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MAYO CLINIC PROCEEDINGS:
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ORIGINAL ARTICLE

Consecutive Transcutaneous and Epidural Spinal Cord Neuromodulation to Modify Clinical Complete Paralysis—the Proof of Concept

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Abstract

Objective: To evaluate the effect of transcutaneous (tSCS) and epidural electrical spinal cord stimulation (EES) in facilitating volitional movements, balance, and nonmotor functions, in this observational study, tSCS and EES were consecutively tested in 2 participants with motor complete spinal cord injury (SCI).

Participants and Methods: Two participants (a 48-year-old woman and a 28-year-old man), both classified as motor complete spinal injury, were enrolled in the study. Both participants went through a unified protocol, such as an initial electrophysiological assessment of neural connectivity, consecutive tSCS and EES combined with 8 wks of motor training with electromyography (EMG) and kinematic evaluation. The study was conducted from May 1, 2019, to December 31, 2021.

Results: In both participants, tSCS reported a minimal improvement in voluntary movements still essential to start tSCS-enabled rehabilitation. Compared with tSCS, following EES showed immediate improvement in voluntary movements, whereas tSCS was more effective in improving balance and posture. Continuous improvement in nonmotor functions was found during tSCS-enabled and then during EES-enabled motor training.

Conclusion: Results report a significant difference in the effect of tSCS and EES on the recovery of neurologic functions and support consecutive tSCS and EES applications as a potential therapy for SCI. The proposed approach may help in selecting patients with SCI responsive to neuromodulation. It would also help initiate neuromodulation and rehabilitation therapy early, particularly for motor complete SCI with minimal effect from conventional rehabilitation.

 ■ Mayo Clin Proc Inn Qual Out 2024;8(1):1-16

Spinal cord injury (SCI) impairs communication between the brain and sublesional circuitry, leading to motor, sensory, and autonomic dysfunctions.[1] Patients with complete motor and sensory American Spinal Injury Association Impairment Scale (AIS-A) or complete motor (AIS-B) injuries consist of 50%-60% of all SCI cases.[2,3] Within this population, if no changes are observed within the first year, patients have extremely low chances for improvement,[4] even with the most advanced rehabilitation and neuromodulation programs.[5-9] Epidural electrical spinal cord stimulation (EES) was successfully implemented to restore motor function in animal models.[10-19] A combination of EES and rehabilitation therapy in subjects with motor complete SCI led to the unexpected restoration of voluntary control over paralyzed limbs,[20] recently replicated by several groups.[8,9,21,22] Although a combination of spinal cord stimulation (SCS) and motor rehabilitation provides optimal outcomes and integration across injury fibers,[5,23] recent works report a significant level of EES-enabled motor function restoration without intensive rehabilitation.[22,24] Another neuromodulation approach, noninvasive

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transcutaneous electrical stimulation (tSCS), showed great potential as a therapy for chronic SCI with a significant effect on multiple systems.[25-28] The effect of tSCS and EES in patients with SCI facilitated multiple studies, facing the large variability across patients' populations and experimental approaches.[8,9,20-22,29,30] Therefore, it is critical to compare the effects of noninvasive and invasive neuromodulation applied to the same subjects. Here, we compare the effect of consecutively applied tSCS and EES on enabling voluntary motor control, balance, and restoration of nonmotor functions after complete paralysis and test the hypothesis that tSCS and EES have different but complementary effects on functional restoration after motor complete SCI.

PARTICIPANTS AND METHODS

Subjects' information, procedures, and timeline: All procedures described herein were performed with the approval of the Kazan Federal University institutional review board (review board decision June 10th, 2019, protocol №16) and internal ethics committee in accordance with the World Medical Association Declaration of Helsinki, 1964. This investigation was carried out as a proof-of-concept study. Two participants (P1 and P2) with traumatic SCI were prescreened with inclusion or exclusion criteria (see Supplemental Materials, available online at http://www.mcpiqojournal.org)[31,32] and signed an informed consent. Participant 1 (P1), a 48-year-old woman with SCI at Th7 classified as AIS-A, and participant 2 (P2), a 28-year-old man with SCI at Th4 classified as AIS-B. Then, neurological evaluation and comprehensive electrophysiological assessment were performed (Figure 1) (for details, see Supplemental Materials). Scales and questionnaires to assess the quality of life, mental health, bowel, bladder, and sexual function are presented in Table.

Assessment of translesional neural connectivity was performed used Neuro MEP-8 (Neurosoft) and the following tests were used (Figure 2):

(a) Spinal cord somatosensory evoked potentials (SSEP): electrical pulses were delivered to the tibial nerve bilaterally at the ankle area while in a supine position with freely hanging feet. Stimulation intensities corresponded to the visual threshold of the motor response of the muscles (flexion of the toes). Stimuli consisted of monophasic rectangular electrical pulses of 0.3 ms duration at 3 Hz and 1.5× of the threshold of visual motor response (visible contraction). The SSEPs were recorded at 5 locations (popliteal region, L2-3, Th12-L1, Th8-9, and C5-6). The average response was calculated from 800 consecutive stimulus pulses.

b) M-response and H-reflex: M-response and H-reflex were assessed by stimulation of the right and left posterior tibial nerve in the popliteal region using a stainless-steel bipolar electrode with a ball-contact of 0.5-cm in diameter (Neurosoft). Responses were recorded from the soleus (SOL) muscle bilaterally using a bipolar EMG surface electrode. The stimulation frequency was 0.1 Hz with a pulse duration of 1 ms delivered by a Neuro MEP-8 stimulator (Neurosoft), and stimulation intensity was gradually increased by a step of 1 mA until the M-response amplitude was no longer increased.

(c) Jendrassik maneuver (JM): To assess the influence of supraspinal signaling on the H-reflex and spinally evoked motor potentials (SEMPs), the testing with reinforcement maneuver (Jendrassik maneuver) was implemented, as described previously.[33] Participants were positioned supine on the couch with feet suspended and asked on the command 'pull' to exert an effort with the fingers of both hands in a 'lock' in front of the chest and sustain it for a few seconds.[34] Participants were instructed to remain relaxed in all muscles other than those participating in the maneuver. To minimize muscle fatigue, a 3-4 min resting period was maintained between the trials. The first experimental session studied changes in the SOL muscle H-reflex bilaterally with or without the JM. Recruitment curves were constructed by plotting the magnitude of the M-response and H-reflex against increasing stimulation intensity. To assess the effects of the JM on H-reflex amplitude, stimulation intensity that produces minimal response

