratio of the s.d. to the mean expressed as a percentage. For all statistical results reported, means (±s.d.) were calculated and significant differences were determined by a two-tailed Mann–Whitney test (GraphPad Software Inc., La Jolla, CA). *P* values greater than 0.001 are reported as exact values.

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

Code availability. Custom-written MATLAB script used to process datasets is available from the corresponding author upon request.

Data availability

Raw and processed datasets are available from the corresponding author upon request.

References

27. Rejc, E., Angeli, C. A., Bryant, N. & Harkema, S. J. Effects of stand and step training with epidural stimulation on motor function for standing in chronic complete paraplegics. *J. Neurotrauma* **34**, 1787–1802 (2017).
38. Behrman, A. L. & Harkema, S. J. Locomotor training after human spinal cord injury: a series of case studies. *Phys. Ther.* **80**, 688–700 (2000).
39. Harkema, S. J., Behrman, A. L. & Barbeau, H. in *Locomotor Training: Principles and Practice,* 54–84 (Oxford University Press, Oxford, UK, 2011).
40. Sayenko, D. G., Angeli, C., Harkema, S. J., Edgerton, V. R. & Gerasimenko, Y. P. Neuromodulation of evoked muscle potentials induced by epidural spinal-cord stimulation in paralyzed individuals. *J. Neurophysiol.* **111**, 1088–1099 (2014).
41. Sayenko, D. G. et al. Spinal segment–specific transcutaneous stimulation differentially shapes activation pattern among motor pools in humans. *J. Appl. Physiol.* **118**, 1364–1374 (2015).
42. Sayenko, D. G. et al. Effects of paired transcutaneous electrical stimulation delivered at single and dual sites over lumbosacral spinal cord. *Neurosci. Lett.* **609**, 229–234 (2015).

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Reporting Summary

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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).

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
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☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	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	EMG and video data were collected using Labchart Version 7 software (ADInstruments, Colorado Springs, CO) synchronized to in-shoe pressure sensors (Tekscan, South Boston, MA) and two-dimensional electronic goniometers (Noraxon U.S.A. Inc., Scottsdale, AZ). EMG was recorded from the skin bilaterally over the RF, VL, MH, TA, MG, and SOL muscles. Standard clinical magnetic resonance imaging and computed tomography was used to visualize the spine, spinal cord, and injury site. Paralysis was measured via the American Spinal Injury Association Impairment Scale (AIS). Transcranial magnetic stimulation (TMS) motor evoked potentials (MEPs) were recorded to evaluate descending connectivity from the brain across the injury. Somatosensory evoked potentials (SSEPs) were recorded to detect ascending sensory signals across the injury. The Modified Ashworth scale was used to evaluate spasticity below the level of injury.
Data analysis	Analyses of motor activities were performed by importing raw data files from respective sources, then all files were synchronized, filtered for noise and artifacts, and processed to create visual summaries of results using a custom written MATLAB script (The MathWorks Inc). Statistical analyzes of summary data were performed using GraphPad (GraphPad Software Inc., La Jolla, CA).

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.

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- Accession codes, unique identifiers, or web links for publicly available datasets
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Field-specific reporting

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Life sciences study design

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

Sample size	Results were generated from one adult male human. Changes in muscle activity and step cycle characteristics during EES-enabled treadmill and over ground stepping were extracted from 10 steps of each leg.
Data exclusions	Stepping bouts of less than 10 steps were excluded from over ground stepping performance (Figure 3a, MMR weeks 25-42). Bouts that lasted less than 10 steps were mostly due to subject fatigue or suboptimal EES voltage intensities.
Replication	Not applicable to this case report.
Randomization	Not applicable to this case report.
Blinding	Blinding the subject, or research staff from the presence of EES was not performed. Communication between the subject and research staff was essential to the rehabilitation paradigm used.

Reporting for specific materials, systems and methods

Materials & experimental systems

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

Methods

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

Human research participants

Policy information about studies involving human research participants

Population characteristics	One male human, 26 years of age, and diagnosed with complete loss of function (AIS A) below the sixth thoracic spinal segment due to spinal cord injury three years prior to study enrollment.
Recruitment	The subject was recruited from Mayo Clinic's database and research registry.

Magnetic resonance imaging

Experimental design

Design type	Clinical MRI protocol to visualize spinal structure integrity from a saggital aspect.
Design specifications	An MRI protocol was not specifically designed for this study.
Behavioral performance measures	Not applicable.

Acquisition

Imaging type(s)	Structural
Field strength	1.5 Tesla
Sequence & imaging parameters	T2-weighted, non-diagnostic spine imaging protocol.
Area of acquisition	Spine with region of interest focused on the eighth thoracic vertebrae.

Diffusion MRI ☐ Used ☒ Not used

Preprocessing

Preprocessing software	MRI files were visualized using Quick query Radiographs and photographs Electronic Analysis and Display Station (QREADS) software that is used clinically at Mayo Clinic.
Normalization	Not applicable.
Normalization template	Not applicable.
Noise and artifact removal	Not applicable.
Volume censoring	Not applicable.

Statistical modeling & inference

Model type and settings	Not applicable.
Effect(s) tested	Not applicable.

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference (See Eklund et al. 2016)	Not applicable.
Correction	Not applicable.

Models & analysis

n/a	Involved in the study
☒	☐ Functional and/or effective connectivity
☒	☐ Graph analysis
☒	☐ Multivariate modeling or predictive analysis