Neuromodulation in Lower Limb Amputees

NCT 04543786

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Background:
Limb amputation results in an extreme form of peripheral nerve injury. Damage to peripheral nerves, such as with neuropathy, crush injuries, nerve transection, or limb amputation often results in chronic pain, which may be associated with altered excitability of spinal sensorimotor pathways. These spinal pathways become hyperexcitable due to a lack of sensory input, which causes tonic disinhibition of descending circuits and spontaneous activity in the dorsal root ganglia. Spinal excitability can be measured using the H-reflex, in which electrical stimulation of muscle spindle Ia afferents activates spinal motoneurons via the myotatic reflex, as well as the posterior root-muscle (PRM) reflex, which is elicited by transcutaneous stimulation over the dorsal roots and is considered to be half of the H-reflex, excluding the peripheral primary afferents, but with multiple root activation. Spinal excitability has not been measured in amputees but may offer a potential biomarker for phantom limb pain (PLP). Neuromodulation may restore normal spinal excitability and reduce PLP, thus offering the potential to improve the quality of life in individuals with a lower limb amputation. The results of this study will provide the foundation for future development of a neuroprosthesis to restore spinal excitability and reduce PLP in individuals with a lower limb amputation.

Objectives:
The overall goal of this work is to investigate the changes in the spinal cord resulting from limb amputation. Specifically, we measured spinal cord excitability using posterior root-muscle (PRM) reflexes in people with a transtibial amputation. We hypothesized that spinal reflexes would be hyperexcitable, indicated by lower thresholds to evoke the reflexes, because of the neuropathic pain state. We applied tSCS each day for 5 days, targeting the dorsal roots corresponding to the distal limbs. We hypothesized that, after 5 days of tSCS, spinal reflex hyperexcitability and PLP would decrease.

Design:
Participants underwent 5 testing and stimulation sessions in 1 week.

We recruited individuals with a unilateral transtibial amputation and who experienced phantom limb pain. To measure spinal cord excitability, we studied PRM reflexes, which were recorded using electromyography (EMG) electrodes placed on the residual limb. We placed bipolar electromyography (EMG) electrodes on the lateral gastrocnemius (LG), medial gastrocnemius (MG), tibialis anterior (TA), and vastus lateralis (VL) muscles and a high-density EMG electrode grid across the putative gastrocnemius muscles. We positioned a ground electrode onto the patella of the residual limb. We recorded EMG data at a sampling rate of 4000 Hz and streamed the data into MATLAB. We delivered stimulation using a DS8R stimulator with a firmware update to allow frequencies up to 10 kHz.

We attempted to elicit H-reflexes by electrically stimulating the tibial nerve of the residual limb. Stimuli consisted of a 1-ms long monophasic, cathodic, square wave pulse. We varied the stimulation amplitude to determine the thresholds for the M-wave as well as the maximum amplitude of the M-wave. We elicited the PRM reflex in the residual limb by electrically stimulating the spinal dorsal roots. We placed round adhesive electrodes paravertebrally of the T12-L1 spinous processes. We placed return electrodes on each anterior superior iliac spine. We wrapped

the participant's torso using Coban wrap and placed a small piece of foam between the tSCS electrodes and the back of the chair. The stimulation pulses to evoke the PRM reflex were 1-ms long monophasic, cathodic, square wave pulse. For the purpose of evoking PRM reflexes in the residual limb, we stimulated through the tSCS electrode ipsilateral to the residual limb only. We determined the stimulation threshold for evoking a PRM reflex in the gastrocnemius muscles, followed by the maximum PRM reflex amplitude. The maximum PRM reflex amplitude was at either the stimulation amplitude past which the magnitude of the PRM reflex no longer increased or the maximum stimulation amplitude tolerated by the participant. In this study, we did not exceed a stimulation amplitude of 180 mA. We varied the stimulation amplitude to obtain recruitment curves for the M-waves PRM reflexes. Specifically, we stimulated 15 amplitudes between 5 mA below threshold and 10-15 mA above the M-wave maximum (or PRM reflex maximum) if tolerated or up to 180 mA in a random order. Each amplitude was repeated four times and stimuli were delivered 10 s apart.