Mayo Clin Proc Inn Qual Out ■ February 2024;8(1):1-16 ■ https://doi.org/10.1016/j.mayocpiqo.2023.09.006
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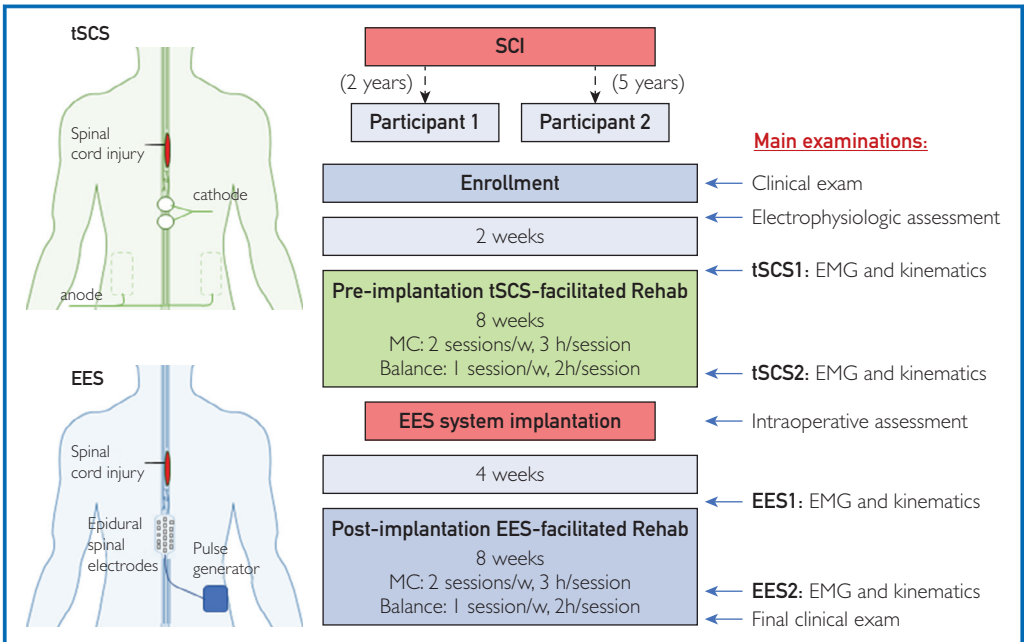

FIGURE 1. Timeline of the study. Two participants (P1, AIS-A at T7 and P2, AIS-B at T4) with 2 and 5 years after SCI, correspondingly, were enrolled in this study. After the initial clinical exam, the electrophysiological assessment was performed, and both participants were tested with a 2-week trial of tSCS with the following assessment (tSCS1). During the following 8 weeks participants received tSCS-enabled motor training with the following assessment (tSCS2). Then, both participants were implanted with EES system with intraoperative electrophysiological assessment and were tested after 4-week rest period after surgery, demonstrating an ability to control legs movements with the EES (EES1). Then, both participants received EES-enabled motor training for 8 weeks with the following evaluation (EES2). The schematic position of the electrodes for tSCS and EES presented on the left side, and the main examinations are outlined on the right side of the figure.

with an amplitude ranging from 30%-50% of Hmax was identified. The second experimental session studied changes in the amplitude of the SEMPs evoked by tSCS (Th12-L1) with or without the JM at the supine position with stimulation intensity adjusted the same way.

(d) Spinally evoked motor potentials: SEMPs were recorded using surface EMG electrodes (Kendall, Meditrace—100; Ag/AgCl, diameter of 22 mm) placed over the rectus femoris (RF), biceps femoris (BF), tibialis anterior (TA), and SOL muscles bilaterally. To evoke SEMPs, active gel adhesive electrodes (TensCare, CM25, diameter of 25 mm) were placed at the midline, in between the Th11-Th12, spinous processes, and 2 reference electrodes (4×2 cm) were placed over the lower abdominal area. Electrical stimulation was performed with monophasic rectangular pulses, with a pulse duration of 1 ms every 10 sec. Stimulation intensity was increased from 30 to 100 mA or to the maximum tolerable intensity. Five stimuli were delivered at each stimulation intensity. At maximum stimulation amplitude (95 mA for P1 and 100 mA for P2), paired pulses, each at an interstimulus interval of 50 ms, were applied.[35]

(e) Transcranial magnetic stimulation (TMS): TMS was used to assess the functional integrity of the cortico-spinal tracts.[36-38] Stimulation with a circular coil 150 mm in diameter (Neurosoft) centered over Fz point (10-20 system) at the projection of lower extremities was used. Stimulation

TABLE. Participants' Assessment Information

	Participant					
	P1			P2		
Scales	BE	tSCS	ESS	BE	tSCS	ESS
ISNCSCI						
NLI		T5			T4	
AIS		A			B	
LEMS		0			0	
UEMS		50			50	
VAC	No	No	No	Yes	No	No
DAP	No	No	No	No	No	No
Pathological reflexes		−			+	
Spasm frequency (PSFS)	0	1	2	2	1	3
Pain (VAS)		80mm	20mm		NA	
Pain management		Lyrica			no	
Rehab (during 1 y)		yes			no	
Level of lesion		Th7			Th4	
Length of lesion		N/A			N/A	
Blood presence		no			No	
ASAF						
AC of the heart		N			N	
AC of the BP	N	160-180 mm Hg	160-180 mm Hg	N	140-160 mm Hg	140-160 mm Hg
AC of sweating		N		HBL#	N	
Temperature regulation		N			N	
AC and SC of BPS		N			N	
Bladder management		0			0	
Bowel management		1			2	
Sexual function		2			1	
BP at rest		128/79			135/80	
HR at rest		63			60	
Orthostatic test (lying down—sit)		NEG			NEG	
History of AD		yes			No	
Medications		no			No	
SCIM						
Self care (out of 20)		16	18		18	
Respiration and sphincter management (out of 40)		6	37		38	
Mobility (out of 40)		37	12		19	
Total (out of 100)		59	67		75	

Continued on next page

frequency was 0.5 Hz, and intensity was gradually increased from 40% to 100% of the maximal intensity (2.2T). Motor-evoked potentials were registered over TA and SOL muscles. As the areas of cortical stimulation were determined, the subthreshold TMS followed by the tSCS was applied. Intervals between the conditioning stimulus and testing stimulus (C-T) ranged from 0 to 180 ms with 20 ms increasing increment.[39,40] The TMS intensity was set at the rate of 100% as MEP was not detected in both subjects. The amplitude characteristics of the MEP recorded from the TA and SOL muscles were analyzed without and with tSCS combined with TMS, and 5 samples were averaged at each time point.

(f) EMG evaluation during attempts of voluntary movements. EMG registration was performed during voluntary general flexion of the hips and knees of both legs without and then in combination with JM. Surface EMG was recorded from the distal (tibialis anterior (TA), medial gastrocnemius (MG), extensor digitorum brevis (EDB), and flexor digitorum brevis (FDB), and proximal (rectus femoris (RF), vastus lateralis (VL), medial hamstring (MH) muscles, and abdominal muscles (RA) bilaterally in supine position. The registration settings were set before the beginning of the assessment and remained unchanged throughout the test. In addition, a 3-min relaxation was performed to differentiate between involuntary (spasticity) and voluntary movements.

After the initial assessment, noninvasive tSCS was used to enable motor training. tSCS-enabled motor training was performed for 8-wk tSCS-enabled motor training (2 sessions per week, 3 h per session) and training in a sitting position (1 session per week and 2 h per session)[8,19] (Supplemental Figure 1, available online at http://www.mcpiqojournal.org), using BioStim-5 (Kosima). Stimulating electrodes (cathode) with a diameter of 3.2-cm (PG479, Fiab) were placed along the midline of the spine between the

Mayo Clin Proc Inn Qual Out ■ February 2024;8(1):1-16 ■ https://doi.org/10.1016/j.mayocpiqo.2023.09.006
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spinous processes at 2 levels Th11-12 and Th12-L1 and at the low abdominal areas (anode). After 8 wk of tSCS-enabled motor training, both participants received implantation of SCS system (RestoreSensor, Sure-Scan MRI, Medtronic).[8,40,41] A stimulator was surgically implanted and connected to a 16-contact electrode array (Specify 5-6-5, Medtronic) positioned on the dorsal epidural surface of the lumbosacral spinal cord below the injury and titanium construction for vertebrae fixation at the T12 vertebra level, confirmed with intraoperative fluoroscopy. The SEMPs were recorded with 2 configurations to ensure the electrode position over the lumbosacral enlargement of the spinal cord.[8,17,42] After 4 wk of rest, participants received 8 wk of EES-enabled motor training with the same configurations for P1 and P2. The subsequent testing with EES and final clinical examination was performed at the end of the study (Figure 1).

