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Supplementary information

The neurons that restore walking after paralysis

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Supplementary Information guide

Description of the clinical trial STIMO
Clinicaltrials.gov: NCT02936453

Study context. All experiments were carried out as part of the ongoing clinical feasibility study STIMO ("Stimulation Movement Overground"), which investigates the effects of spatiotemporal EES combined with weight-supported overground locomotor training on the recovery of motor function after SCI. This study was approved by the Swiss ethical authorities (Swissethics protocol number 04/2014 ProjectID: PB_2016-00886, Swissmedic protocol 2016-MD-0002) and was conducted in accordance with the Declaration of Helsinki. All participants signed a written informed consent prior to their participation. More information is provided at clinicaltrials.gov (NCT02936453). All surgical and experimental procedures were performed at the Lausanne University Hospital (CHUV) and have been previously described in detail[19].

Study Objectives. Primary objective

Address the feasibility in terms of efficacy and safety of overground, robot-assisted neurorehabilitation in combination with spinal EES to reduce the need of assistance required to walk and to increase the speed of walking, i.e., facilitate motor control, in chronic, SCI patients.

Secondary objective

Address the efficacy of overground, robot-assisted neurorehabilitation in combination with spinal EES to improve independence in activities of daily living and to improve endurance during walking, i.e., facilitate motor control, in chronic, SCI patients.

Study Endpoints. Primary endpoints

The overground, robot-assisted neurorehabilitation in combination with spinal EES will result in less assistance required to walk and faster speed of walking. This will be calculated within each individual and across the group (12 patients). *Chosen measures: WISCI II Score, 10-Meter Walk Test, Weight Bearing Capacity (WBC).*

Secondary endpoints

The overground, robot-assisted neurorehabilitation in combination with spinal EES will result in more independence in activities of daily living and an improved endurance during standing and walking. This will be calculated within each individual and across the group (12 patients). *Chosen measures: SCIM III Score, 6-Minute Walk Test.*

Inclusion Criteria. Patients fulfilling all of the following inclusion criteria may be enrolled in the study:

- Age 18-65 (women or men)
- Sensorimotor or motor complete and incomplete SCI graded as AIS A, B, C & D
- Level of lesion: T10 and above, based on AIS level determination by the PI, with preservation of conus function

- The intact distance between the cone and the lesion must be at least 60 mm.
- Focal spinal cord disorder caused by either trauma or epidural, subdural or intramedullary bleeding
- Minimum 12 months post-injury
- Completed in-patient rehabilitation program
- For ASIA C and D, able to stand with walker or 2 crutches
- Stable medical, physical and psychological condition as considered by Investigators
- Able to understand and interact with the study team in French or English
- Adequate care-giver support and access to appropriate medical care in patient's home community
- Agree to comply in good faith with all conditions of the study and to attend all required study training and visit
- Must participate in two training sessions before eligibility is confirmed
- Must provide and sign Informed Consent prior to any study related procedures

Exclusion Criteria.

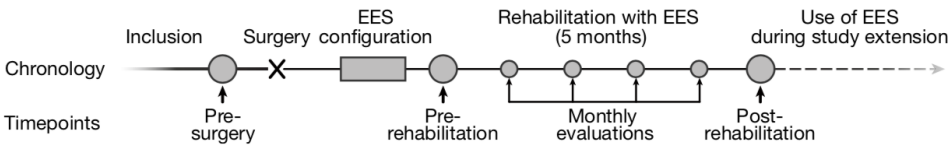
The presence of any one of the following exclusion criteria will lead to exclusion of the subject:

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

the study or to comply with follow-up requirements, or impact the scientific soundness of the study results.

- Patient is unlikely to survive the protocol follow-up period of 12 months.

Study timeline.



The study involves assessments before surgery, the surgical implantation of the neurostimulation system, a one-month period during which EES protocols are configured, and a five-month rehabilitation period with physiotherapists taking place four to five times per week for one to three hours. The rehabilitation program is personalized based on the current capacities and improvements displayed by each participant. At the end of the rehabilitation period, the participants are given the opportunity to be enrolled in a study extension phase during which they can continue using the neurostimulation system at home. Participants enrolled in this extension phase are followed-up on a regular basis by the study team for up to six years.

