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# Reporting Summary

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## Statistics

For all statistical analyses, confirm that the following items are present in the 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 |
| ☐ | ☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☐ | ☒ The statistical test(s) used AND whether they are one- or two-sided<br>*Only common tests should be described solely by name; describe more complex techniques in the Methods section.* |
| ☒ | ☐ A description of all covariates tested |
| ☐ | ☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| ☐ | ☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| ☐ | ☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted<br>*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 |

*Our web collection on statistics for biologists contains articles on many of the points above.*

## Software and code

Policy information about availability of computer code

| | |
|---|---|
| Data collection | Data were collected using the following software: custom MATLAB R2020a/b and R2021 a/b code interfacing with an NI Data Acquisition Board (PCI-6255) and an interprocess communication framework written in MATLAB (Drangonfly; Velliste, 2013) for synchronously recording stimulus pulse timing, EMG, and experimental performance parameters. We used Dexterit-E 3.8 and 3.9 (KINARM) for collecting planar reaching kinematics, the HUMAC2015 v15.000.0276 (HUMAC NORMS) software environment for collecting joint torque data, and VICON Nexus 2.12.1 for acquiring 3D reaching kinematics. Intra-operative CMAPs were recorded using EPWorks software running on the XLTEK Protektor32 (Natus). MRI images were acquired using Prisma System VEC11C (Siemens). |
| Data analysis | Data analysis of all kinematics, joint torques, electromyography, functional performance, recruitment curves, and clinical assessments were performed using custom analysis code written in MATLAB R2020a/b and R2021a/b. Data from intra-operative electrophysiology were extracted manually from propriety data files using the EPWorks v6.1.2.38 software (Natus). MRI lesions were extracted and visualized using MRIcron v1.0.20190902 and MRIcroGL v1.2.20211006. High definition fiber tracking was performed using DSI-Studio. All figures were rendered in Adobe Illustrator CC v26.0 - v26.3.1. |

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 and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

## Data

Policy information about availability of data

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

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

All software and anonymized data will be available on the NIH Brain Initiative Data Server or upon reasonable request to the corresponding author.

# Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

# Life sciences study design

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

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|---|---|
| Sample size | No statistical methods were used to pre-determine sample sizes. All data was analyzed in all subjects independently and no formal statistical comparison between populations was performed. Data comes from the first two enrolled subjects in our clinical trial and no other subject was excluded from the analysis. Number of specific datapoints and repetitions is similar to other studies using similar measures (Wagner 2018, Nature, Gill 2018 Nature Med) |
| Data exclusions | No data was excluded |
| Replication | The reproducibility of experimental finding was confirmed across two subjects ( therapeutic effects of stimulation were assessed for each animal) and across multiple sessions ( therapeutic effects of stimulation were assessed several times for each subjects as reported in the manuscript) Due to the variability of stroke lesions, not all functional tasks were tested in both subjects. |
| Randomization | No randomization was introduced in our experiments. Since no formal statistical comparisons were required in our study and all animals were independently analyzed, no randomization was necessary and the same protocol was performed on all animals. |
| Blinding | The investigators were blinded to experimental condition while processing the data. |

# Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

### Materials & experimental systems

| n/a | Involved in the study |
|---|---|
| ☒ | ☐ Antibodies |
| ☒ | ☐ Eukaryotic cell lines |
| ☒ | ☐ Palaeontology and archaeology |
| ☒ | ☐ Animals and other organisms |
| ☐ | ☒ Human research participants |
| ☐ | ☒ Clinical data |
| ☒ | ☐ Dual use research of concern |

### 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 | In this work we report results from the first 2 subjects participating in our trial who were both caucasian females. SCS01 (31 years) had a right thalamic hemorrhagic stroke secondary to a cavernous malformation 9 years prior to participation in the study. Her interim history involved several bleeding events with eventual ablation of the malformation with gamma knife radiosurgery. At the time of her participation in our trial, her post stroke residual was a left-sided spastic hemiparesis for which she was receiving botulinum injections in her biceps, brachioradialis, and pronator teres. Botulinum treatments were |

suspended starting 6 months prior to the study period and continuing through the end of the study. In the years between her initial infarct and participation, she also underwent a C5-6 anterior cervical discectomy and fusion to treat cervical stenosis as well as a flexor tendon lengthening surgery due to spasticity and suffered arm and wrist fractures in where affected arm. For SCS01, we included in this work analysis of 138 isometric force tests repetitions at multiple joints (54 stim off and 84 stim on) and 36 planar reaches (18 with SCS and 18 without SCS). The results of simulated activity of daily living and other motor tasks that were performed over the course of 3 sessions and reported in Figure 4.