TABLE. Continued

Scales	Participant					
	P1			P2		
	BE	tSCS	ESS	BE	tSCS	ESS
NBDS						
Total		2			5	
GS (out of 10)		8			7	
NBSS						
Incontinence (out of 29)	26	22	22		10	
Storage/voiding (out of 22)	14	13	13		4	
Consequence (out of 23)	6	6	6		8	
QOL (out of 10)	4	5	5		2	
	D	D	D		B	
PHQ9		5	0		0	
MAS	0	1	1+	1+	1+	2

Abbreviations: **AC**, autonomic control; AIS, ASIA (American spinal cords injury association) Impairment Scale; ASAF, autonomic standard assessment form; BE, before enrollment in study; BP, blood pressure; BPS, broncho-pulmonary system; DAP, deep anal pressure Pathological reflexes; HR, heart rate; HBL, hyperhidrosis below lesion; History of AH, arterial hypertension; ISNCSCI, International Standard for neurological classification of spinal cord injury; LEMS, lower extremities motor sub-scores; MAS, Modified Ashworth Scale; MBDS, the neurogenic bowel dysfunction score (GS-general satisfaction); N, normal; NLI, neurological level of lesion; NEG, negative; NBSS, the Neurogenic Bladder Symptom Score (QOL—quality of life); OT, orthostatic test; PSFS, Penn spasm frequency scale; PHQ9, patient health questionnaire-9; SC, somatic control; SF, spasm frequency; SF-36, the short form-36; SCIM III, spinal cord independence measure; UEMS, upper extremities motor sub-scores; VAC, voluntary anal sphincter contraction; VAS, visual analog scale.

Data Processing and Analysis

EMG activity was recorded and processed using Neuro MEPΩ (Neurosoft) and LabChart software (ADInstruments). The EMG data were filtered using a 50-Hz notch filter and a bandpass filter of 20-1000 Hz. Data were sampled at 4 kHz, exported, and analyzed using MATLAB software (The Math-Works Inc). The magnitudes of H-reflex and M-response were calculated as peak-to-peak amplitude. The H/M ratio was the maximum of H-reflex (Hmax) divided by the maximum of M-response (Mmax). Peak-to-peak SEMP amplitudes and latencies were measured in a window of 5-30 ms stimulation artifact using MATLAB script. The EMG data recorded during intraoperative monitoring sets were analyzed separately for each electrode configuration on the midline (Supplemental Figure 3, available online at http://www.mcpiqojournal.org). EMG data with reinforcement maneuver (JM) were analyzed as follows: 5 responses were averaged for each stimulation trial, and the control amplitude values (1.5 of thresholds) were expressed as 100% for each muscle. Then, the rest of the amplitudes collected during JM were expressed as percentage (±SD) of the control value. EMG activity was recorded during tSCS and EES across the study and then compared.

Kinematics

Kinematic data were recorded at 30 fps using a HD web camera with 1280×960 resolution (C310 Logitech). Seven reflective markers were placed on the lower limb laterally while the subject was in a side position at the 8th rib, iliac crest, thigh, knee, tibia, ankle, and 5th toe. The knee and ankle angles were analyzed using Kinovea software. Parameters were calculated as the average of the values obtained in 15 complete gait cycles.

Balance assessment was performed during motor tasks while sitting with the camera placed on the right side at a 2 m distance from the subject and 1.5 m from the ground. From 60-sec video, 6 frames (every 10 sec) were collected and analyzed. Video analysis of balance was consistent of 11 metrics (2

angles, 2 squares, and 7 segments) used to calculate trunk and abdominal curvatures, and arms and head position (for details, see Supplemental Materials).

STATISTICAL ANALYSES

The normal distribution and the variation within each group of data were verified by using the SigmaPlot 11.0 software. Statistical comparisons were made using paired *t* test and one-way repeated measures ANOVA (Student−Newman−Keuls) to compare the amplitudes of responses. In all cases, $P < .05$ was considered statistically significant. To analyze gait kinematics, one-way repeated measures ANOVA (Tukey test) was used for comparing the range of movement before, during, and after stimulation. To assess balance before and during stimulation Mann-Whitney U Test and *t* test were used. All results are presented as means ± standard deviation (SD). $P < .05$ is considered as significant.

RESULTS

Electrophysiological Assessment

Somatosensory evoked potentials during stimulation of the tibial nerve in P1 were found below the Th12-L1 level and in P2 below the Th8-9 level bilaterally, indicating no ascending connectivity above the injury (Figure. 2A).