Supplementary Tables and Legends

Participants	**BT001**		**DM002**		**GO004**		**ST006**		**MB007**		**HT008**		**GF009**		**GJ010**		**MR012**	
Gender	m		m		m		m		m		m		m		m		m	
Age at study enrolment (y)	53		28		34		47		23		32		32		41		29	
Years after SCI at study enrolment	3 years and 2 months		5 years and 11 months		5 years and 5 months		4 years and 3 months		3 years and 8 months		14 years and 3 months		8 years and 11 months		1 year and 3 months		2 years and 10 months	
Assessment at study enrolment (Pre) and after rehabilitation period (Post)	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
American Spinal Injury Association Impairment Scale (AIS)	D	D	C	D	C	D	C	C	C	C	C	D	A	A	A	A	B	C
Neurological level of injury													T4	T3	T3	T3	T7	T7
Lower Extremity Motor Score																		
L2, hip flexors (right | left)	2 | 3	4 | 4	2 | 0	4 | 2	2 | 2	3 | 2	0 | 0	0 | 1	0 | 0	1 | 1	0 | 2	2 | 4	0 | 0	0 | 0	0 | 0	0 | 0	0 | 0	1 | 2
L3, knee extensors (right | left)	4 | 4	4 | 4	2 | 0	4 | 3	4 | 4	4 | 4	0 | 0	1 | 0	0 | 0	0 | 0	1 | 2	4 | 4	0 | 0	0 | 0	0 | 0	0 | 0	0 | 0	1 | 1
L4, ankle dorsiflexors (right | left)	2 | 2	3 | 2	4 | 0	4 | 1	3 | 3	4 | 4	0 | 0	0 | 1	0 | 0	0 | 0	0 | 1	2 | 4	0 | 0	0 | 0	0 | 0	0 | 0	0 | 0	0 | 1
L5, long toe extensors (right | left)	4 | 4	0 | 0	4 | 0	4 | 2	1 | 1	2 | 4	0 | 0	0 | 1	0 | 0	3 | 3	1 | 4	0 | 4	0 | 0	0 | 0	0 | 0	0 | 0	0 | 0	0 | 0
S1, ankle plantar flexors (right | left) (max. 5 per side)	2 | 2	4 | 4	2 | 0	4 | 2	4 | 4	5 | 4	0 | 0	0 | 0	0 | 0	0 | 0	0 | 1	0 | 2	0 | 0	0 | 0	0 | 0	0 | 0	0 | 0	0 | 0
Total (max. 25 | 25)	14 | 15	4 | 5	14 | 0	20 | 0	14 | 14	18 | 18	0 | 0	1 | 3	0 | 0	4 | 4	2 | 10	8 | 18	0 | 0	0 | 0	0 | 0	0 | 0	0 | 0	2 | 4
Deep anal pressure (DAP)													No	No	No	No	Yes	Yes
Voluntary anal contraction (VAC)													No	No	No	No	No	No
Light-Touch Sensory score																		
L1-S2 dermatomes subscore (right | left)	9 | 9	9 | 9	9 | 9	9 | 9	2 | 10	2 | 14	2 | 5	4 | 7	9 | 9	0 | 0	9 | 9	9 | 8	0 | 0	0 | 0	0 | 0	0 | 0	0 | 0	0 | 0
Total (max. 112)	42 | 41	42 | 42	38 | 37	38 | 37	21 | 32	27 | 44	26 | 29	28 | 29	41 | 42	35 | 31	43 | 43	45 | 43	23 | 23	22 | 22	23 | 23	21 | 22	30 | 31	30 | 31
Pin Prick Sensory Scores																		
L1-S2 dermatomes subscore (right | left)	0 | 1	1 | 4	0 | 0	0 | 0	0 | 13	3 | 17	0 | 0	0 | 0	0 | 0	0 | 0	9 | 0	9 | 0	0 | 0	0 | 0	0 | 0	0 | 0	0 | 0	0 | 0
Total (max. 56 | 56)	32 | 33	31| 35	17 | 16	15 | 15	27 | 41	36 | 50	13 | 15	15 | 13	32 | 28	30 | 31	43 | 34	44 | 34	22 | 22	21 | 22	24 | 24	21 | 21	29 | 30	30 | 30