SCS02 (47 years) had a right ischemic middle cerebral artery stroke secondary to a right carotid dissection resulting in a large MCA territory infarct 3 years prior to participation in the study. Her post stroke residual at the time of participation was a left-sided spastic hemiparesis complicated by a left wrist flexion contracture despite treatment with splinting.
For SCS02 we included in this work analysis of 42 isometric force tests repetitions (21 stim off and 21 stim on) and 57 planar reaches (38 with SCS and 19 without SCS) that were obtained across multiple days during the course of the study. The results of simulated activity of daily living and other motor tasks that were performed over the course of 3 sessions and reported in Figure 4.

| | |
|---|---|
| Recruitment | Subjects signed an informed consent whose content were approved by the IRB of the University of Pittsburgh. SCS01 was recruited through referral via clinical collaborator, SCS02 contacted the study via the Pitt+me platform of the University of Pittsburgh. As there was only one study group, recruitment was not subject to self-selection bias. |
| Ethics oversight | All experimental protocols were approved by the University of Pittsburgh Institutional Review Board (Protocol STUDY19090210) under abbreviated IDE. Our study is published on ClinicalTrial.gov number NCT04512690 |

Note that full information on the approval of the study protocol must also be provided in the manuscript.

## Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

| | |
|---|---|
| Clinical trial registration | NCT04512690 |
| Study protocol | The full study protocol is available for viewing at ClinicalTrials.gov (NCT04512690). |
| Data collection | All surgical procedures were performed at UPMC Presbyterian Hospital in Pittsburgh, PA.<br><br>All data were collected in the laboratories of Dr. Marco Capogrosso and Dr. George Wittenberg.<br><br>Each subject came into the lab for 2-3 days of pre-implant testing during the 1-2 months leading up to the implant date. After the implant procedure, participants were given a full weekend to recover before data collection began. During the 30 day implant period, participants engaged in data collection for 4 hours per day on 5 days per week for a period of 4 weeks.<br><br>Post-study follow up data collection was obtained in the laboratory 1 month following explant. |
| Outcomes | Primary Outcome Measures :<br>Adverse Events [ Time Frame: 29 days ]<br>Study is considered successful if no serious adverse events related to the use of electrical stimulation are reported<br><br>Discomfort and Pain [ Time Frame: 7, 14, 21, 29 days ]<br>We will assess the relative level of discomfort and/or pain that is associated to the delivery of stimulation to the spinal cord. After each stimulation trains patients will be asked to report their perceived discomfort level using a 10 value subjective scale. Low values will be assigned to low discomfort, and high values to high discomfort.The study is considered successful if 70% of recruited subjects does not report discomfort or pain at stimulation amplitudes that are required to obtain motor responses in the muscles of the arm and hand<br><br>Secondary Outcome Measures :<br>Motor Impairment [ Time Frame: 15, 29 days ]<br>The Fugl-Meyer Assessment (FMA) is a stroke-specific, performance-based impairment index. It is designed to assess motor functioning, balance, sensation and joint functioning in patients with post-stroke hemiplegia. It is applied clinically and in research to determine disease severity, describe motor recovery, and to plan and assess treatment. The upper extremity motor function score ranges from 0 to 66 points. Minimal Detectable Change (MDC) is 5.2 points. The MCID (Minimally Clinically Important Difference) is 4.25 to 7.25.<br><br>Dexterity / Function: Action Research Arm Test [ Time Frame: 7, 29 days ]<br>The investigators will use the Action Research Arm Test (ARAT) assessment to quantify functional hand and arm dexterity. Performances will be compared with SCS-on against SCS-off. The investigators will consider as a minimally acceptable improvement an increase in the affected arm total score of >4 points. Comparison will be done per patient between Stim-on, Stim-off and pre-study baselines. Maximum score on the test is 57 points, minimum score is zero points, with a higher value indicating better dexterity/function.<br><br>Single Joint Force [ Time Frame: 7, 14, 21, 29 days ]<br>Isometric torque: measure the isometric torque produced by the subject at the shoulder, elbow and wrist joints. Comparison of SCS-on with SCS-off performance. Success Criteria: ≥20% increased torque production over SCS-off baseline as measured during single- |