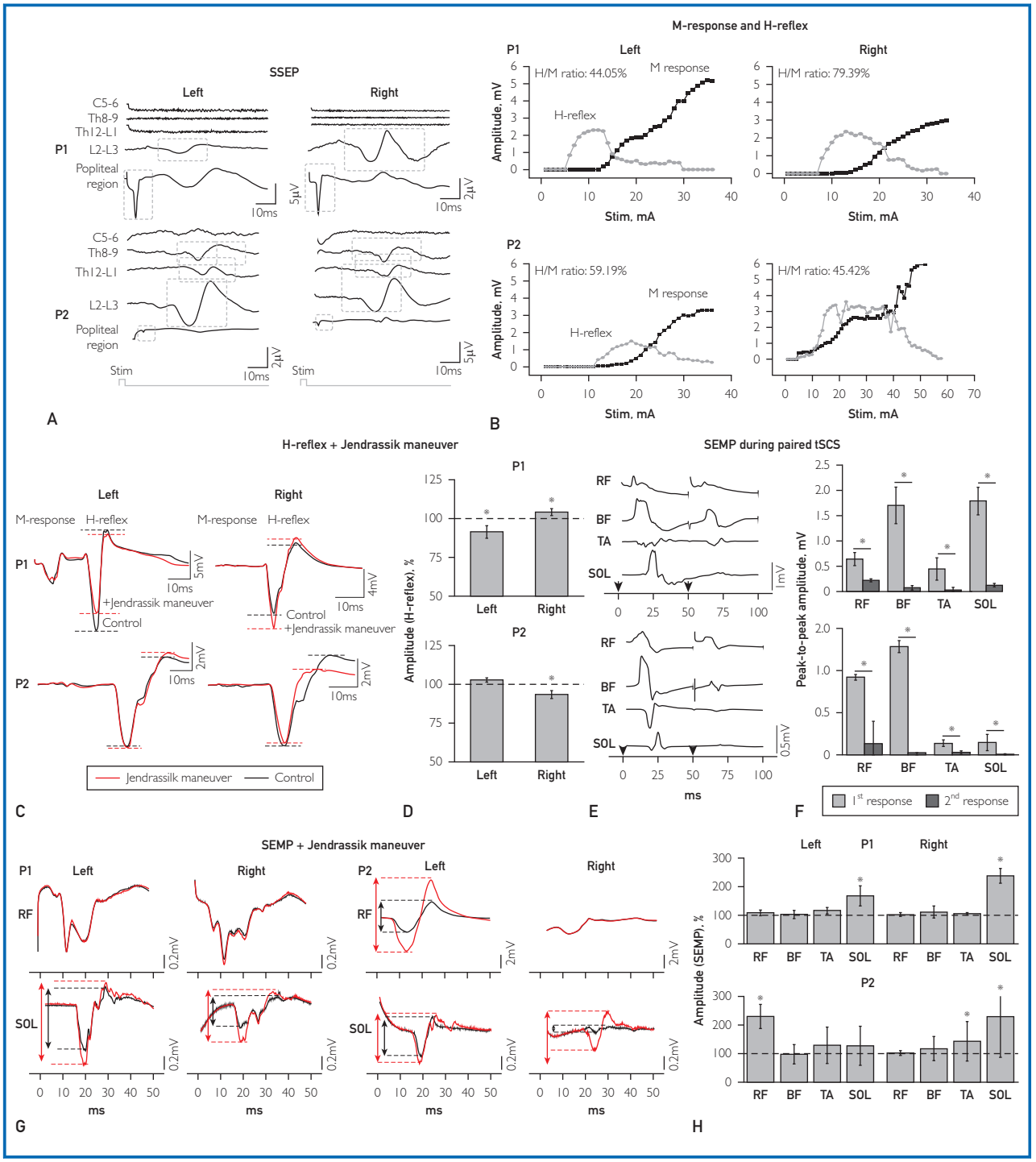
M-response and H-reflex: The recruitment curves of M-response and H-reflex recorded from the SOL muscle on both legs are presented in Figure 2B. For P1 the H/M ratio was lower on the left leg (44.05%—left leg vs 79.39%—right leg) and for P2 was lower on the right leg (59.19%—left leg vs 45.42%—right leg). H-reflex was assessed with and without JM (Figure 2C). In P1 JM facilitated H-reflex to 104.3±2.14% of the control values on the right side (2.66±0.062 with JM mV vs 2.54±0.10 mV without JM; $P=.05$) with some inhibition of H-reflex on the left side to 91.54±4.05% (2.40±0.053 mV with JM vs 2.49±0.051 mV without JM; $P=.02$). In P2 JM inhibited H-reflex on the right side to 93.60±2.52% (4.25±1.56 mV with JM vs 4.54±0.33 mV without JM; $P=.04$) with no significant effect on H-reflex on the left side (Figure 2D).

Spinally evoked motor potentials were tested with tSCS (T12-L1) applied as a single or paired pulse with 50ms intervals.[43-46] During paired stimulation, the amplitude of SEMP was affected by the postactivation depression in all tested muscles in both participants. (Figure 2E and F). In P1, the difference in RF was 0.42±0.07mV ($P=.002$), in BF 1.63±0.26mV ($P=.02$), in TA 0.43±0.16mV ($P=.007$), and in SOL 1.67±0.22mV ($P=.005$). In P2, the difference in RF was 0.89±0.20mV ($P=.005$), in BF 0.98±0.62mV ($P=.001$), in TA 0.10±0.02mV ($P=.001$), and in SOL 0.13±0.05mV ($P=.02$). The examples of the SEMPs to tSCS (Th11-12) in RF and SOL without (black line) and with JM (red line) presented in Figure 2G. In P1, the amplitude of SEMP in RF was not significantly changed, although, in SOL SEMPs were facilitated on both legs during JM. In P2, SEMP amplitude during JM was increased in left RF and in SOL on both sides (Figure 2G). The effect of JM on SEMP amplitude across all tested muscles is presented in Figure 2H. In P1, JM facilitated SEMP in left and right SOL (167.90±34.68% and 238.66±25.48%; $P=.001$). In P2, JM facilitated SEMP in left RF (230.39±42.23%; $P=.006$), in right TA (142.01±68.93%; $P=.05$), and in right SOL (228.76±143.06%; $P=.009$) (Figure 2H).

Transcranial magnetic stimulation reported no responses to TMS in tested muscles on both sides in both participants. Also, no significant changes were observed in SEMP evoked by tSCS (Th11-12) during conditioning with TMS (Supplemental Figure 1).

Volitional EMG activity: Attempts to perform general flexion on both legs reported no changes in EMG when applied without JM (Supplemental Figure 2A, available online at http://www.mcpiqojournal.org). Some increase in EMG amplitude was found during attempts of flexion with JM in P2 (Supplemental Figure 2B).

In summary, electrophysiological assessment alone reported no functional connectivity through the ascending or descending pathways across the injury, whereas in combination with JM, H-reflex, and SEMPs reported modulation in both participants, indicating on discomplete character of injuries.



Volitional Motor Control With tSCS and EES

Volitionally initiated rhythmic activity in legs with tSCS and EES was observed in both participants (Figure 3 and Videos 1 and 2). Initial trial of tSCS (P1: 30 Hz, 90-105 mA and P2: 30 Hz, 110 mA) (tSCS1) (Supplemental Table, available online at http://www.mcpiqojournal.org) reported minimal restoration of voluntary movements in both participants. Then, tSCS-enabled rehabilitation with the same parameters of stimulation was performed for 8 wk with the following testing with tSCS (tSCS2) and clinical examination. After EES system was implanted, EES (P1: 20 Hz bilaterally, 7.5 V and P2: 20

Hz bilaterally, 4.5 V) (EES1) (Supplemental Table) reported EES-enabled volitional motor control and robust rhythmic movements already on the 2nd postoperative day. After 4 wk of rest, participants received 8 wk of EES-enabled motor rehabilitation with the subsequent testing with EES (EES2) and final clinical examination, reporting variations between both participants in the effect of neuromodulation with tSCS and EES.

In P1, during the 1st assessment with tSCS (tSCS1), reported increased movements in the left knee and right knees during tSCS (15.47±3.3°and 10.11±1.8°) compared with before (6.22±4.3° and 2.87±1.3°) and after tSCS (7.82±1.3°and 3.14±1.3°, respectively) (P=.001) (Figure. 3E). Movements in left ankle were increased during (7.05±4.2°) compared with before (3.68±3.1°) and after tSCS (2.95±1.8°) (P=.003), although, in right ankle they were not different between during, before, and after tSCS1 (Supplemental Figure 4A, available online at http://www.mcpiqojournal.org). During the 2nd assessment with tSCS (tSCS2), P1 reported increased movements in left knee during (16.01±7.0°) compared with before (10.50±5.0°) (P=.02) and after tSCS (6.65±3.8°) (P=.001) and in right knee during (7.13±4.2°), compared with before (0.40±0.2°) and after tSCS2 (3.14±1.3°) (P=.05) (Figure 3E). Movements in left ankle were higher after tSCS (16.78±4.1°) compared with before (6.47±3.4°) and movements in right ankle were higher after (2.79±1.5°) compared with before (0.61±0.3°) tSCS2 (P=.05) (Supplemental Figure 4A). After 8 weeks of tSCS-enabled training in P1, led to improvement in left leg movements, both in knee (6.22±4.3° before vs 10.49±4.9° after training) and in ankle (3.67±3.1° before vs 6.47±3.3° after training), whereas on the right leg P1 reported no improvement in knee and in ankle when tested without tSCS.