Supplementary Table 1.
Neurological status of participants before and after EES^{REHAB}

		exemplary factor loadings	
TEMPORAL GAIT FEATURES		**PC1**	**PC2**
1 Cycle duration (s)		-0.1576717	-0.0191449
2 Cycle velocity (cm/s)		0.71765717	0.29440406
3 Stance duration (s)		0.33582429	-0.3705423
4 Swing duration (s)		-0.4934916	0.32363859
5 Relative stance duration (% of gait cycle)		0.71023334	-0.359373
6 Double stance duration (%)		0.60312357	-0.2502677
LIMB TRAJECTORIES			
7 Stride Length (cm)		0.6316849	0.23280816
8 Step Length (cm)		0.5614388	0.34622112
9 3D toe path length (cm)		0.5916267	0.43111612
10 Maximal backward position of foot (cm)		-0.5463889	0.59951176
11 Maximal forward position of foot (cm)		-0.6926062	0.53389382
12 Step Height (normalised)		0.72236662	0.10545746
13 Step Height (cm)		0.69784106	0.20966774
14 Max speed during swing (cm/s)		0.89591401	0.21245806
15 Time of max velocity during swing (% duration)		-0.1168049	0.07212136
16 Acceleration at swing onset (cm/s^2)		0.78410584	0.26677412
17 Endpoint velocity (cm/s)		0.62851802	0.15385477
18 Velocity vector at swing onset (deg)		-0.0894592	0.08398648
DRAG			
19 Drag duration (s)		-0.5417939	0.3603316
20 Drag duration (%)		-0.8373842	0.17578048
STABILITY			
21 Lateral displacement during swing (cm	Base of Support	0.05691657	0.00178616
22 Stance width (cm)	Base of Support	0.4236455	-0.2945496
23 Pelvis max vertical movement	Base of Support	0.32070531	0.34089065
24 Pelvis min vertical movement	Base of Support	-0.146866	0.25713701
25 Amplitude of pelvis vertical movement	Base of Support	0.6460952	0.16661574
26 Variability of saggital trunk oscillation	Trunk Stability and Oscillations	0.53231482	0.01633459
27 Velocity of saggital trunk oscillation	Trunk Stability and Oscillations	0.76830774	0.27624926
28 Variability of vertical hip oscillation	Trunk Stability and Oscillations	0.66372851	0.06598395
29 Variability of medio-lat hip oscillation	Trunk Stability and Oscillations	0.58622279	0.11184576
30 Variability of hip rotations	Trunk Stability and Oscillations	0.6548397	0.17146907

		exemplary factor loadings	
JOINT ANGLES AND SEGMENTAL OSCILLATIONS		**PC1**	**PC2**
31 Crest oscillations (deg)	Backward	0.00455681	-0.1268226
32 Thigh oscillations (deg)	Backward	0.17662466	-0.5597325
33 Shank oscillations (deg)	Backward	-0.3169744	-0.0503729
34 Foot oscillations (deg)	Backward	0.23729717	-0.6326635
35 Whole limb oscillations (deg)	Backward	-0.1183546	-0.4490518
36 Crest oscillations (deg)	Forward	0.39236189	-0.0480982
37 Thigh oscillations (deg)	Forward	0.65639533	-0.46911
38 Shank oscillations (deg)	Forward	0.46698448	-0.2054473
39 Foot oscillations (deg)	Forward	0.68490153	-0.5701471
40 Whole limb oscillations (deg)	Forward	0.71750685	-0.5190953
41 Hip Joint (deg)	Extension	-0.1734601	0.59996864
42 Knee Joint (deg)	Extension	-0.0337904	0.34799307
43 Ankle joint (deg)	Extension	-0.2004252	0.63830011
44 Whole limb abduction (deg)	Abduction	0.03433273	0.28954361
45 Foot abduction (deg)	Abduction	0.08665734	0.39377111
46 Hip Joint (deg)	Flexion	-0.5538434	0.39348434
47 Knee Joint (deg)	Flexion	-0.5989274	0.34107686
48 Ankle joint (deg	Flexion	-0.6415763	0.473096
49 Whole limb adduction (deg)	Adduction	-0.2250942	0.13517821
50 Foot adduction (deg)	Adduction	-0.1538514	0.0444364
51 Crest oscillations (deg)	Oscillation Amplitudes	0.59139626	0.0952798
52 Thigh oscillations (deg)	Oscillation Amplitudes	0.73214101	0.02554458
53 Shank oscillations (deg)	Oscillation Amplitudes	0.7759825	-0.1850331
54 Foot oscillations (deg)	Oscillation Amplitudes	0.68378825	-0.1976867
55 Whole limb oscillations (deg)	Oscillation Amplitudes	0.79581363	-0.2813106
56 Hip Joint (deg)	Oscillation Amplitudes	0.54185671	0.40830478
57 Knee Joint (deg)	Oscillation Amplitudes	0.64190896	0.03090613
58 Ankle joint (deg)	Oscillation Amplitudes	0.64712855	0.08329771
59 Whole limb medio-lat oscillation (deg)	Oscillation Amplitudes	0.5193659	0.28178655
60 Foot medio-lat oscillation (deg)	Oscillation Amplitudes	0.1960371	0.26750767