joint isometric torque.

Joint Velocity [ Time Frame: 7, 14, 21, 29 days ]
The investigators will use the KINARM robot to quantify joint velocity. The investigators will measure 2D kinematics of the arm during several different horizontal reaching tasks. The investigators will also quantify joint velocity in 3D while subjects perform reach and grasp tasks unsupported. Subjects will be tasked to reach to targets or objects and manipulate objects while 3D videos of their arm and hand movements are recorded. Arm and hand kinematics will then be analyzed offline in parallel to EMG analysis of arm and hand muscles. Comparison will be done per patient between Stim-on and Stim-off at different time-points. Given the scientific nature of this task no minimal acceptable improvement is defined and data will be used to understand effects of SCS on arm kinematics.

Movement Smoothness [ Time Frame: 7, 14, 21, 29 days ]
The investigators will use the KINARM robot to quantify movement smoothness. The investigators will measure 2D kinematics of the arm during several different horizontal reaching tasks. The investigators will also quantify movement smoothness in 3D while subjects perform reach and grasp tasks unsupported. Subjects will be tasked to reach to targets or objects and manipulate objects while 3D videos of their arm and hand movements are recorded. Arm and hand kinematics will then be analyzed offline in parallel to EMG analysis of arm and hand muscles. Comparison will be done per patient between Stim-on and Stim-off at different time-points. Given the scientific nature of this task no minimal acceptable improvement is defined and data will be used to understand effects of SCS on arm kinematics.

Time to Target [ Time Frame: 7, 14, 21, 29 days ]
The investigators will use the KINARM robot to quantify time to target. The investigators will measure 2D kinematics of the arm during several different horizontal reaching tasks. The investigators will also quantify time to target in 3D while subjects perform reach and grasp tasks unsupported. Subjects will be tasked to reach to targets or objects and manipulate objects while 3D videos of their arm and hand movements are recorded. Arm and hand kinematics will then be analyzed offline in parallel to EMG analysis of arm and hand muscles. Comparison will be done per patient between Stim-on and Stim-off at different time-points. Given the scientific nature of this task no minimal acceptable improvement is defined and data will be used to understand effects of SCS on arm kinematics.

Sensory motor integration: success-rate [ Time Frame: 7, 14, 21, 29 days ]
The investigators will use the KINARM robot to quantify functional sensory acuity and sensory-motor integration. The investigators will measure 2D kinematics of the arm during different exercises where subjects will reach to defined targets with and without visual feedback. These tasks are designed to assess proprioception acuity and sensory-motor integration. Success-rate will be quantified offline. Comparison will be done per patient between Stim-on and Stim-off at different timepoints. Given the scientific nature of this task no minimal acceptable improvement is defined and data will be used to understand effects of SCS on sensorimotor integration processes.

Sensory motor integration: displacement error [ Time Frame: 7, 14, 21, 29 days ]
The investigators will use the KINARM robot to quantify functional sensory acuity and sensory-motor integration. The investigators will measure 2D kinematics of the arm during different exercises where subjects will reach to defined targets with and without visual feedback. These tasks are designed to assess proprioception acuity and sensory-motor integration. Displacement error from true target location will be quantified offline. Comparison will be done per patient between Stim-on and Stim-off at different timepoints. Given the scientific nature of this task no minimal acceptable improvement is defined and data will be used to understand effects of SCS on sensorimotor integration processes.