In P1, during the 1st assessment with EES (EES1) reported no difference in left knee movement compared with before and after EES1. In right knee movements significantly increased during EES1 (22.86±5.6°) compared with before (4.68±2.1°) and after EES1 (5.32±2.3°) (P=.001) (Figure 3E). During the 2nd assessment with EES (EES2), P1 reported increased movements in left knee during (57.29±3.9°) and after EES2 (64.91±7.4°) compared with before EES2 (23.97±14.4°) (P=.001) and in right knee during (32.01±7.6°) and after (41.26±11.2°) compared with before EES2 (6.89±2.2°) (P=.001) (Figure 3E). Movements in left ankle during EES2 were not different from movements before or after EES and in the right ankle movements during (2.2±1.0°) were higher compared with before EES2 (1.06±0.6°) (P=.003) (Figure 4A). After 8 weeks of EES-enabled training in P1, led to improvement in volitional movements in left knee (15.42±4.0° before vs 23.96±14.4° after training) and right knee (4.68±2.1°before vs 6.88±2.2° after training).

In P2, during the 1st assessment with tSCS (tSCS1), reported increased movements in left

FIGURE 2. Assessment of translesional connectivity in P1 and P2. (A) Examples of SSEPs from the five locations (popliteal region, L2-3, Th12-L1, Th8-9, and C5-6) during bilateral stimulation of the tibial nerve (each line represents an average from 800 responses). Dash-line squares indicate the SSEP at the Th8-9, Th11-12, L2-3, and popliteal region. (B) Examples of recruitment curves of the M-response. (black lines) and the H-reflex (light grey lines) for participants 1 and 2. (C) Examples of M-response and H-reflex recorded from right and left soleus muscles without (black lines) and with Jendrassik maneuver (JM) (red lines). Grey squares indicate the M response area at the soleus muscle. (D) The amplitudes of the H-reflex recorded from right and left Soleus muscle during JM presented as % from H-reflex without JM (dashed line) (n = 5). Asterixis are indicate significant difference between peak-to-peak H-reflex amplitude with control (100%) and with Jendrassik maneuver (*, P<.05). (E) Examples of SEMPs during paired tSCS at Th11-12 with a 50-ms interstimulus interval (onset of each stimulus presented with black arrow). (F) Responses to the 1st and 2nd stimuli demonstrate that responses to the 2nd stimulus (dark grey bars) are lower compared to responses evoked by the first stimulus (light grey bars) for both participants (n=6, P<.05). (G) Examples of the SEMPs recorded from RF and SOL during stimulation at Th11-12 without (black lines) and with JM (red lines). (H) The amplitudes of the SEMPs recorded from left and right proximal (RF and BF) and distal (TA and SOL) muscles during tSCS at Th11-12 with JM, presented as % from SEMP recorded without JM (dashed line) (n=5). Asterixis are indicate significant difference between peak-to-peak SEMPs amplitude with control (100%) and with Jendrassik maneuver (*, P<.05). The error bars represent the standard error of the mean (SD).

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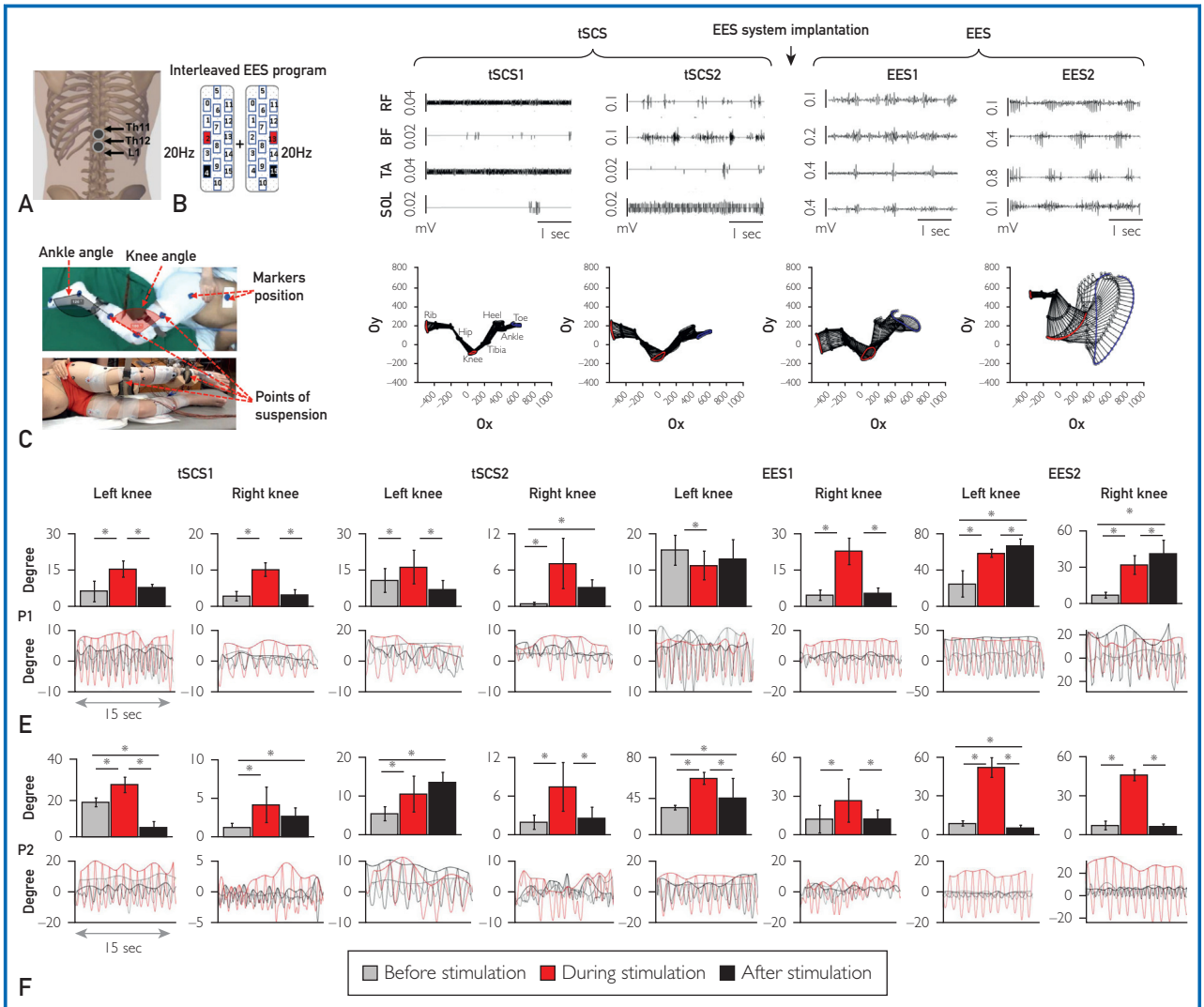
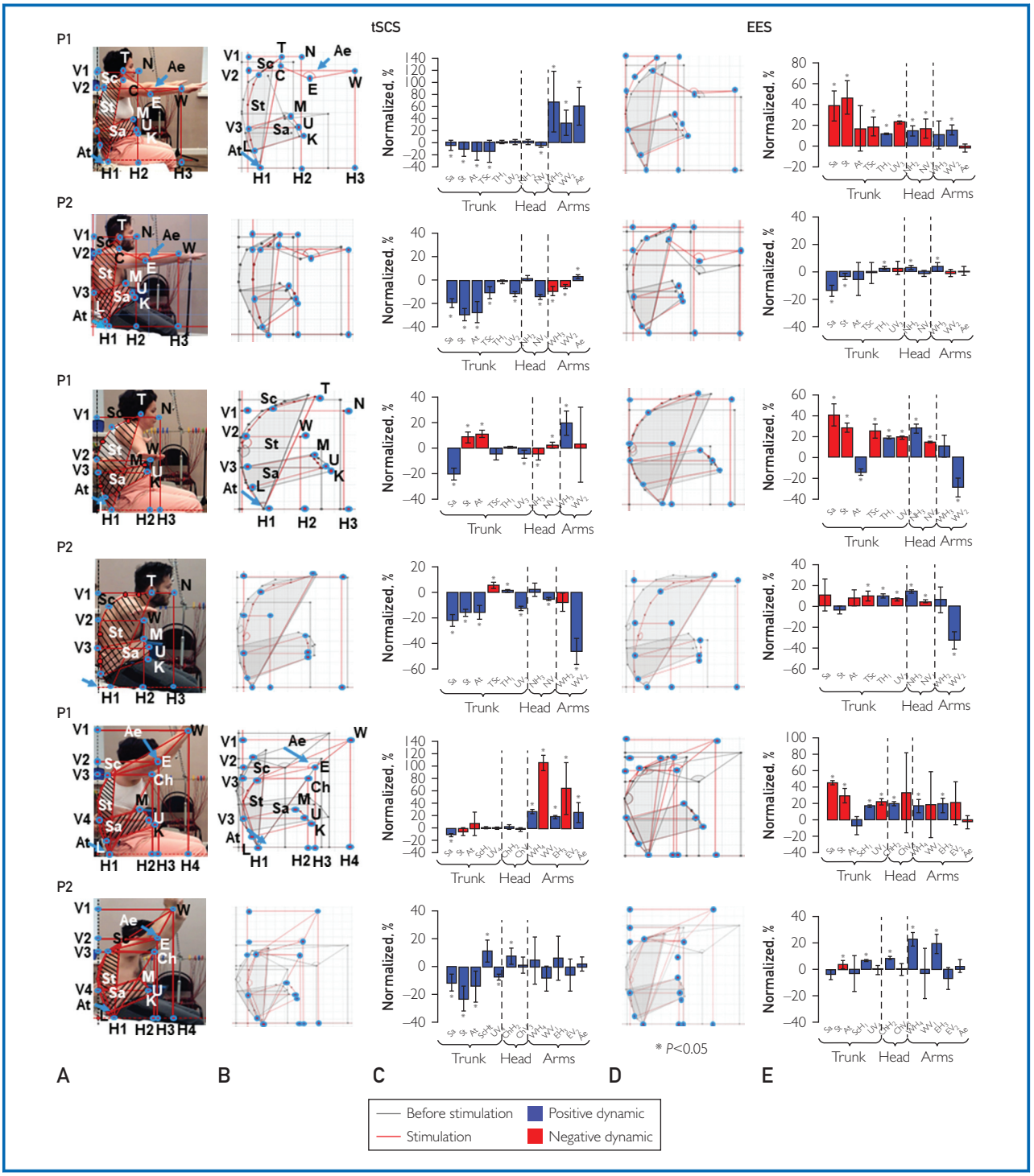