We performed the pain pressure threshold test using an algometer. The pain pressure threshold test measures the minimum amount of pressure that the participant can tolerate at a specific location. We pushed the rubber tip (1 cm diameter) of the algometer onto the skin over muscle (not pushing on bone) on several locations of both the residual and intact limbs. The participant reported when the pressure became painful, at which point we removed the algometer and recorded the pressure magnitude.

We asked participants to rate their PLP in the last 24 hours using a visual analog scale (VAS) from 0 and 10, where 0 indicated no pain at all, and 10 indicated the worst pain imaginable. Participants completed the short-form McGill Pain Questionnaire to describe their pain prior to participation in the study as well as throughout the week. The McGill Pain Questionnaire evaluates the sensation, temporal changes, and strength of pain. The total McGill Pain Questionnaire score indicates the intensity and affect the pain has on their life, and a 5-point change in McGill Pain Questionnaire is considered clinically meaningful.

We delivered bilateral tSCS for neuromodulation continuously for 30 minutes, with a break at 15 minutes to inspect the stimulation site. The tSCS consisted of 1 ms long pulses with a 10 kHz carrier frequency, delivered at 30 Hz. We started with a low amplitude of stimulation (approximately 10-20 mA) and slowly increased the stimulation beyond PRM reflex threshold, according to the comfort of the participant.

All data were collected while the participant sat comfortably in a chair. At the beginning of each day, the participant rated their PLP over the last 24 hours using the VAS. We marked the location of the stimulation and EMG electrodes with a permanent marker to ensure consistent placement across the 5 days. Every day, we performed the M-wave and PRM reflex measures, followed by high-frequency tSCS for 30 minutes. At the beginning of the first and fifth days, the participant completed the McGill Pain Questionnaire, and we performed the pain pressure threshold test.

Study Protocol for study using transcutaneous spinal cord stimulation in lower limb amputees

Before participant comes in:

1. AO0 = control port on stimulator
2. PO0 = trigger port on stimulator
3. AO1 = DIGI port on TMSi
4. TMSi cables: HDEMG, 2x BPEMG, IMU
5. PW on stimulator = 1000 us; 100%, 10 us; Monophasic, negative; 10 V = 180 mA
6. Make sure Stimulator output is **disabled**

Pain Assessments:

1. Give participant MPQ, GQPLA Day 1: ☐ Day 5: ☐
2. Give participant VAS Day 1: ☐ Day 2: ☐ Day 3: ☐ Day 4: ☐ Day 5: ☐
3. Use algometer to get pain threshold in the following regions:

Residual limb	**Day 1 (lbf)**	**Day 5 (lbf)**
Bottom of stump		
Anterior 5 cm above stump		
Posterior 5 cm above stump		
Medial 5 cm above stump		
Lateral 5 cm above stump		
Anterior 10 cm above stump		
Posterior 10 cm above stump		
Medial 10 cm above stump		
Lateral 10 cm above stump		
Mid-quad		
Mid-hamstring		
Intact limb		
On heel		
Ball of foot		
Top of foot		
Back of ankle		
Mid-shin (TA)		
Mid-calf		
Mid-quad		
Mid-hamstring		
Left arm		
Mid-biceps		
Right arm		
Mid-biceps		

Prep Participant:

1. Prep skin on amputated limb
2. Place and tape EMG electrodes
    a. HDEMG: SOL; BP1: LG; BP2: MG; BP3: TA; BP4: Q
    b. Orientation of grid: _____
3. Place IMU on lateral side of stump
4. Place tibial nerve electrodes
5. Wrap the leg in Coban
6. Place tSCS electrodes: **2** round adjacent to T12 (L1 = belly button, L4 = IC); **2** rect on ICs
7. Turn on TMSi

Record Impedances:

1. Run reflex GUI (*StimHreflexTMSiv15.m*)
2. Change name of file to "SubjectID_ DayX_Impedacnes_R1", reset counter to 1
   Day 1: ☐ Day 2: ☐ Day 3: ☐ Day 4: ☐ Day 5: ☐

tSCS threshold:

3. Place foam behind participant's back
4. Make sure stimulator PW is 1000 us
5. Change name of file to "SubjectID_DayX_tSCSthresh_ R1", reset counter to 1
6. Make sure GUI in PRM-reflexes mode
7. Calibrate stimulator
8. **Enable** stimulator
9. Set stimulation amplitude to 10 mA
10. Stimulate single pulses
11. Increase in 5 mA increments until have PRM-reflex in 1 of 3 stimulations
12. Note down tSCS threshold:
    Day 1: occurred at _____mA in _____ muscle
    Day 2: occurred at _____mA in _____ muscle
    Day 3: occurred at _____mA in _____ muscle
    Day 4: occurred at _____mA in _____ muscle
    Day 5: occurred at _____mA in _____ muscle

tSCS PRM max:

1. Change name of file to "SubjectID_DayX_tSCSmax_R1", reset counter to 1
2. Stimulate at 5 mA above threshold and observe PRM response
3. Increase by 5-10 mA until have maximum amplitude
4. Note down tSCS max:

Day 1: occurred at _____mA in _____ muscle. Max defined by: _____
Day 2: occurred at _____mA in _____ muscle. Max defined by: _____
Day 3: occurred at _____mA in _____ muscle. Max defined by: _____
Day 4: occurred at _____mA in _____ muscle. Max defined by: _____
Day 5: occurred at _____mA in _____ muscle. Max defined by: _____

5. **Disable** stimulator

<u>tSCS recruitment curve:</u>

1. Calibrate stimulator
2. **Enable** stimulator
3. Change name of file to "SubjectID_ DayX_tSCSRC_ R1", reset counter to 1
4. Set limits to below threshold and above max
    a. Amplitude range:
    Day 1: _____mA to _____mA, notes: _____
    Day 2: _____mA to _____mA, notes: _____
    Day 3: _____mA to _____mA, notes: _____
    Day 4: _____mA to _____mA, notes: _____
    Day 5: _____mA to _____mA, notes: _____
5. Stimulate RC (default settings: 10 s between points, 4 reps each, 15 sweeps)
    *Note: this takes 10 minutes to complete – warn the participant
6. 1.3x threshold:

Day 1 = _____mA, Day 2 = _____mA, Day 3 = _____mA, Day 4 = _____mA, Day 5 = _____mA

<u>tSCS rate-dependent depression:</u>

1. Change name of file to "SubjectID_ DayX_tSCSRDD_R1", reset counter to 1
2. Set min and max amplitudes to 1.3x threshold
3. Stimulate **5** pulses with the time between pulses equal to 10 s (0.1 Hz)
    Day 1 notes: _____
    Day 2 notes: _____
    Day 3 notes: _____
    Day 4 notes: _____
    Day 5 notes: _____
4. Stimulate **5** pulses with the time between pulses equal to 1 s (1 Hz)
    Day 1 notes: _____
    Day 2 notes: _____
    Day 3 notes: _____
    Day 4 notes: _____
    Day 5 notes: _____
5. **Disable** stimulator

H-reflex threshold:

1. Strap leg to put pressure on electrodes
2. Change name of file to “SubjectID_ DayX_Hthresh_R1”, set counter to 1
3. Make sure GUI in H-reflex mode
4. Calibrate stimulator
5. **Enable** stimulator
6. Set stimulation amplitude to 5 mA, stimulate single pulse
7. Increase in 1-5 mA increments until have H-reflex in 1 of 3 stimulations
8. Note down H-reflex threshold:
   Day 1: occurred at _____mA in _____ muscle
   Day 2: occurred at _____mA in _____ muscle
   Day 3: occurred at _____mA in _____ muscle
   Day 4: occurred at _____mA in _____ muscle
   Day 5: occurred at _____mA in _____ muscle