Spasticity [ Time Frame: 7, 15, 21, 29 days ]
The investigators will quantify spasticity scores using the Modified Ashworth Scale (MAS) for the shoulder, elbow and wrist joint and compare values with SCS-on and SCS-off. The investigators will consider as a minimally acceptable improvement a decrease of MAS >1, if available for the specific joint. Comparison will be done per patient between Stim-on and Stim-off and pre-study baselines. Maximum score on the MAS is 4, minimum score is 0, with a lower number indicating less spasticity.

Sensorimotor Network Function [ Time Frame: 29 days ]
The investigators will perform resting state and motor-task functional MRI of the brain and spinal cord to quantify neural network activation at rest and during the execution of simple motor tasks.

Sensorimotor Network Structure Integrity [ Time Frame: 29 days ]
The investigators will perform High-definition Diffusion Weighted Imaging to quantify Fractional Anisotropy as a measurement of axon integrity in the brain and spinal cord pre and post study.

Cortico-spinal Tract Integrity [ Time Frame: 29 days ]
The investigators will measure muscle evoked potential consequent to Transcranial Magnetic Stimulation of the cortico-spinal tract to assess integrity of the cortico-spinal tract. They will also explore SCS responses when conditioned by a TMS pulse and vice-versa.

Spinal Circuit Excitability [ Time Frame: 7, days ]
The investigators will measure H-reflexes of arm muscles obtained during stimulation of the peripheral nerves to quantify excitability of spinal motoneurons to stimulation of primary sensory afferents pre and post-study. Expected Result: The main scientific hypothesis is that SCS will change sensori-to-motoneuron excitability that can be measured via H-reflex responses pre and post-implant.

Motoneuron Firing Rates [ Time Frame: 7, 14, 21, 29 days ]
The investigators will use high-density EMGs on arm muscles to calculate firing rates of single spinal motoneuron discharge during isometric maximal voluntary contractions.

# Magnetic resonance imaging

## Experimental design

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|---|---|
| Design type | Anatomical Scan T1-Weighted 3D and Diffusion Weighted Imaging |
| Design specifications | No trial, single session |
| Behavioral performance measures | Subjects did not perform and behavioral tasks while undergoing MRI. |

## Acquisition

| | |
|---|---|
| Imaging type(s) | Structural; Diffusion |
| Field strength | 3T |
| Sequence & imaging parameters | Structural- (MPRAGE) sequence (TR = 2300 ms; TE = 2.9 ms; FoV = 256 × 256 mm2; 192 slices, slice thickness = 1.0 mm, in-plane resolution = 1.0 × 1.0 mm); Diffusion: diffusion spectrum imaging scheme to capture a total of 257 diffusion samples. The maximum b-value used was 4000 s/mm² and the in-plane resolution and slice thickness were 2 mm. |
| Area of acquisition | Whole brain scan |

Diffusion MRI ☒ Used ☐ Not used

Parameters: Diffusion: diffusion spectrum imaging scheme to capture a total of 257 diffusion samples. The maximum b-value used was 4000 s/mm² and the in-plane resolution and slice thickness were 2 mm.

## Preprocessing

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|---|---|
| Preprocessing software | MRIcro_GL (NITRC) was used to visualize and export the resulting segmented overlays. |
| Normalization | The diffusion data were reconstructed in the MNI space using q-space diffeomorphic reconstruction |
| Normalization template | The diffusion data were reconstructed in the MNI space using q-space diffeomorphic reconstruction |
| Noise and artifact removal | Scans provided strong signal to noise ratio and no artifacts were observed. |
| Volume censoring | Subjects remained sufficiently still, no volume censoring was required. |

## Statistical modeling & inference

| | |
|---|---|
| Model type and settings | Does not apply, only DTI analysis was performed |
| Effect(s) tested | No effects were tested, analysis was performed at the individual subject level |

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

| | |
|---|---|
| Statistic type for inference (See Eklund et al. 2016) | No statistical inferences were used, analysis was performed at the individual subject level |
| Correction | Only the cerebrospinal tract was analyzed |

## Models & analysis

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