FIGURE 3. The restoration of voluntary leg movements was tested in a side-line position. (A) Schematic localization of transcutaneous electrodes (cathodes) used for tSCS in relation to the vertebrae levels. (B) Schematic diagrams of active electrodes on epidural electrode array (Medtronic, 5-6-5) used for evaluation of the volitional control with EES. (C) An example of reflective markers location for kinematic data collection and joint angle evaluation during legs' movements. (D) Examples of EMG activities generated in left legs' muscles—rectus femoris, biceps femoris, tibialis anterior, and SOL, during side position training with tSCS and EES across the study in participant 2 (P2). Examples of legs' kinematics collected with a video capture system (Vicon) from the left leg in P2 during tSCS and EES corresponding to EMG samples. (E) The mean range of movements in the knee joint in participant 1, collected from 15 complete gait cycles during tSCS1 with examples of knee flexion-extension before, during, and after tSCS1, tSCS2, EES1, and EES2 with a kinematic graphical representation of knee flexion-extension before (grey), during (red), and after stimulation (black), presented below as color lines (15sec duration). Y-axis reflects the degree of max to min joint angle movements. (F) The mean range of movements in the knee joint in P2, collected from 15 complete gait cycles during tSCS1 with examples of knee flexion-extension before, during, and after tSCS1, tSCS2, EES1, and EES2 with the kinematic graphical representation of knee flexion-extension before, during, and after stimulation. Abbreviations similar to E. Asterisks indicate significant difference in range of movements before, during and after stimulation (*;$P<.05$). The error bars represent the standard error of the mean ±SD.



and right knees during (26.85±3.9° and 4.14±2.2°) compared with before (17.79±2.1° and 1.23±0.5°) and after tSCS1 (4.76±3.0° and 2.66±1.0°) (*P*=.001) (Figure 3F). Movements in the left ankle during (24.25±1.54°) and before (22.79±2.64°) were higher compared with after tSCS1 (4.01±0.48°) (*P*=.001). Right ankle movements were higher during (15.89±2.53°) compared with before (5.01±1.11°) and after

Mayo Clin Proc Inn Qual Out ■ February 2024;8(1):1-16 ■ https://doi.org/10.1016/j.mayocpiqo.2023.09.006
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tSCS1 (2.62±0.56°) (*P*=.001) (Supplemental Figure 4B). During the 2nd assessment with tSCS (tSCS2), P2 reported an increase in the left knee movement during (10.44±4.6°) and after tSCS2 (13.48±2.5°) compared with before tSCS2 (5.38±1.8°) (*P*=.05). Movements in right knee were increased with tSCS2 (7.41±3.7°) compared with before (1.92±1.1°) and after tSCS2 (2.5±1.7°) (*P*=.05) (Figure 3F). Movements in left ankle were higher with tSCS2 (23.52±10.9°) compared with before (9.73±5.5°) and after tSCS2 (1.59±0.8°) (*P*=.05), whereas movements in the right ankle during tSCS2 (2.14±1.4°) were lower compared with movements before (8.79±3.6°) and after tSCS2 (5.48±2.0°) (*P*=.05) (Supplemental Figure 4B). After 8 weeks of tSCS-enabled training in P2, led to minimal improvement in right knee movements (1.23±0.5° before vs 1.9±1.1° after training) and in the right ankle (5.01±4.3° before vs 8.79±3.6° after).