M-max on peripheral nerve:

1. Change name of file to “SubjectID_ DayX_Mmax_R1”, reset counter to 1
2. Set stimulation amplitude to 5 mA above H-reflex threshold
3. Stimulate single pulse, observe motor response
4. Stimulate at higher amplitudes until motor response no longer increasing
   a. Note: Pk-pk amp in *EMGpk* variable
5. Note down M-max:
   Day 1: occurred at _____mA in _____ muscle
   Day 2: occurred at _____mA in _____ muscle
   Day 3: occurred at _____mA in _____ muscle
   Day 4: occurred at _____mA in _____ muscle
   Day 5: occurred at _____mA in _____ muscle
6. **Disable** stimulator

Peripheral recruitment curve:

1. Calibrate stimulator
2. **Enable** stimulator
3. Change name of file to “SubjectID_ DayX_PeriphRC_R1”, reset counter to 1
4. Set limits to below H-reflex threshold and above M-max
   a. Amplitude range:
      Day 1: _____mA to _____mA, notes: _____
      Day 2: _____mA to _____mA, notes: _____
      Day 3: _____mA to _____mA, notes: _____
      Day 4: _____mA to _____mA, notes: _____
      Day 5: _____mA to _____mA, notes: _____

5. Stimulate RC (default settings: 10 s between points, 4 reps each, 15 sweeps)
   *Note: this takes 10 minutes to complete – warn the participant
6. 1.3x threshold:

Day 1 = _____mA, Day 2 = _____mA, Day 3 = _____mA, Day 4 = _____mA, Day 5 = _____mA

<u>Peripheral rate-dependent depression:</u>

1. Change name of file to "SubjectID_periphRDD_DayX_R1", reset counter to 1
2. Set min and max amplitudes to 1.3x threshold
3. Stimulate 20 pulses with the time between pulses equal to 10 s (0.1 Hz)
   Day 1 notes: _____
   Day 2 notes: _____
   Day 3 notes: _____
   Day 4 notes: _____
   Day 5 notes: _____
4. Stimulate 20 pulses with the time between pulses equal to 1 s (1 Hz)
   Day 1 notes: _____
   Day 2 notes: _____
   Day 3 notes: _____
   Day 4 notes: _____
   Day 5 notes: _____
5. **Disable** stimulator
6. Close reflex GUI

<u>tSCS neuromodulation:</u>

1. Ask participant if they need a break or to use the washroom
2. Set stimulator pulse width to 40 us; output to biphasic
3. Change control of output to the dial
4. Connect bilateral stimulation lead wires
5. Open neuromod GUI (*tSCSguiControlStimv6.m*)
6. **Enable** stimulator
7. Set duration to 15 minutes (900 s), start stimulation, with low amplitude set on the dial
8. Slowly increase stimulation as they get used to the feeling and contractions
9. Set amplitude to
   Day 1: _____ mA (engagement of back muscles)
   Day 2: _____ mA (engagement of back muscles)
   Day 3: _____ mA (engagement of back muscles)
   Day 4: _____ mA (engagement of back muscles)
   Day 5: _____ mA (engagement of back muscles)
10. Check skin under stimulation electrodes

11. Begin second stimulation (15 minutes (900 s))
    Day 1: _____ mA (engagement of back muscles)
    Day 2: _____ mA (engagement of back muscles)
    Day 3: _____ mA (engagement of back muscles)
    Day 4: _____ mA (engagement of back muscles)
    Day 5: _____ mA (engagement of back muscles)
12. **Disable** stimulator
13. Disconnect bilateral tSCS lead

REPEAT REFLEXES – **Make new folder**

Record Impedances:

1. Run reflex GUI (*StimHreflexTMSiv15.m*)
2. Change name of file to "SubjectID_ DayX_Impedacnes_R2", reset counter to 1
   Day 1: ☐ Day 2: ☐ Day 3: ☐ Day 4: ☐ Day 5: ☐

tSCS threshold:

1. Make sure stimulator PW is 1000 us, set to control mode
2. Place foam behind participant's back
3. Change name of file to "SubjectID_ DayX_tSCSthresh_R2", reset counter to 1
4. Make sure in PRM-reflexes mode
5. Calibrate stimulator
6. **Enable** stimulator
7. Set stimulation amplitude to 10 mA
8. Stimulate single pulses
9. Increase in 5 mA increments until have PRM-reflex in 1 of 3 stimulations
10. Note down tSCS threshold:
    Day 1: occurred at _____mA in _____ muscle
    Day 2: occurred at _____mA in _____ muscle
    Day 3: occurred at _____mA in _____ muscle
    Day 4: occurred at _____mA in _____ muscle
    Day 5: occurred at _____mA in _____ muscle

tSCS PRM max:

1. Change name of file to "SubjectID_ DayX_tSCSmax_R2", reset counter to 1
2. Stimulate at 10 mA above threshold and observe PRM response
3. Increase by 5-10 mA until have similar amplitude in response as M-max
4. Note down tSCS max:

Day 1: occurred at _____mA in _____ muscle. Max defined by: _____
Day 2: occurred at _____mA in _____ muscle. Max defined by: _____
Day 3: occurred at _____mA in _____ muscle. Max defined by: _____
Day 4: occurred at _____mA in _____ muscle. Max defined by: _____
Day 5: occurred at _____mA in _____ muscle. Max defined by: _____

5. **Disable** stimulator

<u>tSCS recruitment curve:</u>

1. Calibrate stimulator
2. **Enable** stimulator
3. Change name of file to "SubjectID_ DayX_tSCSRC_R2", reset counter to 1
4. Set limits to below threshold and above max
    a. Amplitude range:
        Day 1: _____mA to _____mA, notes: _____
        Day 2: _____mA to _____mA, notes: _____
        Day 3: _____mA to _____mA, notes: _____
        Day 4: _____mA to _____mA, notes: _____
        Day 5: _____mA to _____mA, notes: _____
5. Stimulate RC (default settings: 10 s between points, 4 reps each, 15 sweeps)
    *Note: this takes 10 minutes to complete – warn the participant
6. 1.3x threshold:

Day 1 = _____mA, Day 2 = _____mA, Day 3 = _____mA, Day 4 = _____mA, Day 5 = _____mA

<u>tSCS rate-dependent depression:</u>

1. Change name of file to "SubjectID_DayX_tSCSRDD_R2", reset counter to 1
2. Set min and max amplitudes to 1.3x threshold
3. Stimulate **5** pulses with the time between pulses equal to 10 s (0.1 Hz)
    Day 1 notes: _____
    Day 2 notes: _____
    Day 3 notes: _____
    Day 4 notes: _____
    Day 5 notes: _____
4. Stimulate **5** pulses with the time between pulses equal to 1 s (1 Hz)
    Day 1 notes: _____
    Day 2 notes: _____
    Day 3 notes: _____
    Day 4 notes: _____
    Day 5 notes: _____
5. **Disable** stimulator

H-reflex threshold:

1. Change electrodes to tibial nerve electrodes, put strap on the leg
2. Change name of file to “SubjectID_ DayX_Hthresh_R2”, set counter to 1
3. Make sure GUI in H-reflex mode
4. Calibrate stimulator
5. **Enable** stimulator
6. Set stimulation amplitude to 5 mA, stimulate single pulse
7. Increase in 1-5 mA increments until have H-reflex in 1 of 3 stimulations
8. Note down H-reflex threshold:
   Day 1: occurred at _____mA in _____ muscle
   Day 2: occurred at _____mA in _____ muscle
   Day 3: occurred at _____mA in _____ muscle
   Day 4: occurred at _____mA in _____ muscle
   Day 5: occurred at _____mA in _____ muscle

M-max on peripheral nerve:

1. Change name of file to “SubjectID_DayX_Mmax_R2”, reset counter to 1
2. Set stimulation amplitude to 5 mA above H-reflex threshold
3. Stimulate single pulse, observe motor response
4. Stimulate at higher amplitudes until motor response no longer increasing
   a. Note: Pk-pk amp in *EMGpk* variable
5. Note down M-max:
   Day 1: occurred at _____mA in _____ muscle
   Day 2: occurred at _____mA in _____ muscle
   Day 3: occurred at _____mA in _____ muscle
   Day 4: occurred at _____mA in _____ muscle
   Day 5: occurred at _____mA in _____ muscle
6. **Disable** stimulator

Peripheral recruitment curve:

1. Calibrate stimulator
2. **Enable** stimulator
3. Change name of file to “SubjectID_ DayX_PeriphRC_R2”, reset counter to 1
4. Set limits to below H-reflex threshold and above M-max
   a. Amplitude range:
      Day 1: _____mA to _____mA, notes: _____
      Day 2: _____mA to _____mA, notes: _____
      Day 3: _____mA to _____mA, notes: _____
      Day 4: _____mA to _____mA, notes: _____
      Day 5: _____mA to _____mA, notes: _____

5. Stimulate RC (default settings: 10 s between points, 4 reps each, 15 sweeps)
   *Note: this takes 10 minutes to complete – warn the participant
6. 1.3x threshold:

Day 1 = _____mA, Day 2 = _____mA, Day 3 = _____mA, Day 4 = _____mA, Day 5 = _____mA

<u>Peripheral rate-dependent depression:</u>

1. Change name of file to “SubjectID_periphRDD_DayX_R2”, reset counter to 1
2. Set min and max amplitudes to 1.3x threshold
3. Stimulate 20 pulses with the time between pulses equal to 10 s (0.1 Hz)
   Day 1 notes: _____
   Day 2 notes: _____
   Day 3 notes: _____
   Day 4 notes: _____
   Day 5 notes: _____
4. Stimulate 20 pulses with the time between pulses equal to 1 s (1 Hz)
   Day 1 notes: _____
   Day 2 notes: _____
   Day 3 notes: _____
   Day 4 notes: _____
   Day 5 notes: _____
5. **Disable** stimulator
6. Close reflex GUI

Notes:

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Statistical Analysis Plan

We tested for normality using the Shapiro–Wilk test and assessed the homogeneity of variance using Levene's test. A p-value < 0.05 was considered significant.

Grouped threshold data contain the mean threshold across all channels of the HD-EMG grid. We performed a linear correlation between the PRM reflex threshold for each electrode on the HD-EMG grid with its impedance value. We expressed reflex thresholds in units of charge (μC), obtained by multiplying the stimulation amplitude (in mA) by the pulse width (1 ms). To compare the PRM reflex thresholds over 5 days, we performed a one-way analysis of variance (ANOVA) with Bonferroni post-hoc tests.

We created recruitment curves for the PRM reflexes, M-waves, and F-waves by plotting the mean peak-to-peak amplitude of the response from each electrode on the HD-EMG grid as a function of stimulation amplitude. The slope of the recruitment curve corresponded to the slope of the line between the inflection points across the steepest part of the curve.

We determined the change in PPT over time by subtracting the PPT at each location on Day 1 from Day 5. We normalized the PPT at each location to the maximum PPT value recorded for each participant. We expressed the change in PPT on a scale between -1 and 1, where -1 indicated a maximal decrease in PPT, 1 indicated a maximal increase in PPT, and 0 indicated no change in PPT. We also compared the average PPT across all tested locations on each limb between day 1 and day 5 using a paired t-test.

We used repeated measures ANOVA to compare the mean VAS score from all participants across the 5 days. A decrease in the VAS score by 50% and at least 1 point is considered clinically meaningful. We summated the responses from each subsection of the MPQ to obtain a total score. A clinically meaningful decrease in MPQ score is a 5-point decrease.