		exemplary factor loadings	
VELOCITIES		**PC1**	**PC2**
61 Whole limb oscillation velocity (deg/s)	Minimum angle velocities	-0.8464141	-0.18285
62 Hip oscillation velocity (deg/s)	Minimum angle velocities	-0.759858	-0.3463828
63 Knee oscillation velocity (deg/s)	Minimum angle velocities	-0.8054906	-0.3284002
64 Ankle oscillation velocity (deg/s)	Minimum angle velocities	-0.6920943	-0.1568301
65 Whole limb oscillation velocity (deg/s)	Maximum Angle velocities	0.90190547	0.04548126
66 Hip oscillation velocity (deg/s)	Maximum Angle velocities	0.76680173	0.39026841
67 Knee oscillation velocity (deg/s)	Maximum Angle velocities	0.8515911	0.14651785
68 Ankle oscillation velocity (deg/s)	Maximum Angle velocities	0.81309484	0.25109988
69 Whole limb oscillation velocity (deg/s)	Amplitude of angle velocities	0.91926192	0.09321898
70 Hip oscillation velocity (deg/s)	Amplitude of angle velocities	0.79560219	0.37980295
71 Knee oscillation velocity (deg/s)	Amplitude of angle velocities	0.8825796	0.25975487
72 Ankle oscillation velocity (deg/s)	Amplitude of angle velocities	0.76572477	0.19610856
INTRALIMB TEMPORAL COUPLING			
73 Correlation between pelvis and thigh	proximal distal	0.24428247	-0.3228218
74 Correlation between thigh and shank	proximal distal	0.1160031	-0.4449952
75 Correlation between shank and foot	proximal distal	0.00752821	-0.5059836
76 Correlation between hip and knee	proximal distal	-0.0175302	-0.1634833
77 Correlation between knee and ankle	proximal distal	-0.0274252	-0.1351518
78 Correlation between ankle and foot	proximal distal	0.06456071	-0.14037
ROBOTIC SUPPORT FEATURES			
79 Body Weight Support (%)		-0.8008253	-0.112497
80 Horizontal Support Force (%)		-0.7955145	-0.0546379

Supplementary Table 2.
Gait parameters calculated from kinematic recordings during walking in mice

Group	n	Condition	Had SCI?	Had EES^{REHAB}?	Terminal condition	Therapeutic feature modelled
1	3	Uninjured	No	No	None	Uninjured
2	3	SCI	Yes	No	None	SCI
3	3	EES^{REHAB}	Yes	Yes	None	EES^{REHAB}
4	3	$SCI^{\rightarrow EES::walking}$	Yes	No	EES^{ON} for 30 minutes while walking	Walking with EES after SCI
5	3	$EES^{REHAB\rightarrow EES::walking}$	Yes	Yes	EES^{ON} for 30 minutes while walking	Walking with EES after EES^{REHAB}
6	3	$EES^{REHAB\rightarrow EES}$	Yes	Yes	EES^{ON} for 30 minutes (no walking)	Immediate effect of EES in the absence of walking after EES^{REHAB}
7	3	$EES^{REHAB\rightarrow cortex}$	Yes	Yes	Optogenetic stimulation of the motor cortex for 30 minutes to model voluntary control (no walking)	Immediate effect of residual descending input in the absence of EES after EES^{REHAB}
8	3	$EES^{REHAB\rightarrow EES::cortex::walking}$	Yes	Yes	Optogenetic stimulation of the motor cortex and EES^{ON} for 30 minutes while walking	Walking with residual descending input and EES after EES^{REHAB}

Supplementary Table 3.
Description of experimental conditions