In P2, during the 1st assessment with EES (EES1), reported increased movements in left knee (58.04±6.02°) compared with before (28.01±2.4°) and after EES1 (37.88±19.9°) (*P*=.001) and in right knee during (26.55±16.6°) compared with before (12.15±10.8°) and after EES1 (12.19±7.2°) (*P*=.05). Movements in left and right ankle were higher during (9.97±4.3° and 17.76±13.3°) compared with before (0.46±0.3° and 8.07±5.1°) and after EES1 (0.5±0.3° and 8.33±3.8°) (*P*=.008 and *P*=.001, respectively) (Figure 4B). During the 2nd assessment with EES (EES2), P2 reported increased movements in left knee during (51.91±7.5°) compared with before (8.66±2.0°) and after EES2 (5.16±2.2°) (*P*=.001). Movements in right knee where higher with EES2 (45.77±4.2°) compared with before (6.80±3.3°) and after ESS2 (6.04±1.9°) (*P*=.001). Left ankle movements were higher during (20.20±8.0°) compared with before (0.50±0.3°) and after EES2 (0.63±0.6°) (*P*=.001). Movements in right ankle during EES2 (8.89±6.0°) were higher compared with the movements before (0.92±0.6°) and after EES2 (1.05±0.3°) (*P*=.001) (Supplemental Figure 4B). Eight weeks of EES-enabled training in P2, led to no change or decrease in volitional movements when tested without EES and significant improvement in performance with EES.

Balance Control With tSCS and EES

Balance control was evaluated while participants were sitting with arms forward, sideward, and upright based on metrics of head, arms, and trunk position during tSCS and then during EES. With tSCS, P1 reported improvement of trunk, hands, and head control in hands forward position, although, with hands sideward and hands upward, only some parameters were improved with tSCS. With EES P1 reported a decline in balance in all 3 positions compared with before stimulation. With tSCS, P2 reported improvement in trunk, hands, and head control in the hands forward and hands sideward positions and improvement of trunk control in the hands upright position. With EES, P2 reported less trunk control with minimal improvement in arms and head control in all 3 positions (Figure 4, and Videos 3 and 4).

With P1 sitting with both hands forward, with tSCS reported improvement in all metrics of trunk, head, and arms control. In regards of the trunk control tSCS led to decrease of abdominal curvature (Sa) in −5.0±8.4%

FIGURE 4. Balance control while sitting with hands forward, sideward, and upward. (A) The perturbations in the arm position relative to the main horizontal and vertical lines were assessed based on specific anatomic landmarks. Main horizontal line (red dash line) goes across the point of contact between pelvis and supporting surface, and main vertical line (black dash line) goes through the most prominent point of spine curve. In arms up position with hands covering nose, chin apex was used instead of nose apex. 11 metrics (2 angles, 2 squares, and 7 segments) were calculated to assess the balance control without and with stimulation (tSCS and EES) in P1 and P2. Main lines and metrics to evaluate trunk, head, and arms position with hands forward, sideward, and upward are described in detail in the balance assessment (see methods and Table 3). (B) Representative reconstruction of head, arms, and trunk position during testing before (grey line) and during stimulation (red line) tSCS. (C) Normalized values out of 6 frames were recorded every 10 sec in 60 sec periodbefore and during stimulation (SD, *P*<.05). Zero represents 100% of values recorded without tSCS. (D) Representative reconstruction of head, arms, and trunk position during testing with (red line) and without (grey line) EES. (E) Normalized values from 6 frames were recorded every 10 sec in 60 sec periodbefore and during stimulation (SD, *P*<.05). Zero represents 100% of values recorded without EES.

(*P*=.02), decrease of spinal curvature (St) in −11.1±11.6% (*P*=.03), decreased low back inclination from the vertical line (At) in −15.34±14.8% (*P*=.002), decreased the distance between tragus and scapular spine (TSc) up to −14.69±17.6 (*P*=.005), compared with assessment before stimulation. With EES both Sa and St were increased in 38.4±14.5% and 46.5±15.7%, respectively (*P*<.001), Tragus and scapular spine increased in 18.8±8.9% (*P*=.002), TH1 increased in 11.56±0.7% (*P*<.001), and UV3 increased in 22.723±1.2% (*P*=.001), overall indicating disturbance in trunk control. Head position control was improved with tSCS with the line connecting nose and vertical line at V1 (NV1) decreased in -4.2±4.4% (*P*=.008) and no changes in distance between nasal apex and main horizontal line at H2 (NH2). With EES NH2 increased in 14.53±4.8% (*P*=.001), whereas NV1 increased in 16.9±8.7% (*P*=.004), indicating disturbance in head control. Participant 1 reported improvement in arms control during tSCS with increase the distance between wrist and horizontal line at H3 (WH3) in 67.7±51.0% (*P*=.001), increase the distance between wrist and vertical line at V2 (WV2) in 32.7±21.1% (*P*=.001), and angle indicating elbows position (Ae) in 60.66±32.0% (*P*=.001). With ESS WV2 increased only in 15.4±4.7% (*P*=.001), whereas WH3 and Ae did not change (Figure 4, P1 A-F and Table 3).

Participant 2 sitting with both hands forward, with tSCS reported improvement in trunk and head control with decreased Sa in −19.47±3.9% (*P*=.001), St in −29.13±5.1%, At in −27.25±8.8% (*P*=.03), TSc in −10.34±4.8 (*P*=.004), UV3 in −11.55±2.3 (*P*=.001), and no changes in TH1.

The EES also reported improvement with Sa and St decreased in −13.96±4.1% (*P*=.001) and −3.73±2.2% (*P*=.012), TH1 increased in 2.64%±1.3 (*P*=.004), and no changes in At, TSc, and UV3. Head position control improved during tSCS with NV1 decreased in −14.15±2.2% (*P*=. 03) and during EES with NH2 increased in 3.04±1.4% (*P*=.004).

Participant 2 reported some decline in arms control during tSCS with WH3 decreased in −9.42±3.8% (*P*=.002) and WV2 decreased in −5.34±1.7% (*P*<.001), whereas Ae increased in 2.91±1.6% (*P*=.007) compared with no stimulation. During ESS WH3 increased in 3.64±3.1% (*P*=.05) and WV2 did not change (Figure 4, P2 A-F and Table 3). Extended results of balance assessment during sitting with hands sideward and upward presented on Figure 4.

The tSCS and EES effect on nonmotor symptoms: clinical evaluation before and after 8 wk of tSCS-enabled training reported improvement in nonmotor functions. Participant 1 reported feeling of the abdominal wall and fulness of the bladder after 5-6 wk of tSCS-enabled training and throughout the EES-enabled rehabilitation program. With EES P1 further reported improvement in bladder control and regained capacity to induce urination with maneuvers. The Neurogenic Bladder Symptom Score (NBSS) in P1 decreased from 46 points to 41 points, and the quality-of-life score increased from 4 to 5. Participant 1 initially reported low muscle tone, gradually increasing with tSCS-enabled training from 0 to 1+ on the Modified Ashworth Scale (MAS). The frequency of spasms increased from 0 to 1 during tSCS and to 2 during ESS. Participant 1 also reported an increase in neuropathic pain in both legs from 3 of 10 to 6 of 10 on the right leg and from 6 of 10 to 8/ of 10 on the left leg on VAS (information from self-control diary) that was well controlled with Pregabalin (75 mg). Participant 2, before enrollment, described hyperhidrosis below the level of injury on the left side and reported improvement after about 6 wk of tSCS-enabled training. Furthermore, during tSCS, P2 observed some sensation below the level of injury, mainly on the left side of his flank and hip and in the right leg. During EES-enabled motor training, P2 continued to report similar sensations, and later, it became consistent even without EES. With EES, P2 reported an increase in muscle tone from 1 to 2 points on MAS. Although both participants reported increased muscles tone during EES-enabled motor training, it did not affect their motor performance. Both participants reported some variation in blood pressure (BP) during the initial period of tSCS and EES with the episodic increase of BP up to 160-180 mm Hg in P1 and up to 140-160

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mm Hg in P2 with their normal BP 120-130mm Hg. The BP was normalized after 20 min of rest. After 2-3 days of training, both participants reported a normal range of BP.

DISCUSSION

This study compares the effect of consecutively applied tSCS and EES on the restoration of neurologic functions after motor complete SCI, reporting the predominant role of EES in the restoration of volitional motor control and tSCS in the improvement of balance. tSCS, similar to previous reports,[26] facilitated minimal volitional control, still essential for initiation of tSCS-enabled motor training, whereas EES immediately facilitated high-amplitude voluntary-controlled movements. Both participants were diagnosed with motor complete SCI and electrophysiological assessment reported evidence of translesional connectivity when combined with JM. Clinically complete SCI associated with evidence of translesional connectivity, is known as discomplete.[37,47-49] Although most of the clinically complete patients with SCI have residual anatomical connectivity,[50] instrumental assessment alone is ineffective in identifying the role of these fibers. Facilitation of H-reflex and SEMP with JM and increase in EMG activity during attempts to flex legs with JM, are similar to our previous report, where electrophysiological assessment in combination with JM facilitated subfunctional connectivity in AIS-A subject.[33] Electrophysiological assessment and following trial of tSCS confirmed a discomplete injury in both participants. The EES facilitated high-amplitude volitional movements already during the first attempt on the second day after implantation of the EES system, suggesting that the stronger effect of EES is likely related to more selective activation of the spinal structures. Comparison of motor performance without stimulation before and after 8 weeks of tSCS-enabled and EES-enabled training reported only minimal improvement, primarily in knee angle, which is inconsistent with recently reported significant restoration of volitional control even without stimulation.[29,30] This difference could be because of shorter compared with other studies stimulation-enabled training or individual differences between participants. The SEMP was previously studied in subjects with SCI applying tSCS and EES, suggesting that both types of stimulation activate the common neuronal structures at the lumbosacral segments sharing similar latency, amplitude, and shape.[35] Here, SEMPs amplitude was higher when tested with tSCS. This could be because of the different electrodes (surface EMG electrodes during tSCS vs subdermal needle electrodes during EES).[51,52] The different current flow and distribution of the electrical field with tSCS and EES are another factors to consider.[53-55]

The effect of tSCS to improve balance was reported previously, although without direct comparison to EES.[28,56] Our results show that tSCS has a stronger effect on balance improvement than EES, suggesting modulation of the spinal circuitry with tSCS, activating multi-segmental projections necessary to engage the balance control. This effect could be attributed to the wider current field with tSCS and facilitation of the afferent systems not activated with EES.[56] The tSCS effect on balance can be related to direct neuro-muscular activation, like in studies with improved balance through increased trunk stiffness because of low-intensity FES.[57] Similarly, current distribution during EES with more lateral electrode contacts could mediate the FES-type effect through direct motor axons activation.[30]

The difference between tSCS and EES raises several key questions. The optimal duration of tSCS-enabled training to improve and maintain the balance achieved with tSCS, particularly when it follows with EES, still needs to be determined. The effect of EES is likely to be mediated through the circuitry in the lumbosacral segments.[58-60] The tSCS and EES can facilitate the common neuronal structures.[35] However, widespread electrical field during tSCS[61] may activate additional sensory afferents in peripheral nerves, dorsal root ganglias, and spinal roots across several segments. After SCI, a reduced number of fibers responsible for precise coordination of the multiple muscles cannot provide the same level of accuracy, although the capacity to initiate-terminate, and modulate rhythmic activity with EES remains with limited connectivity in discomplete SCI. The question is—whether the limited fibers could provide accuracy and precision of movements and if this improvement can be achieved at all after motor complete SCI? One possibility is that

with consecutive tSCS-enabled and EES-enabled rehabilitation, the sublesional circuitry will be tuned to respond to supralesional commands, leading to more accurate control. This hypothesis is supported by our observation that subjects with paraplegia and EES regained volitional movement in single joints at a lower range of motion compared with the high-amplitude rhythmic leg movements,[8] and were able to walk with minimal assistance, although with a smaller amplitude and slower movements.[9] Another possibility for improvement in motor control after SCI may come from the integration of sublesional and supralesional components of spinal network. Recent findings indicate the importance of ascending signaling across SCI[23,62] and report the benefits of translesional stimulation.[63,64] By activating supralesional circuitry and facilitating sublesional network, neuromodulation may further improve motor and sensory control, compensating for the limited connectivity across the injury.[23] Another opportunity may come from segment-specific stimulation tuned to the main stimulation targets.[65,66] In support of this concept, a recent report found that individually adjusted electrode configuration leads to fast functional restoration.[30] The following critical step should elaborate advanced assessment of activated fibers[33] and segment-specific stimulation[66] for optimal activation of the spinal circuitry leading to further improvement. Both participants reported improvement in nonmotor functions started during the tSCS trial and continued throughout this study. Nonmotor effects of tSCS-related and EES-related mechanisms still need to be explored and connected with specific neural subtract.[67,68]

CONCLUSION

The restoration of neurologic functions in patients who are paralyzed with SCS is an impressive outcome of recent studies. This work reports that this effect can be attributed to both tSCS and EES applied even years after SCI. The dormant spinal circuitry can be re-engaged by the consecutive combination of noninvasive (tSCS) and invasive (EES) neuromodulation, providing different functional outcomes on volitional movements and balance. The extent of improvement achieved with external or implantable stimulating devices and further optimization of neuromodulation and rehabilitation therapy will determine the directions for future studies, translating new findings into effective therapy.

POTENTIAL COMPETING INTERESTS

The authors have no competing interests.

ACKNOWLEDGMENTS

Far Eastern Federal University and DNA Research Clinical Center KFU for their support in this study. The authors thank Dr Totorkulov for assisting during surgical procedures and Dr Kantur for assisting during experimental procedures and for subjects' evaluation. We want to thank both participants for their commitment and their trust in this research efforts. Drs Mukhametova and Militskova contributed equally to this work.

SUPPLEMENTAL ONLINE MATERIAL

Supplemental material can be found online at http://www.mcpiqojournal.org. Supplemental material attached to journal articles has not been edited, and the authors take responsibility for the accuracy of all data.

Abbreviations and Acronyms: **AIS**, ASIA (American spinal cords injury association) Impairment Scale; **EMG**, electromyography; **EES**, epidural electrical spinal cord stimulation; **JM**, Jendrassik maneuver; **SCI**, spinal cord injury; **SEMP**, spinally evoked motor potentials; **SSEP**, spinal cord somatosensory evoked potentials; **TMS**, transcranial magnetic stimulation; **tSCS**, transcutaneous electrical spinal cord stimulation

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Grant Support: This work was supported by the Ministry of Science and Higher Education of the Russian Federation under the agreement #075-15-2020-921 to support the development of a world-class research center "Pavlov Center for Integrative Physiology for Medicine, High-tech Healthcare, and Stress Tolerance Technologies", and by the Subsidy Allocated to Kazan Federal University for the

State Assignment in the Sphere of Scientific Activities (project FZSM-2022—0020).

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